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College of Health and Allied Sciences
University of Cape Coast, Ghana

A Message from the Editor

Bridging Tradition and Science in Herbal Medicine

The evolution of man and that of plants have an inseparable connection. Since time immemorial, the birthplace of man has also been the birthplace of medicine, where the harmonious interplay of nature and need gave rise to what is today known as African Traditional Medicine (ATM). As we embark upon this special issue on Traditional and Herbal Medicine: Bridging Ancient Wisdom and Modern Health Research by the Integrated Health Research Journal (IHRJ), we stand at an important crossroad. We are not only preserving the knowledge of our forefathers but also integrating their botanical legacy into contemporary medical practice through rigorous scientific investigation.

The African Ecological Imperative

Africa is not simply a continent; it is an evolutionary laboratory of remarkable resilience. The diverse landscapes of Africa include dense rainforests with their misty canopies in the Congo region, arid areas in the Sahel zone, and fynbos vegetation in the southern cape. These ecosystems have provided Africa with its botanical richness, which is impressive, yet astonishingly understudied.

The "peculiar" nature of African plants, which has captured the interest of pharmacologists worldwide, is attributed to the peculiar climatic conditions in Africa. Plants indigenous to Africa have adapted to the harsh environmental conditions, such as intense ultraviolet exposure, long periods of drought, and high temperatures. To sustain themselves, these plants produce intricate secondary metabolites with significant bioactivity that provide protection for the plant while offering medicinal value to humans.

The possible anti-malarial activity in Cryptolepis sanguinolenta and the immune-modulatory effects in the African baobab tree, among many other promising natural products, show that Africa's "green gold" is still an under-explored source of molecular diversity. However, even though Africa harbors around 25% of all the plants on earth, only a few have been investigated scientifically.

A Global Perspective

While Africa's botanical diversity is indeed exceptional, traditional and herbal medicine is a practice that transcends borders. We are also proud to present contributions from other parts of the world, including India, Indonesia, and Pakistan, where traditional medicine systems have deep historical roots and have long been practiced. In these regions, herbal medicine is not only a part of cultural heritage but also a living practice, supported by generations of knowledge and experience.

These global perspectives enrich this issue by highlighting how traditional and herbal medicine is being integrated into modern healthcare systems worldwide, particularly in countries where it has been practiced for centuries.

Bridging the Gap: The Role of CoHAS and IHRJ

At CoHAS, we are fully aware that when talking about "integration" under Integrated Health, this is not merely a buzzword; rather, this is a scientific requirement. Herbal medicine is no longer just an alternative treatment but is actually the only existing treatment method for many Africans.

One of the objectives of the Integrated Health Research Journal is to create the academic background needed to shift traditional medicine practices into standardized medicine practice. Although the theme for this special issue was centered around three primary concerns in herbal medicine, namely validation, standardization, and conservation, not

all submitted manuscripts tackled the topic explicitly. Nonetheless, our efforts continue to explore these three issues as integral elements of integrated health care.

From Folklore to Formal Science

Indeed, one of the main problems associated with African traditional herbal medicine was always the absence of clinically documented information, as well as the lack of standardized preparation protocols. The specific climatic conditions of the continent imply that an herb collected in the wet season in Ghana will have a completely different chemical composition compared to an herb grown in the arid areas of its neighbors. It is in studying these factors that lies the future of African ethnopharmacology.

This collection of papers sheds light on these factors and discusses how to utilize the unique properties of local flora to tackle the ever-growing problem of non-communicable diseases, antibiotic resistance, and new viruses. Indeed, by stressing the importance of endemicity, the authors draw attention to the solutions lying right outside our doors.

A Call to Action

As we embark on this unique issue, we urge the academic, medical, and indigenous communities to look at the plants in Africa from an awe-filled, but equally scientific perspective. At UCC, we are proud of our interdisciplinary focus, creating a synergy between lab work and the environment.

There is no doubt about the "African advantage" in research into botanical medicine—the biological diversity of our continent, not to mention the depth of its traditional wisdom, is without parallel. The challenge before us is to make sure that this abundant resource can be made available for the benefit of all.

Our sincere wish is that the papers contained here would spark a new era of botanical discovery period during which we pay homage to the ecology of our land even as we strive for scientific excellence.

Acknowledgment of Guest Editors and Reviewers

We would like to convey our sincere gratitude to the guest editors who have made a significant contribution: Dr. Malik Suliman Mohamed from the Department of Pharmaceutics, College of Pharmacy, Jouf University, Saudi Arabia; Dr. Isaac Tabiri Henneh from the Department of Pharmacotherapeutics and Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, University of Cape Coast, Ghana; Dr. Ahmed Arbab from the Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum, Sudan; and Dr. Amina Dirar from the Natural Products Research Department, National Centre for Research Khartoum, Sudan.

Our sincere thanks go out to all those who served as reviewers for their tremendous help in making this issue a reality. Your valuable feedback has played an important role in bringing about this publication, and we truly thank you for your efforts.

Prof. Konozy EH.,

Editor-in-Chief, IHRJ

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COMMENTARY

Documentation of medicinal plants of The Sudan, methods applied and future prospects

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In an attempt to establish a national work plan or program for studying medicinal plants and their therapeutic uses, it is primarily important to identify and document all available information in the field. Documentation of medicinal plants is considered crucial in preserving the loss of orally transmitted indigenous cultural heritage, identifying new therapeutic compounds for further pharmacological studies, securing the intellectual property rights for local communities, and facilitating conservation processes to overwhelm overexploitation through sustainable utilization.

Sudan is located in northeast Africa, bordered by seven countries and has a substantial coastline along the Red Sea. It is bounded by Egypt to the north, the Red Sea to the northeast, Eritrea and Ethiopia to the east, South Sudan to the South, Central African Republic to the southwest, and Chad to the west and Libya to the Northwest. Such a location places the country at the intersection of sub-Saharan Africa, Middle East and stretches across the Red Sea.

The vegetation of the country follows a north-south moisture gradient and an east-west elevation gradient [1]. The north-south gradient, shifting from arid desert, semi-arid, Sahelian shrublands and further into Sudanian Wooded Savanna. These vegetation belts extend across most West African countries. The east-west gradient, transitioning from the Ethiopian Highlands towards the Nile.

Sudan with such characteristic location, diverse vegetation, climatic conditions and different ethnic communities retain a unique blend of indigenous cultures with East and West African, Arabian and Egyptian cultures. As a result, The Sudan harbours a high diversity of medicinal plants that will continue to play an important role in the health sector by providing natural sources for traditional remedies and modern pharmaceuticals.

Documentation of medicinal plants in The Sudan started early in the 19th century by "Wellcome Tropical Research Laboratories Reports (1906-1911)", followed by "Wellcome Chemical Laboratories Reports (1964-1960)" and Broun and Massey [2] working with the flora of The Sudan. Since then, great efforts were done to document the wealth of the country in the field of medicinal plants in a systematic way to cover all parts of The Sudan.

"Atlas of Medicinal Plants of The Sudan", is a project fostered by Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan. It is a long-term project proposed in 1972 with the establishment of the "Medicinal and Aromatic Herbs Research Unit, Medical Research Council, National Council for Research". The project was designed to document primary information on the medicinal plants and their folkloric uses in the different Regions/States of The Sudan. Medicinal plants of each Region/ State will be published in a separate part. In the last phase of the project, all published parts will encompass the "Atlas of Medicinal Plants of The Sudan". It was planned that this Atlas will follow recent scientific approaches in applying up-to-date Latin botanical names using the international online databases e.g. "International Plant Names Index" – IPNI (https://www.ipni.org) and "Plants of the World Online" – POWO (https://powo.science.kew.org). Habit and habitat of each plant, local/ vernacular names and

line illustrations are also included to attract a wide array of users. To permanently preserve the plants claimed to possess therapeutic activities, samples from all parts of the plants should be pressed, dried, mounted in sheets and deposited at the herbarium of the Institute in the form of voucher specimens. These specimens must be accompanied by detailed data including the collector's name, specific geographical location, date of collection and habitat information. The informants recruited in the semi-structured questionnaires should also be identified and included in subsequent publications and patents to prevent misappropriations of their intellectual property rights.

In over fifty years, wide areas of the country were surveyed including Erkowit (Red Sea State) [3], Eastern Nuba Mountains (South Kordofan State) [4], White Nile State [5], Northern Kordofan [6], Khartoum State [7], Ingassana (Blue Nile State) [8], Red Sea State [9], River Nile State 10, and Northern State [11,12].

At present, medicinal plants of Gezira State are understudy and will follow the same methodology applied in the previous parts of the "Atlas". An initial pilot surveys were already done to gather preliminary data and to establish base-line information for further field work. Literature surveys were conducted as well on geography, demography and flora of the State. Lists of the herbalists who will participate in the ethnobotanical studies involving interviews and questionnaires with local community were also prepared. Unfortunately, the process of the project was suspended by the present civil war in the country.

Besides the areas covered by the "Atlas of Medicinal Plants of The Sudan", a considerable number of researches were reported to document the medicinal plants of different regions of The Sudan. These regions include: West Kordofan [13], South Kordofan [14], Darfur [15], Fangoga area (Sennar State) [16].

Both wild native plants and exotic plants play an important role in herbal remedies and were put in consideration in the documentation of the medicinal plants of The Sudan. Wild plants are non-cultivated plants growing in natural habitats, contributing to biodiversity and ecological interactions, and are progressively threatened by climatic changes and habitat loss. Exotic plants on the other hand are alien, non-native highly valued plants introduced intentionally and mainly utilized for cultural purposes, food and agriculture, and as ornamental landscaping [17].

Exotic medicinal plants play an important role in the traditional primary healthcare, to treat different human and animal ailments alongside with wild species and to the management of their health. The use of exotic medicinal plants as an alternative source of medicinal remedies could alleviate harvesting pressure of wild indigenous plants thereby enhancing biodiversity [18]. In addition, although there is strong belief among indigenous population that wild plants have more effective therapeutic benefits, these exotic plants fill the gaps not met by wild plants, diversify the local repertoire of medicinal plants of the area [19], and address modern health challenges [20].

The use of exotic plants in traditional medicine by indigenous population is well accepted among local communities in many African countries e.g. Angola [21], Ghana [22], Ethiopia [23], Kenya [24], South Africa [18]. It is worth mentioning that the use of exotic plants accumulated wide acceptance in some communities in Angola (Songo City) and the majority of medicinal plants used as indigenous remedies are exotic (71 %) compared to native plants (29 %) [21].

In the Sudan, wild and exotic herbal medicines are widely used for treating and managing ailments by rural and urban populations with different frequencies. In Khartoum State, the capital city, accommodating urban population, exotic species represent about (67 %) of the total species claimed to possess therapeutic properties [7], whereas in Northern State, wild species represent (55 %) of the total species reputed to acquire therapeutic potentials [11,12], highlighting that urban inhabitants rely mostly on exotic species.

Members of the families Fabaceae (Leguminosae), Asteraceae, Euphorbiacae, Capparaceae and Cucurbitaceae, constitute major sources of indigenous remedies in Sudan. Although The Sudan has no formal pharmacopoeia, a number of wild plants were recorded in International "Herbal Pharmacopoeia". These plants include: *Abrus precatorius* L., *Boscia integrifolia* J.St.-Hil., *Calotropis procera* (Aiton) W.T. Aiton, *Citrullus colcynthis* (L.) Schrad, *Ricinus communis* L., *Senegalis senegal* (L.) Briton, *Senna alexandrina* Mill., *Tamarindus indica* L., *Terminalia leiocarpa* (DC.) Baill. and *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. In the absence of formal pharmacopoeia, traditional herbal practitioners rely mainly on indigenous knowledge, and various curative methods of preparation, administrative routs, specialized techniques (cutting, stripping/ scraping, uprooting, etc.) of therapeutic recipes are applied. These practitioners claim to be able to cure a wide range of conditions including: abdominal colic, arthritis, diabetes, diarrhea, dysentery, epilepsy, gallstones, hemorrhoids, jaundice, leprosy and nephritis. In a systematic bibliographic investigation on the medicinal plants of The Sudan [25], analyzing numerous publications covering diverse sources, websites and research engines, Sudanese medicinal plants have been documented to exert anticancer, antimicrobial, protozoal, insecticidal, molluscicidal, toxicological, physiological and pharmacological activities.

Various parts of the plant are used in the preparation of herbal remedies including whole plants, leaves, roots/ tubers/rhizomes, bark, seeds, flowers and fruits. Medicinal properties derived from plants can also come from specialized exudates. These exudates are plant derived natural bioactive compounds discharged externally to

their surfaces by specialized cells in different plant parts. They are categorized into resins, gums, mucilage and sap based on their physico-chemical characteristics. The main benefit of harvesting plant exudates is that the gathering is safe and can be utilized over the lifespan of the plant. Over-collection and harvesting of roots or whole plants in the preparation of herbal remedies presents great threat to viability of the plant and ecosystem, and will provoke unsustainable development of plant-based products. These potential threats could be addressed by cultivation, sustainable foraging, community engagement and the use of exotic plant to alleviate harvesting pressure on wild indigenous plants.

In The Sudan, numerous barriers are faced in utilizing and effective dissemination of indigenous knowledge due to certain traditional beliefs. This knowledge is regarded as family heritage or legacy and should be transferred only from fathers to sons. Others considered this knowledge as top confidential or tricks of the trade and worry if revealed it will lose its activities and become ineffective in remedies. Due to such attitudes, herbalism is recognized as a "stingy profession" by a wide array of ethnobotanical personals. They are encouraged to unveil their knowledge by referring to our affiliation to a "National Research Institute" and these knowledge if found genuine their intellectual property rights will be secured.

The present study showed that The Sudan is highly bio diverse and rich in ethnobotanical heritage and that herbal remedies, utilizing both wild and exotic plants, are widely used in different rural and urban populations with different frequencies. Documentation of such diverse medicinal plants is crucial in health sector by providing natural sources for traditional remedies and in identifying potential plants to be addressed for future investigations for novel bioactive compounds.

It is urgent to document the indigenous knowledge of all States/Provinces of the country before being perished by urbanization/ modernization and conservative strategies should be put forwards for sustainable harvesting. Future investigations should be carried out on all potential sources to provide quality standards by studying their phytochemistry, pharmacology, microscopic and macroscopic features, potential side effects, drug interactions, etc., and ultimately to adopt the long waiting "Sudanese Herbal Pharmacopoeia".

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RESEARCH ARTICLE

MET-Mediated Drug Resistance in Colorectal Cancer: In Silico Analysis of the Potential of Epicatechin-3-Gallate and Epigallocatechin-3-Gallate as Therapeutic Adjuvants

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Abstract

Background: Colorectal cancer (CRC) is the third most common cancer worldwide by incidence and the second leading cause of cancer-related death. Although chemotherapy has improved survival rates, long-term use often leads to chemoresistance and significant side effects. Therefore, new adjuvant strategies are urgently needed. This study examines the potential of tea catechins (Camellia sinensis), specifically epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG), as adjuvants to overcome chemoresistance in CRC.

Methods: We used a computational approach combining network pharmacology and molecular docking.

Results: Protein target analysis showed that ECG and EGCG specifically target four proteins, including the key hub protein c-Met (MET). The MET protein plays a vital role in CRC chemoresistance, especially in response to anti-angiogenic therapy. This potential is supported by patient survival data indicating a poor prognosis for CRC patients with MET overexpression. The molecular docking results suggest that EGCG and ECG bind strongly to c-Met, with binding energies of -9.2 kcal/mol and -9.1 kcal/mol, respectively. This high affinity supports the idea that ECG and EGCG can directly modulate c-Met's various functions.

Conclusion: This in silico study provides a solid molecular basis for developing tea catechins as chemosensitizers to improve chemotherapy effectiveness and reduce chemoresistance in CRC.

Keywords: Chemoresistance, Colorectal Cancer, c-Met, Epigallocatechin-3-Gallate, Ethnopharmacology

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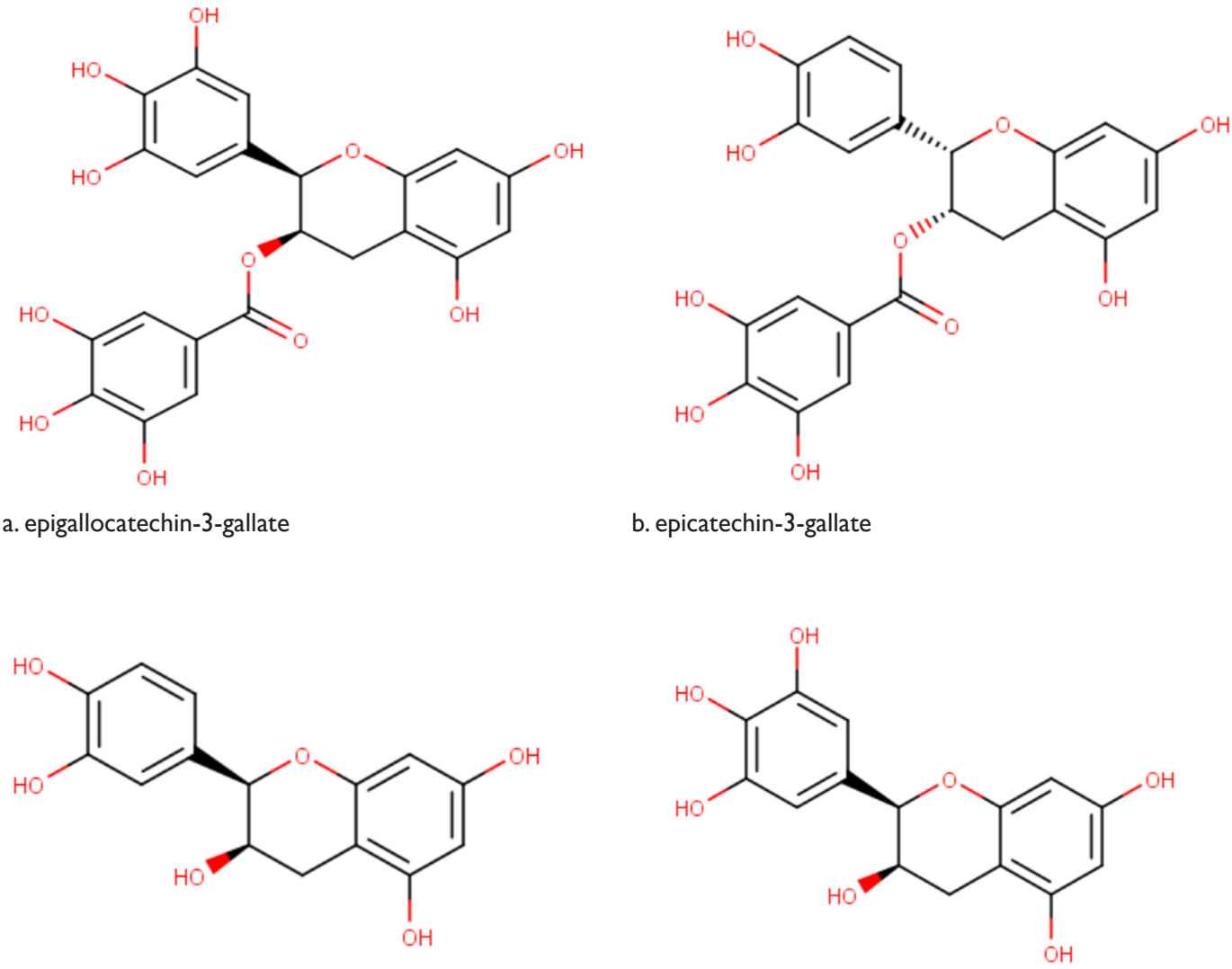
Introduction

Colorectal cancer (CRC) remains a significant global public health challenge; it is currently third in incidence and second in cancer-related deaths [1]. The World Health Organization (WHO) reported about 1.9 million new CRC cases globally in 2020 [1]. In Indonesia, registry data and clinical studies show increasing incidence, geographic differences, and a shift toward younger age groups. Additionally, treatment outcomes in Indonesia are affected by the fact that most patients are diagnosed at an advanced stage [2–4].



a. epigallocatechin-3-gallate

b. epicatechin-3-gallate

c. epicatechin

d. epigallocatechin

Figure 1. Chemical structures of tea catechins: a) epigallocatechin-3-gallate, b) epicatechin-3-gallate, c) epicatechin, and d) epigallocatechin (source: PubChem database).

Although improved screening and treatment have increased CRC survival rates, 5-fluorouracil (5-FU)-based regimens remain the cornerstone of care in both curative and palliative settings [5,6]. These chemotherapeutic agents are often combined with targeted therapies to extend patient survival. However, prolonged chemotherapy use leads to serious issues, such as systemic toxicity and chemoresistance [7,8]. Resistance to these chemotherapeutic agents poses a major obstacle, resulting in treatment failure, disease recurrence, and a poor clinical outlook [9–11]. Along with clinical obstacles, limited access, and the high cost of targeted therapies, these factors restrict their adoption within Indonesia's healthcare financing system, thereby weakening their cost-effectiveness [12]. Therefore, developing new adjuvant strategies to enhance treatment results and reduce the emergence of chemoresistance has become an essential priority.

Natural products hold great potential as adjuncts in cancer treatment due to their multitarget effects, favorable toxicity profiles of certain compounds, and their abundance in diverse regions, such as Indonesia [13–15]. Various natural compounds have shown anticancer effects against CRC cells in preclinical studies [11,16,17]. Among the promising bioactive substances is catechin, a plentiful component of tea (Camellia sinensis). Catechin derivatives, such as epigallocatechin-3-gallate (EGCG), are known for their wide-ranging anticancer effects, including the ability to inhibit proliferation, induce apoptosis, and exhibit anti-angiogenic and anti-metastatic properties, as well as their established role as effective chemosensitizers in various cancer models [13,18–20].

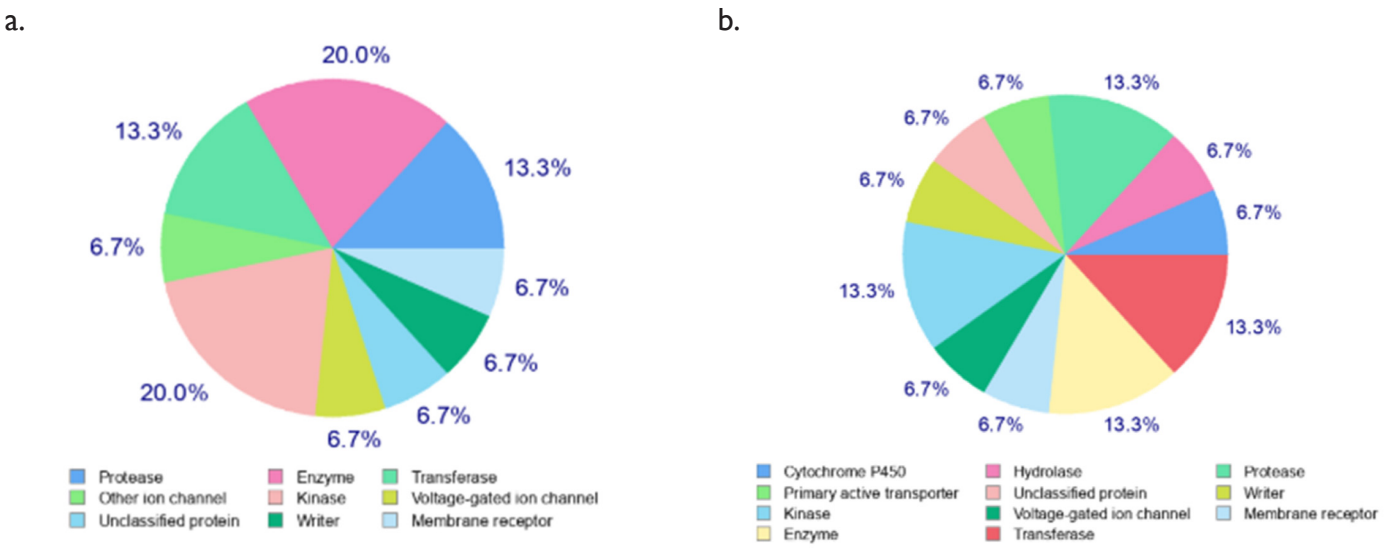


Figure 2. Target prediction results for (a) epicatechin-3-gallate and (b) epigallocatechin-3-gallate. Epicatechin and epigallocatechin did not identify any protein targets in Homo sapiens cells.

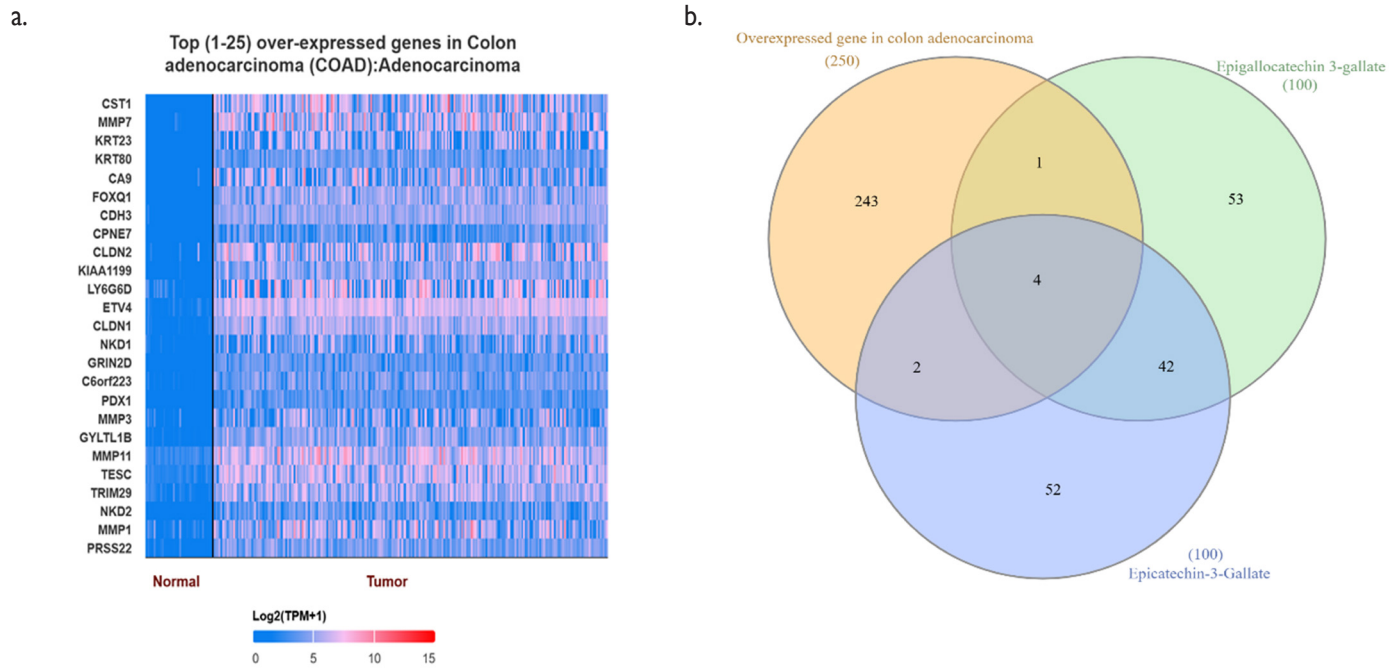


Figure 3. Gene expression analysis in CRC patients (a) revealed that 25 genes, including MMP7, CA9, and FOXQ1, were overexpressed compared to normal tissues. The predicted protein targets of epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) were combined with the overexpressed proteins in CRC to find common target proteins (b).

This study aims to perform a computational analysis and mechanistic review of the potential of tea catechins as adjuvant agents to overcome chemoresistance in colorectal cancer by targeting specific proteins. The results from this in silico analysis are expected to provide a framework for prioritizing relevant molecular targets, thereby supporting the development of more affordable and accessible adjuvant therapy strategies.

Materials and Methods

Structural Acquisition and Pharmacology Analysis

The three-dimensional (3D) structure of tea catechin compounds includes epicatechin (CID:72276), epicatechin-3-Gallate (CID: 65056), epigallocatechin (CID: 72277), and epigallocatechin-3-gallate (CID: 65064) obtained from a public chemical database, PubChem (National Center for Biotechnology Information) at (https://pubchem.ncbi.nlm.nih.gov/) [21]. The physicochemical and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity)

properties of catechin compounds were analyzed using the pKCSM server at https://swissadme.ch/ [22]. The data was accessed in January 2026.

Gene Expression Analysis in Colorectal Cancer Patients

Gene expression data from CRC patients were analyzed to identify clinically relevant protein targets. This data was accessed through the UALCAN website at https://ualcan.path.uab.edu/analysis.html [23], which provides access to The Cancer Genome Atlas (TCGA) data. Proteins that showed overexpression in CRC tissue compared to normal tissue were collected and downloaded as relevant CRC targets. The data was accessed in January 2026.

Target Integration and Protein Interaction Analysis

Potential target proteins of tea catechins in the context of CRC were predicted using a machine-learning-based target prediction server, namely SwissTargetPrediction, available at https://swisstargetprediction.ch [24]. The prediction

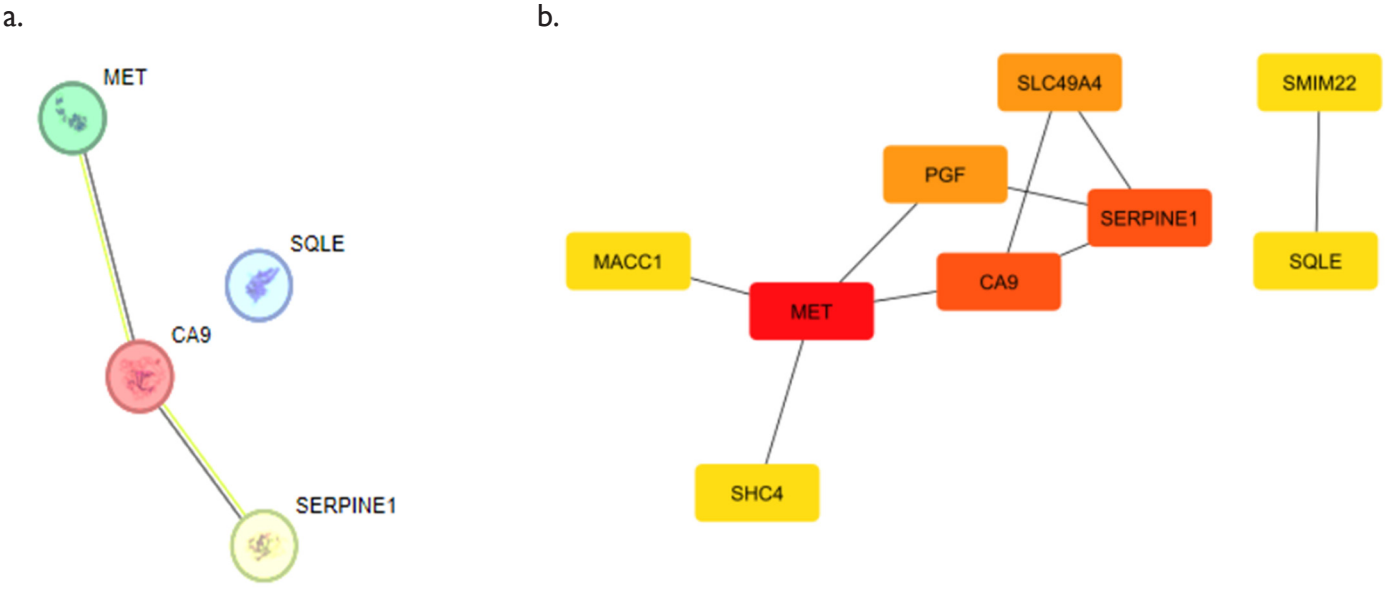


Figure 4. Protein interaction networks created using the STITCH server (a) and Cytoscape software (b).

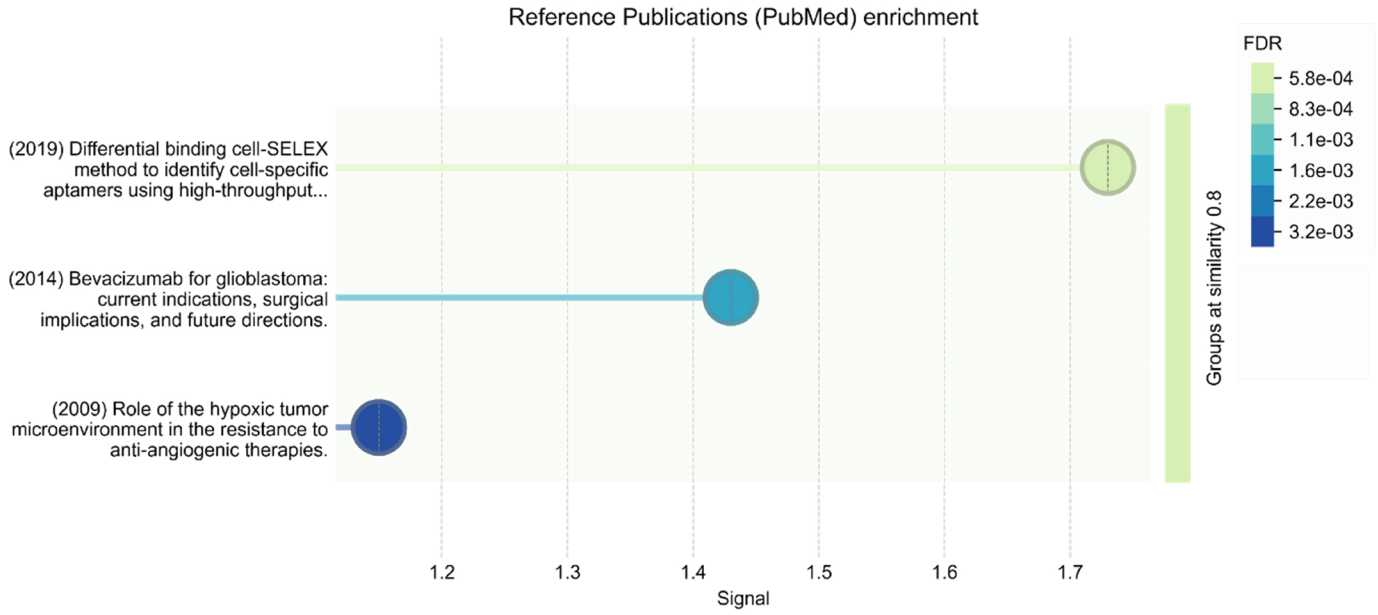


Figure 5. Gene Ontology (GO) and pathway enrichment analyses of the prioritized target proteins.

Table 1. Assessment of the physicochemical properties and drug-likeness of tea catechin derivatives

Description	Compound				
	EC	ECG	EGC	EGCG	Capmatinib
Molecular weight < 500 g/mol	290.271	442.376	306.27	458.375	412.428
Log P < 5	1.54	2.52	1.25	2.23	3.42
Hydrogen bond acceptors < 10	6	10	7	11	6
Hydrogen bond donors < 5	5	7	6	8	1
Rotatable Bonds ≤ 10	1	3	1	3	4
Surface Area Å²	119.662	179.948	124.456	184.742	176.164
Lipinski's Violation	No	1	1	2	No

Table 2. Data on absorption, distribution, metabolism, excretion, and toxicity (ADMET) of tea catechin

Description	Compound				
	EC	ECG	EGC	EGCG	Capmatinib
Huma intestinal absorption (HIA) (%)	68.829	62.096	54.128	47.395	95.104
Caco2 permeability (log pap)	-0.283	-1.264	-0.375	-1.521	1.344
P-gp substrate	Yes	Yes	Yes	Yes	Yes
VDss (L/kg)	10.64	4.61	20.00	6.40	0.39
Fraction unbound (Fu)	0.235	0.158	0.274	0.215	0.216
CYP3A4 inhibitor	No	No	No	Yes	Yes
Total clearance (log ml/min/kg)	0.183	-0.169	0.328	0.292	0.711
AMES toxicity	No	No	No	No	Yes
Max. tolerated dose (log ml/min/kg)	0.438	0.449	0.506	0.441	0.370
Hepatotoxicity	No	No	No	No	Yes
Oral Rat Acute Toxicity (LD50) (mol/kg)	2.5	2.55	2.49	2.52	2.6

results were filtered to include only the species "Homo sapiens" and the highest predicted probability. The list of target proteins was integrated with the list of proteins overexpressed in CRC to identify common targets. Interactions between target proteins were analyzed using the STITCH 5 database (Search Tool for Interactions of Chemicals and Proteins) at https://stitch-db.org/ [25]. The data was accessed in January 2026.

Biological Network Analysis

Protein interaction data were analyzed using Cytoscape software to construct, visualize, and analyze interaction networks. Network topology analysis was performed using the CytoHubba 0.1 plugin in Cytoscape 3.10.3 (https://cytoscape.org/) to obtain protein hubs [26].

Survival Analysis

Survival analysis of colorectal cancer patients expressing specific genes was performed using the GEPIA (http://gepia.cancer-pku.cn/) [27]. The data was accessed in January 2026.

Molecular Docking Analysis

Molecular docking was performed using compounds based on target predictions, namely epigallocatechin-3-gallate (EGCG) (CID: 65064), epicatechin-3-gallate (EGC) (CID: 107905), and capmatinib (control) (CID: 25145656) [28]. These compounds were then optimized using Avogadro 1.2 (https://avogadro.cc/) with the Merck Molecular Force Field 1994 (mmff94) to achieve the lowest score [29]. The structures were converted to the Pdbqt format using Open Babel 2.4.1. The c-Met kinase protein structure was downloaded from the Protein Data Bank (https://www.rcsb.org/) with ID 3RHK, which has a resolution of 1.94 Å—considered good quality as it is below 2 Å—and belongs to Homo sapiens [20]. The protein was prepared using AutoDockTools 1.5.7, in which water molecules were removed, polar hydrogens were added, and Kollman charges were assigned, and then saved in PDBQT format. Docking was performed with AutoDock Vina 1.2.3 at coordinates center_x = -8.266, center_y = 12.334, center_z = -1.420 and box size size_x = 28, size_y = 24, size_z = 26, based on the active site of the protein in ATP binding site attached by ARQ 197, while performing the redocking process of the crystal ligand in the protein structure [30,31]. Redocking and docking analyses were conducted using PyMOL 3.1 to evaluate the Root Mean

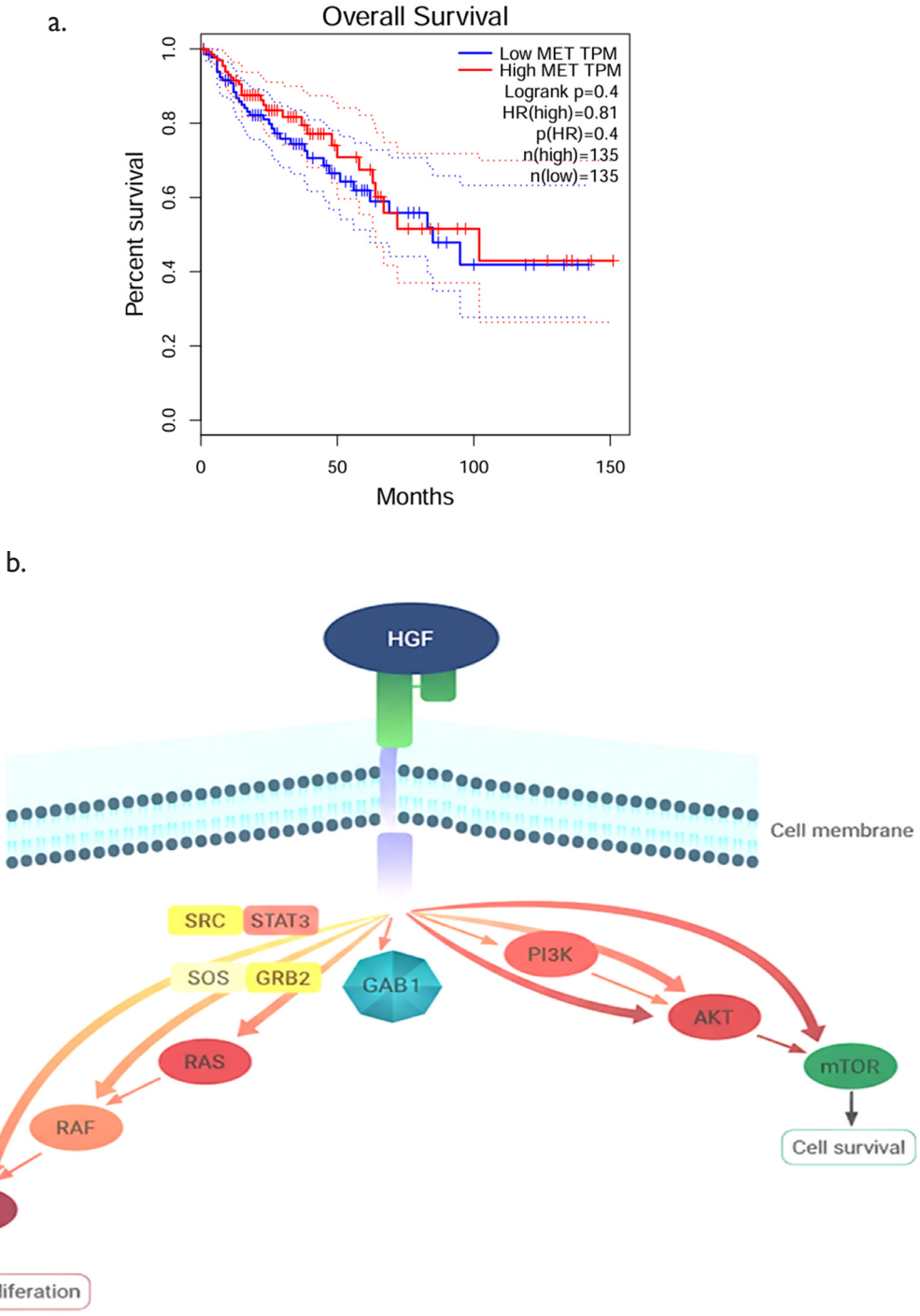


Figure 6. Protein interaction networks created using the STITCH server (a) and Cytoscape software (b).

Square Deviation (RMSD) [32], compound binding values, and interactions, and using Biovia Discovery Studio 2025. The inhibition constant (Ki) calculation was obtained from the binding energy (ΔG), as follows [32]:

$$Ki = \exp\left(\frac{\Delta G}{RT}\right)$$

Note:

$R = 1.985 \times 10^{-3}$ kcal mol^{-1} K^{-1}

T is the temperature (298.15 K)

Results

This study aims to conduct a computational analysis to evaluate the therapeutic potential of tea catechins as adjuvant agents to overcome chemoresistance in colorectal cancer (CRC) by targeting and suppressing key proteins. There are four primary catechins found in tea leaves: epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG) (Figure 1a-d).

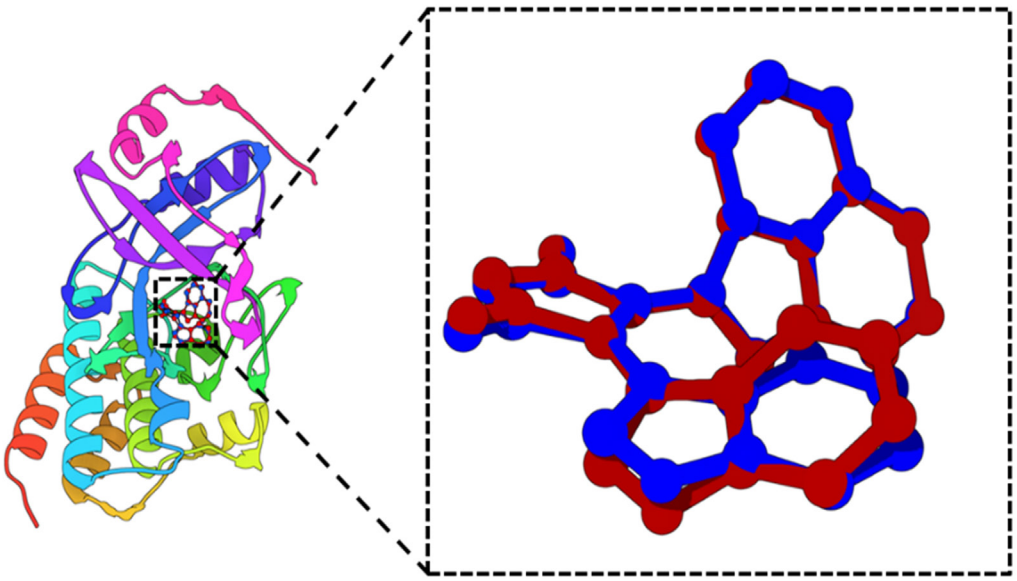


Figure 7. Visualization of the comparison results for re-docking the native ligand (ARQ 197) with protein c-Met. Note: red line (after) and blue line (before).

Physicochemical and ADMET analyses using pkCSM indicate that all evaluated catechins have potential as drug candidates [22]. However, some parameters deviate from Lipinski's Rules of Five (RO5), including molecular weight, Log P, hydrogen-bond acceptors, and hydrogen-bond donors (Table 1). ECG and EGC each have one violation, EGCG has two, and capmatinib has none. The violation from RO5 does not always determine the final result of the compound, but it is just an indicator [33]. In comparison, EC had no violations. ADMET analysis focused on several indicators (Table 2). The highest absorption was observed for capmatinib (95.10%) and EC (68.82%), and the lowest for EGCG (47.39%). This result shows that every compound has good absorption, greater than 30% [34].

Caco-2 permeability ranged from -1.521 to 1.344; this indicator correlates with HIA: the higher the value, the better the result [35]. The P-gp substrate results showed that all compounds are P-gp substrates, indicating that they are recognized by P-gp and actively effluxed from cells, thereby reducing intracellular absorption [45]. The steady-state distribution volume (VDss) ranged from 0.39 to 20.00 L/kg. EGC showed the highest value, whereas capmatinib had the lowest. VDss values greater than 2.8 L/kg indicate that a compound can effectively reach tissues, while values above 3.5 L/kg suggest an even greater capacity for tissue distribution [28].

Human unbound fraction (Fu) ranged from 0.158 to 0.274, a value lower than 0.7, indicating that a moderate proportion of the compounds remains unbound in plasma. These values suggest a considerable level of plasma protein binding while still allowing sufficient free drug to distribute into tissues [35]. CYP3A4 inhibitors identified include EGCG and capmatinib. When a compound acts as a CYP3A4 inhibitor, it can deactivate other CYP3A4 substrates. This mechanism is especially important because CYP3A4 is highly expressed in colon cancer, where it contributes to drug resistance during chemotherapy [36]. EGC had the highest value, and ECG the lowest, with a compound range of -0.169 to 0.328; however, capmatinib showed the highest value. Total clearance indicates how efficiently a drug is eliminated from systemic circulation through metabolic and excretory processes. Compounds with higher total clearance values are generally eliminated more quickly from the body, which may decrease systemic accumulation but can also shorten the duration of pharmacological activity [28].

The maximum tolerated dose (MTD) for all compounds ranges from 0.370 to 0.506; values below 0.477 are considered low, while the highest is EGC. The high MTD value indicates that the compound is better tolerated at higher doses [37]. All compounds tested showed "No" for Ames' toxicity (potential to cause mutagenicity in bacteria) and hepatotoxicity, except capmatinib. Lethal Dose (LD_{50}) values ranged from 2.49 to 2.6, suggesting that all compounds are low-toxicity [28,37].

Epicatechin-3-gallate (ECG) is predicted to target mainly proteins from the enzyme and kinase categories. Meanwhile, epigallocatechin-3-gallate (EGCG) is expected to target a wider range of proteins, mainly kinases, transferases, membrane receptors, and enzymes (Figure 2).

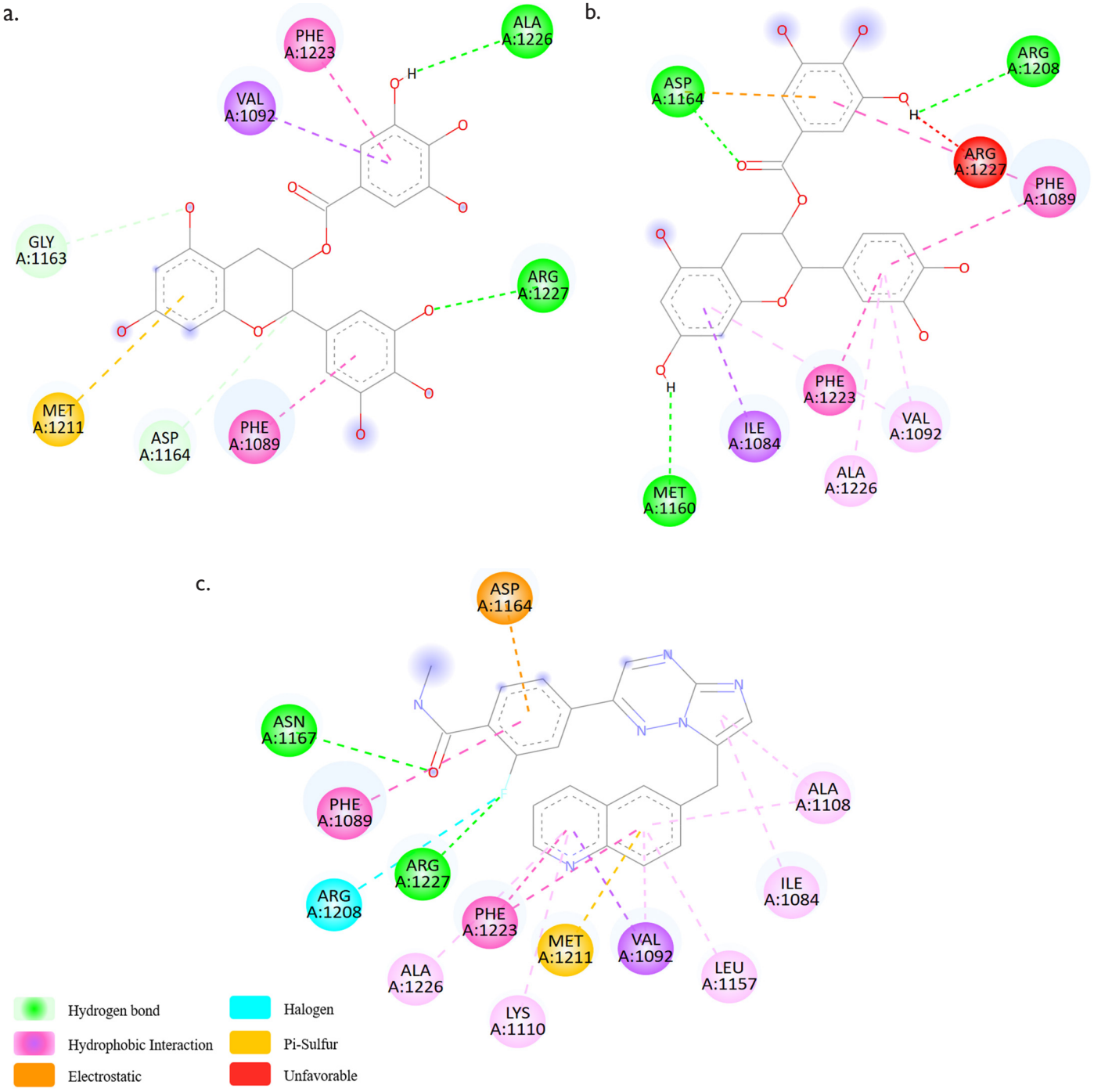


Figure 8. 2D visualization of the molecular docking results for (a) EGCG and (b) ECG with the c-Met protein. Red highlights indicate interactions within the protein's active site that are consistent with those of the native ligand.

Results show that 250 genes are overexpressed in CRC patients, and four common target proteins were identified: CA9, SERPINE1, MET, and SQLE (Figure 3). Protein-protein interaction (PPI) analysis further indicated that MET acts as a central hub within the interaction network. (Figure 4; Table 3).

Functional enrichment analysis reveals that the target proteins significantly contribute to chemoresistance, particularly against anti-angiogenic agents, which are crucial for colorectal cancer progression (Table 3; Figure 5). This result is further supported by survival analysis, which indicates that patients with MET protein overexpression have a poorer prognosis and significantly lower overall survival (Figure 6a). Additionally, the MET protein functions not just as an isolated target but as a central hub that integrates multiple signaling pathways essential for cancer cell survival (Figure 6b). For further analysis, the MET protein was docked with the compounds. The docking validation was performed by re-docking the ligand from the crystal structure (Figure 7), yielding a result of 0.285 Å, whereas valid re-docking results range from 0.0 to 2 Å [32].

Molecular docking results showed that capmatinib and EGCG have the highest binding energies, at -11.3 kcal/mol

Table 3. Network topology analysis using CytoHubba and functional annotation of target proteins

Rank	Gene Symbol	Protein Name	Biological Function
1	MET	MET proto-oncogene, receptor tyrosine kinase	A critical component of signalling pathways regulating cell proliferation, motility, invasion, and angiogenesis.
2	CA9	Carbonic anhydrase 9	An enzyme that catalyses the hydration of carbon dioxide; it maintains intracellular pH homeostasis under hypoxic environments.
2	SERPINE 1	Serpin family E member 1 (PAI-1)	The primary inhibitor of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA); it plays a key role in fibrinolysis.
4	PGF	Placental Growth Factor	A member of the Vascular Endothelial Growth Factor (VEGF) family, it functions as a potent growth factor that stimulates neo-angiogenesis.
4	SLC49A4	Solute Carrier Family 49 Member 4	A solute carrier protein involved in the transmembrane transport of metabolites and nutrients.
6	MACC1	Metastasis-Associated in Colon Cancer 1	A transcription factor that acts as a key regulator of MET signalling.
6	SMIM22	Small Integral Membrane Protein 22	A small integral membrane protein.
6	SQLE	Squalene Epoxidase	A rate-limiting enzyme in the cholesterol biosynthesis pathway.

Table 4. Molecular docking analysis results

Protein	Ligand	Binding affinity (kcal/mol)	Inhibition Constant (μM)	Binding Interactions
c-Met	EGCG	-9.2	0.188	Hydrogen bonds: Pro1158, Met1160 Hydrophobic bonds: Ile1084, Phe1089, Val1092, Ala1108, Lys1110, Leu1157, Phe1223, Ala1226, Arg1227 Electrostatic bonds: Met1211
c-Met	ECG	-9.1	0.221	Hydrogen bonds: Met1160, Asp1164, Arg1208 Hydrophobic bonds: Ile1084, Phe1089, Val1092, Phe1223, Ala1226 Unfavourable bonds: Arg1227
c-Met	Capmatinib (control)	-11.3		Hydrogen: Asn1167, Arg1227 Hydrophobic: Ile1084, Phe1089, Val1092, Ala1108, Lys1110, Leu1157, Phe1223, Ala1226 Electrostatic: Aps1164 Pi-Sulphur: Met1211

and -9.2 kcal/mol, respectively, along with estimated inhibition constants of 0.0051 μM and 0.18 μM (Table 4). Meanwhile, epicatechin-3-gallate (ECG) exhibited a binding free energy of -9.1 kcal/mol and an estimated inhibition constant of 0.221 μM. These negative, thermodynamically favorable values indicate that the ligand-protein interactions occur spontaneously and are highly stable. Further analysis of the ligand-protein complexes revealed that the compounds interact with the active site of c-Met kinase via multiple intermolecular interactions. Important interactions include hydrogen bonding, hydrophobic interactions, and electrostatic interactions, although minor unfavorable interactions were also observed (Table 4; Figure 8). EGCG and EGC bind in the ATP-binding pocket, stabilized by AR 197, as demonstrated by the control capmatinib, with residues Phe1089, Met1211, Phe1223, and Arg1227 playing crucial roles in the protein [28,38]. This interaction profile strongly supports the hypothesis that ECG and EGCG can modulate MET's pleiotropic functions in CRC. Furthermore, these findings provide a molecular basis for the potential chemosensitizing effects of these compounds

on CRC cells during chemotherapy.

Discussion

The MET proto-oncogene encodes the receptor tyrosine kinase (MET), whose activity is regulated by its ligand, hepatocyte growth factor (HGF). Dysregulation or uncontrolled activation of MET in colorectal cancer (CRC) triggers downstream signalling cascades that involve vital pathways such as PI3K/Akt, MAPK, and STAT3 [17,20,39]. These pathways play a crucial role in driving oncogenic processes, including uncontrolled cell proliferation, migration, invasion, and chemoresistance (Figure 6b).

MET overexpression is a primary mechanism of CRC cell resistance to conventional chemotherapy, particularly to cytotoxic agents such as 5-fluorouracil (5-FU) and oxaliplatin [5,6,11,16]. Enhanced MET signalling tends to protect cancer cells from apoptosis, typically induced by cytotoxic drugs, often by promoting the epithelial-mesenchymal transition (EMT) or activating anti-apoptotic proteins [9,20,39]. Furthermore, MET activation—via either ligand-dependent induction by HGF or ligand-independent crosstalk with factors such as EGF and IGF-1—promotes the maintenance of cancer stemness, thereby driving aggressive oncogenic phenotypes and metastatic progression [19,40]. Consequently, suppressing MET signalling is a necessary strategy to overcome treatment failure and improve prognosis in patients with CRC.

Our in-silico results identify MET as a key hub in the CRC signaling network, supporting the development of MET-targeted compounds. Epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG), the main bioactive components of tea (Camellia sinensis), show promise as MET-targeted therapies (Figure 4). Mechanistically, EGCG inhibits MET activation and downstream signaling. In vitro studies confirm EGCG's ability to block these pathways, reducing CRC cell proliferation, invasion, and metastasis. Moreover, EGCG's potential as a chemosensitizing adjuvant is particularly important; combining EGCG with first-line CRC chemotherapeutic agents, such as 5-fluorouracil (5-FU), has been shown to produce synergistic effects [5,6,11]. This combination markedly promotes apoptosis, increases cellular sensitivity to 5-FU, and inhibits cancer cell growth and colony formation by modulating apoptotic pathways and inducing cell-cycle arrest [10,17,20,39].

Although epicatechin-3-gallate and epigallocatechin-3-gallate show promising therapeutic potential, their clinical use faces major hurdles, particularly their low oral bioavailability and rapid breakdown in the gastrointestinal tract, especially at neutral or alkaline pH [11,41,42]. As a result, the therapeutic dose that reaches the MET target within tumor tissues remains insufficient. Future research should focus on creating new nanotechnology-based drug formulations. Preclinical strategies—such as encapsulating tea catechins in pH-responsive nanoparticles, liposomes, or lipid-polymer hybrids—have been demonstrated to improve the stability and bioavailability of these compounds [10,11,41,42].

While the current in silico analysis offers a solid initial framework for identifying epicatechin-3-gallate and epigallocatechin-3-gallate as potential therapeutic adjuvants against MET-mediated drug resistance, certain limitations must be recognized. Primarily, the molecular docking and binding affinity predictions presented here represent a static computational environment; the study lacks molecular dynamics simulation (MDS) data, which is crucial for assessing the structural stability, conformational flexibility, and time-dependent behavior of the protein-ligand complexes under simulated physiological conditions. As a result, although the current findings are enough to generate a prioritized list of candidate phytochemicals, they should be considered a foundational step. Future research will involve conducting high-nanosecond MDS to dynamically validate these interactions, followed by rigorous in vitro and in vivo experimental tests to confirm the biological effectiveness of these compounds in overcoming resistance in colorectal cancer cell lines.

Conclusion

Our in-silico findings identify epicatechin-3-gallate and epigallocatechin-3-gallate as promising colorectal cancer chemosensitizers targeting the c-Met (MET) receptor. Molecular docking simulations showed strong binding affinities (EGCG: -9.2 kcal/mol; ECG: -9.1 kcal/mol), providing a molecular basis for using tea catechins as multitarget inhibitors to block MET-mediated signaling and reduce chemoresistance in CRC.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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RESEARCH ARTICLE

Cassia tora Gum: Structural Modification and Pre-Formulation Studies

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Abstract

Background: Natural gums are biodegradable, biocompatible, non-toxic, easily available, and accessible. However, certain associated disadvantages, such as low swelling ability, viscosity, and flow ability, make them unsuitable for pharmaceutical applications. The study aims to modify *Cassia tora* gum (CTG) through cross-linking by exploring the free hydroxyl groups.

Methods: The CTG obtained from the seeds was taken with the cross-linking reagent and were subjected to the synthetic procedure for the preparation of the cross-linked CTG batches. The reagents utilized as cross-linking agents include Borax (B), Epichlorohydrin (ECH), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The reagents were utilized at varying concentrations of 1%, 2%, and 3%, with the most optimal concentration selected for the final batch preparation. The envisaged cross-linked gums were characterized by spectral techniques, and evaluated for physical and rheological parameters.

Results: The cross-linked gums were found to possess modified parameters. The CTG-B was found to be the most optimized gum among others, in terms of flowability (1.31±0.02), freeze-thaw stability (84.9%), flocculation efficiency (62.43% turbidity removal), and sedimentation volume (2.15±0.28 mL). In addition, the fabricated gums exhibited distinct surface morphology, suggesting enhanced drug-loading capacity.

Conclusion: Thus, a novel bio-polymeric materials was developed in the study, which demonstrates promising formulation properties as compared to the native gum.

Keywords: Biomedical-pharmaceutical applications, borax, carbodiimide, *Cassia tora* gum, cross-linking, epichlorohydrin

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Introduction

Materials science is a rapidly evolving field, with carbohydrate polymers leading synthetic/petrochemical-based polymers in formulation development [1]. They find wide application in agriculture, biomedical, and pharmaceutical industries [2]. In the pharmaceutical industry, they are a paramount constituent used for controlling the viscosity of solutions, stabilization of suspensions, granulation, film-forming/coating, binding, and thickening[3], gel-forming [4], and controlling solubility in controlled delivery systems [5]. These are also employed as agents in water-purification, wastewater treatment [6], and biodegradable packaging [7].

Bio-polymeric materials have the advantage of interacting with skin surface proteins and lipids and display peculiar physicochemical properties [8]. In addition, they are biodegradable, non-toxic, and biocompatible [9]. However, polymers, particularly those of natural occurrence, have certain associated drawbacks, which include water solubility issues, viscosity, and flowability. The expanding industrial utility of natural gums has led to intensified research into existing gums and their modification procedures. The process of chemical modification, such as cross-linking, may help to overcome drawbacks and enhance the acceptability of these polymers [10].

Cassia tora gum (CTG) is one such naturally occurring polymer. The CTG is a hydrophilic colloid that is obtained from the endosperm of seeds of *Cassia tora* and *Cassia obtusifolia*, belonging to the Leguminosae family [11]. The endosperm of the seed contains numerous polysaccharides possessing the molecular structure of galactomannan. The CTG is a high-molecular-weight polysaccharide that primarily consists of linear chains of 1,4-β-D-mannopyranose units, branched with α-D-galactopyranose at every fifth position, with a total of 7% glucose, 15% galactose, and 78% mannose [12]. It swells in normal water, while upon boiling, it forms a highly viscous aqueous colloid. It forms an aqueous gel when combined with carrageenan or xanthan gum, or other gelling or thickening agents. It is widely used as an emulsifier, a binding agent [13] a thickener, a foam stabilizer, a gelling agent, a diluent, a stabilizing additive, and an agent for moisture retention [14, 15], useful in the pharmaceutical industry. Apart from the pharmaceutical industry, the CTG also finds usage in the food industry as a texturing agent for the preparation of frozen dairy desserts, poultry-meat products [16], and cheese [17]. However, the CTG has associated drawbacks like swellability, viscosity, and flowability. The chemical modifications provide an efficient way to not only overcome the drawbacks associated with the CTG but also to improve its compressibility, stability, retrogradation, and flocculation efficiency [18]. The CTG can be modified by carbamylation, carboxymethylation, cyanoethylation, carbamoyl ethylation, graft co-polymerization [6], and cross-linking.

In the present study, a cross-linking strategy was employed using three different cross-linking reagents, like Borax (B), Epichlorohydrin (ECH), and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), to test the improvement in the properties of the CTG. The modifications due to cross-linking may either be within the carbohydrate polymer chains or through intermolecular bonds, thereby increasing the crosslinking density with anticipated tuning in the physicochemical properties for applicative needs [19]. The modified CTGs were evaluated to determine the extent of cross-linking and alterations in flow property, compressibility, swellability, viscosity, retrogradation, freeze-thaw stability, and flocculation efficiency. They were also subjected to particle size determination, Scanning Electron Microscopy (SEM), Thermal Gravimetric Analysis (TGA), and Differential Scanning Calorimetry (DSC). The complete instrumental platform helped to identify the cross-linked gum as a new biomaterial of pharmaceutical importance.

Methods

Collection, identification, authentication, and extraction of plant material

The seeds of *Cassia tora* were collected from the local areas of Lucknow, U.P., India in the month of October, 2018. They were identified and authenticated by the National Botanical Research Institute, Lucknow, under Ref. No: NBRI/CIF/666/2018. All the chemicals used in the research were of analytical grade. CTG was isolated from the seeds and purified using simple methods such as washing, filtration and drying as per the method described by Soni and Pal [10], with a percentage yield of 64%.

Syntheses and optimization of cross-linked CTGs

To synthesize the cross-linked carbohydrate polymers, variable concentrations of all three cross-linking reagents were taken [8] (Figure 1). The most optimal concentration of the crosslinkers, based on crosslinking density, was selected for final batch preparation. In addition to the concentration, two more variables, the rpm (rotations per minute) and the reaction time (in minutes), were taken to design the experiment.

Characterization of CTC and cross-linked CTGs

Micrometric studies

The CTG and cross-linked CTGs, namely borax cross-linked CTG (CTG-B), epichlorohydrin cross-linked CTG (CTG-ECH), and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide cross-linked CTG (CTG-EDC) were evaluated for various micrometric study parameters like bulk density, tapped density, Carr's compressibility index, Hausner's ratio, and angle of repose by the methods reported in the literature [20, 21].

Solubility Studies

The solubility studies of CTG and cross-linked CTGs were performed in purified water and ethanol.

Particle size, zeta potential, and polydispersity index

Particle size polydispersity index were analyzed using dynamic light scattering (DLS) with a Zetasizer (Nano ZS, Malvern Instruments, UK) at 173° detection angle in an optically homogeneous square polystyrene cuvette. The results were the average of 3 runs, with at least 13 measurements. Before the measurements, all samples were diluted with ultra-purified water to generate a suitable scattering intensity [22]. Zeta potential was determined using the Electrophoretic Light Scattering (ELS) technique (Nano ZS, Malvern Instruments, UK) at a conductivity of 0.009 mS/cm, with suitable dilution in ultra-purified water. The result was an average of 3 runs, with at least 12 measurements.

Scanning electron microscopy (SEM)

The surface morphology of the CTG and the cross-linked CTGs was characterized through a scanning electron microscope (JSM 6490, JEOL, Japan) [23]. Gold sputtering was performed for approximately 5 minutes to achieve a homogeneous coating on the sample and facilitate high-quality photographs. Images were captured at a low accelerating voltage (30kV) with a current of about 80 mA.

Table 1. Different micrometric study parameters of CTG, CTG-B, CTG-ECH, and CTG-EDC.

Parameters	Results*			
	CTG	CTG-B	CTG-ECH	CTG-EDC
Bulk Density (g/ml)	0.57 ± 0.01	0.45 ± 0.01	0.31 ± 0.01	0.37 ± 0.02
Tapped Density (g/ml)	0.72 ± 0.01	0.58 ± 0.02	0.37 ± 0.02	0.48 ± 0.01
Carr's Compressibility Index (%)	20.09 ± 0.46	24.78 ± 0.64	19.06 ± 0.69	22.13 ±0.91
Hausner Ratio	1.24 ± 0.02	1.31 ± 0.02	1.20 ± 0.03	1.33 ±0.08
Angle of Repose (°)	38.21 ± 0.32	42.93 ± 0.45	36.82 ± 0.60	41.15 ± 0.66

*The values represent mean ± SD (n = 3).

Table 2. Particle size characterization of CTG, CTG-B, CTG-ECH, and CTG-EDC.

Characterization	CTG	CTG-B	CTG-ECH	CTG-EDC
Size (-µm)	8.38±0.79	3.14±0.41	8.31±0.87	8.51±0.62
PDI	3.16±0.49	1.58±0.21	3.18±0.76	2.60±0.53
Zeta Potential (mV)	-7.02±1.52	-34.90±2.60	-5.43±1.95	-3.40±2.10

Swelling index (%)

A 50 ml measuring cylinder containing 1 g of each gum powder (raw and cross-linked) was tapped 100 times, after which the initial volume (X_i) was noted. 10 ml of distilled water was added to each gum powder. The new volume (X_v) obtained was recorded after 24 h. This method was repeated in triplicate, and the swelling index was calculated as the ratio of final volume to initial volume.[24]

$$\text{Swelling Index}(\%) = \frac{Xv}{Xi} \times 100 \quad (1)$$

where X_v and X_i are the final volumes of the gum powder and the initial volume of the gum powder.

Sedimentation volume

1 gm of each gum powder (raw and cross-linked) was added to 50 ml of distilled water. The pH of the slurry was adjusted to 7.0 using either 5% (w/w) sodium hydroxide or 5% (w/w) hydrochloric acid solution. It was heated in a boiling water bath for 15 minutes, taking care that the total volume of the slurry remained constant. A 10 ml sample was taken from the slurry and transferred to a centrifuge tube, and centrifuged at 4000 rpm for 15 minutes at room temperature. The clear liquid was decanted, and the volume of the clear liquid was determined [25]. The volume obtained was used to calculate the sedimentation volume using the equation:

$$SV = 10\text{-}V \quad (2)$$

where *V* is the volume of the clear liquid (ml).

Flocculation efficiency

The flocculation efficiency of each gum powder (CTG and cross-linked CTGs) and of alum (a commonly used material for purifying turbid water) was studied using the standard jar test procedure. The test protocol was optimized for 1% kaolin suspension in distilled water. Six beakers containing 100 ml kaolin suspension (pH 2.0) each were used, out of which one was kept as a control (without any gum powder), and the other five were fortified with the powdered alum and different doses of the polymers, from 0.5, 1.0, 1.5, and 2.0 ppm. The initial turbidity was measured using a nephelometer/turbidity meter. The solutions were indistinguishably stirred using a jar test apparatus at 300 rpm for 1min, 80 rpm for 5 min, followed by a settling time of 40 min. After 40 minutes of settling, the supernatant samples were drawn from each beaker, and turbidity was measured using a calibrated nephelometer/turbidity meter. Similar experiments on flocculation efficiency were repeated for a kaolin suspension at pH 5.8 (achieved by adding diluted HCl) and 7.0 (achieved by adding dilute NaOH) [26]. The diagrammatic representation of comparative flocculation efficacy has been reported in Figure 4. Based on the initial and final turbidity values at different pH levels, overall flocculation efficiency was calculated as:

$$\%\text{Turbidity removal} = \frac{(\text{Initial turbidity} - \text{final turbidity})}{\text{Initial turbidity}} \times 100 \quad (3)$$

Retrogradation

1% w/w slurry of each gum powder (raw and cross-linked) was mixed with 50 ml distilled water, followed by gelatinization in a water bath for 10 minutes. The volume was maintained throughout the process. The gum paste was then cooled at 25°C, and the transparency of the supernatant was measured at the variable standing times of 20 minutes, 40 minutes, 60 minutes, 80 minutes, and 100 minutes [27].

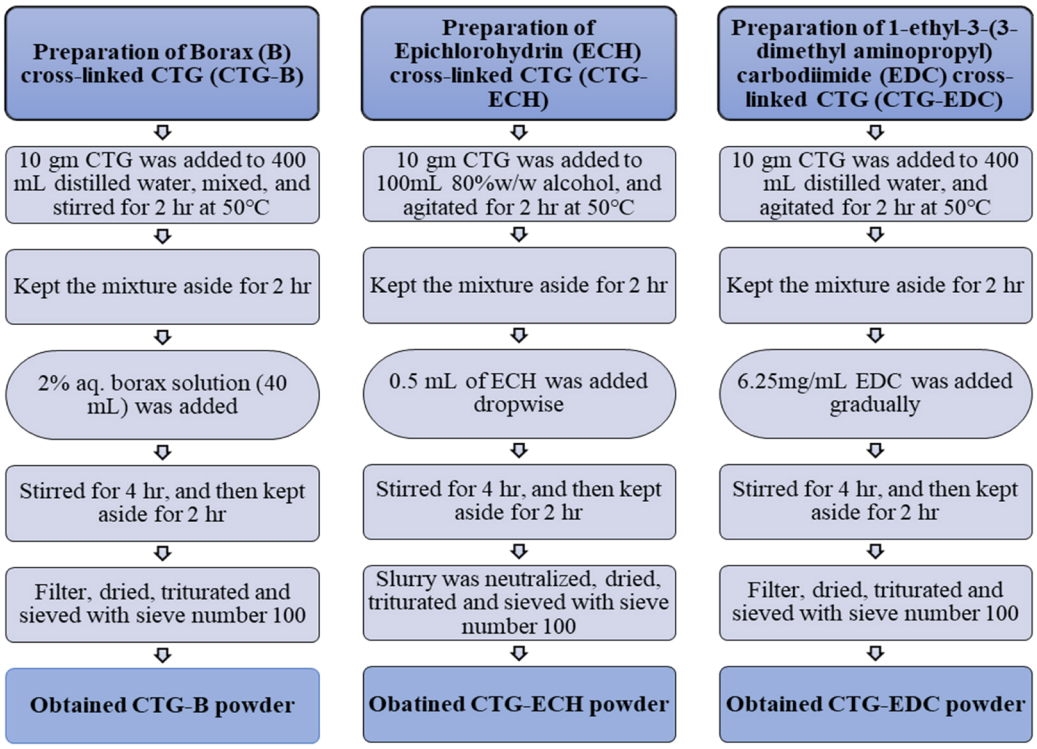

Figure 1: Preparation of the different crosslinked CTG.

Thermal analysis

Thermogravimetric Analysis (TGA)

A sample mass of approximately 5mg was placed in a crucible. The thermogravimetric analysis of CTG and the cross-linked CTGs was performed with a thermogravimetric analyzer (TGA-50 Shimadzu, Japan) at a temperature range of 20-800°C, at a constant heating rate of 10°C/min, under an inert atmosphere (N_2 40 mL/min) [28].

Differential Scanning Calorimetry (DSC)

A sample of approximately 5-10 mg was weighed and placed in a hermetically sealed standard Shimadzu aluminum pan and DSC curves were recorded in the temperature range of 20–300°C at 10°C/min, under an inert atmosphere (N_2 40 mL/min). The analysis was performed in triplicate (DSC-60 plus Shimadzu, Japan) [29]. DSC heat flux mode was used to measure the heat flow difference required to keep the sample and reference at the same temperature, thereby analyzing both endothermic and exothermic events.

Freeze-thaw stability

5 gm of each gum powder (raw and cross-linked) were mixed with distilled water to prepare a paste, which was then gelatinized in a centrifuge tube at 95°C. These were cooled at room temperature for 15 minutes. 10 gm of each gum paste was then taken in a 10 ml centrifuge tube and kept at 4°C for 24 hours, and then at -18°C for another 24 hours. The tubes were removed from the deep freezer and thawed at 50°C in a water bath for 24 hours. It was then centrifuged at 3500 rpm for 15 minutes. The clear liquid obtained was decanted and weighed. The percentage of the separated water was calculated using the given equation [8]:

$$\% \text{ FTS} = \frac{\text{Weight of decanted liquid}}{\text{Total weight of the paste before centrifugation}} \times 100 \qquad (4)$$

Analytical characterization of CTG and cross-linked CTGs

The cross-linked CTG powders were confirmed by comparing the data of the cross-linked CTG powders with that of the raw CTG utilizing spectroscopic techniques. The FTIR spectrometer (Bruker Alpha-II), Water Alliance e2695/HLC-TQ mass spectrometer, and Bruker Advance 400/AivIII- (300) FT-NMR spectrometer was utilized for the purpose.

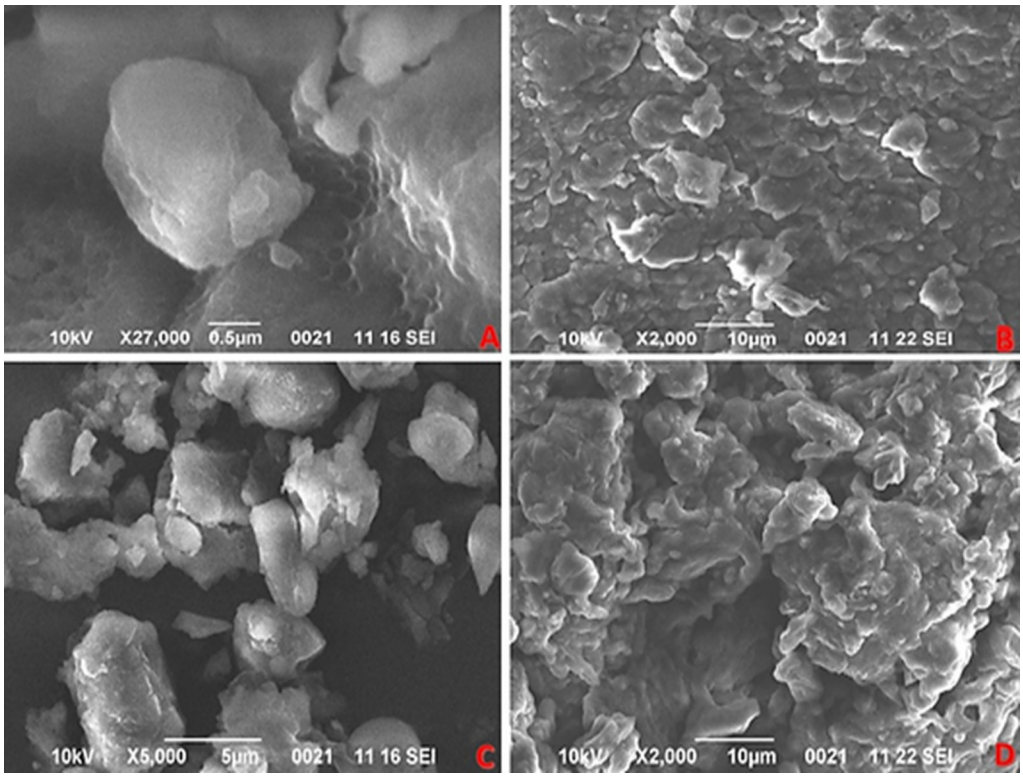

Figure 2: Microscopic morphology (SEM) of (A) CTG, (B) CTG-B, (C) CTG-ECH, and (D) CTG-EDC.

Results

Micrometric Studies

The results of micrometric studies for CTG and cross-linked CTGs are listed in Table 1.

Particle size, zeta potential, polydispersity index (PDI)

The particle size distribution and surface charges of CTG and optimized batches of cross-linked CTGs were determined by the DLS and are as given in Table 2.

Scanning electron microscopy (SEM)

The surface morphology of CTG and cross-linked CTGs was characterized by SEM, and the micrographs obtained are depicted in Figure 2.

Sedimentation volume and percentage swelling index

The calculated sedimentation volume results showed maximum sedimentation of the raw CTG (sedimentation volume = 4.30±0.57 mL). The minimum sedimentation volume was observed in CTG-B (sedimentation volume = 2.15±0.28 mL) as compared to CTG-ECH (sedimentation volume= 3.47±0.5 mL), and CTG-EDC (sedimentation volume = 2.65±0.28 mL). The swelling index was found to be 90.32±1.52, 50.66±0.57, 81.66±0.57, and 63.65±1.15% for CTG, CTG-B, CTG-ECH, and CTG-EDC, respectively, as shown in Figure 3A and 3B.

Retrogradation

The retrogradation of CTG and cross-linked CTGs is shown in Figure 3C. The obtained results showed improved retrogradation values in the cross-linked CTGs. When compared with the data of CTG, it was concluded that the cross-linked CTG powder exhibited stronger retrogradation, which indicates higher cross-linking.

Flocculation efficiency

The flocculation efficiency was calculated as the percent turbidity removal. The experiment was performed at different pHs, viz., 2.0, 5.8, and 7.0, to ensure acidic, neutral, and alkaline conditions, and compared with the standard flocculating agent alum, and the raw CTG, as shown in Figure 4. All the cross-linked CTGs show improved flocculation efficiency at 1.00 ppm as compared to raw CTG. From the data, CTG-B at 1.0 ppm (pH 7.0) was found to be optimal for percent turbidity removal (62.43), and ECH at pH 2.0 exhibited a percent turbidity removal of 18.42 at an optimal concentration of 1.5 ppm.

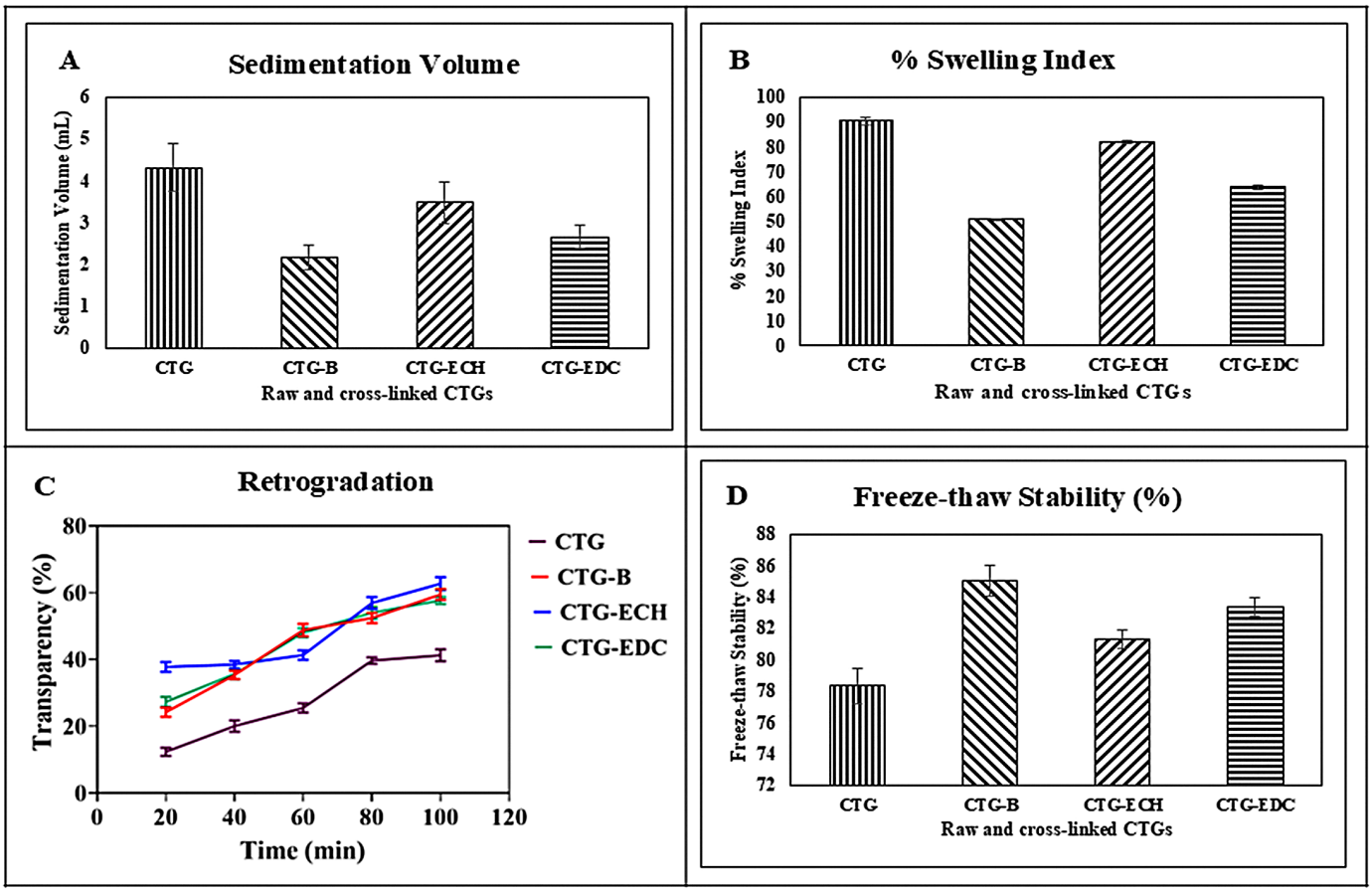

Fig. 3: (A) Sedimentation Volume, (B) Swelling Index, (C) Retrogradation and (D) Freeze-thaw stability studies of CTG, CTG-B, CTG-ECH, and CTG-EDC. The values represent mean ± SD (n = 3).

Freeze-thaw stability

The freeze-thaw stability values for CTG, CTG-B, CTG-ECH, and CTG-EDC were determined as 78.32±1.15, 84.99±1.0, 81.33±0.57, and 83.33±0.57%, respectively, and represented by data given in Figure 3D.

Analytical Characterization of CTG and Cross-linked CTGs

The spectra of the CTG and the cross-linked CTGs as obtained using IR spectroscopy, NMR spectroscopy and Mass spectrometry are as provided in the supplementary materials, S-13 (a) to S-13 (l).

Thermal analysis

Differential Scanning Calorimetry (DSC) and Thermo Gravimetric Analysis (TGA)

According to Figure 5, after crosslinking of CTG, the onset temperature, peak temperature, endset temperature, melting enthalpy, and thermal stability were changed. The onset temperature increased for CTG-EDC, whereas the end-set temperature and melting enthalpy increased for all cross-linked gums.

Discussion

Micrometric studies

The raw CTG and cross-linked CTG-ECH show fair flow, whereas cross-linked CTG-B and CTG-EDC show passable flow, as is indicated by the obtained angle of repose values. The Carr's compressibility index of CTG and CTG-ECH show values of 20.09±0.46 and 19.06±0.69, respectively, denoting that they have fair flowability as compared to CTG-B and CTG-EDC, which have values of 24.78±0.64 and 22.13±0.91, respectively, indicating passable flowability. The Hausner's ratio for CTG and CTG-ECH shows that powders with low inter-particle friction had ratio of 1.24±0.02 and 1.20±0.03, indicating a fair flow property, as compared to the CTG-B and CTG-EDC, which show values of 1.31±0.02 and 1.33±0.08, respectively, indicating passable flow properties. The poor flow properties of cross-linked CTH-B may be due to reduced particle size and increased surface area and roughness, which increases the interparticle friction. Further, solubility studies demonstrate that CTG was soluble in hot water and insoluble in ethanol, whereas crosslinked gums were sparingly soluble in hot water and practically insoluble in ethanol.

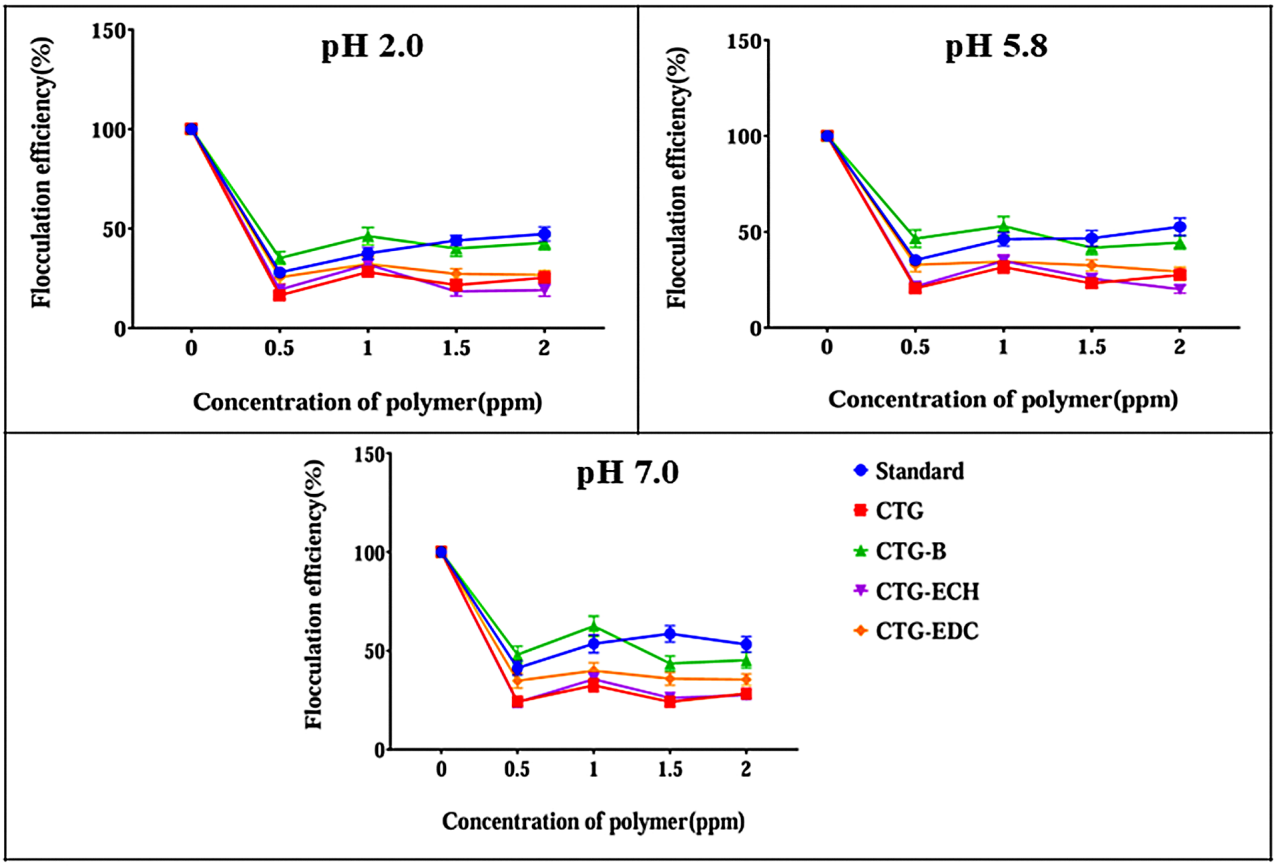

Fig. 4: Comparative flocculation efficiency of standard (alum), CTG, CTG-B, CTG-ECH, and CTG-EDC at different pH (A. pH 2.0; B. pH 5.8; C. pH 7.0)

Particle size, zeta potential, polydispersity index (PDI)

In order to characterize the gums for average particle size and PDI, the DLS method was selected to because of its relatively fast analysis and minimal sample preparation. The optimized batch of CTG-B, compared to the other cross-linked gums, displayed a smaller average particle size of 3.140 µm (Supplementary material, S-12), a zeta potential of -34.90 mV, and a PDI of 1.540 as. The smaller particle size of cross-linked gum exhibits enhanced functional properties due to an increased surface area. The PDI of 1.540 signifies a heterogeneous characteristic of the gum. However, in comparison to the other modified gums and the CTG, it indicates a narrow size distribution (Table 2). The obtained zeta potential greater than ±30 mV indicates a less aggregated, and a more stable crosslinked structure.

Scanning electron microscopy (SEM)

The SEM images of the cross-linked gum reveal a highly irregular and wrinkly surface exhibiting deep crevices (Figure 2), which indicates a high surface-to-volume ratio and an amorphous nature of a porous matrix. Lack of sharp and geometric edges confirms an amorphous nature of the crosslinking that disrupts regular lattice formation, while the dark recessed areas (or voids) visible between the raised section indicate a porous network [30].

Sedimentation volume and percentage swelling index

Sedimentation volume (Figure 3A), is indicative of the extent of the cross-linking in a polymer and is inversely related to it, i.e., the lesser the sedimentation volume of the polymer, the greater will be the cross-linking. This may be because cross-linking hinders water molecules from entering the cross-linked polymer [8]. As observed from the experimental results, the raw CTG displayed maximum sedimentation value of (sedimentation volume = 4.30±0.57 mL), whereas, minimum sedimentation volume was observed in CTG-B (sedimentation volume = 2.15±0.28 mL), indicating a greater extent of crosslinking, compared to CTG-ECH (sedimentation volume = 3.47±0.5 mL), and CTG-EDC (sedimentation volume = 2.65±0.28 mL).

The swelling properties of gum help it be used as an excipient in several formulations. The optimized CTG-B showed a low swelling index as compared to other cross-linked CTGs and raw CTG, which makes it suitable for controlled release [31] formulation by preventing the initial outburst of the drug from formulation due to the high swelling index (Figure 3B).

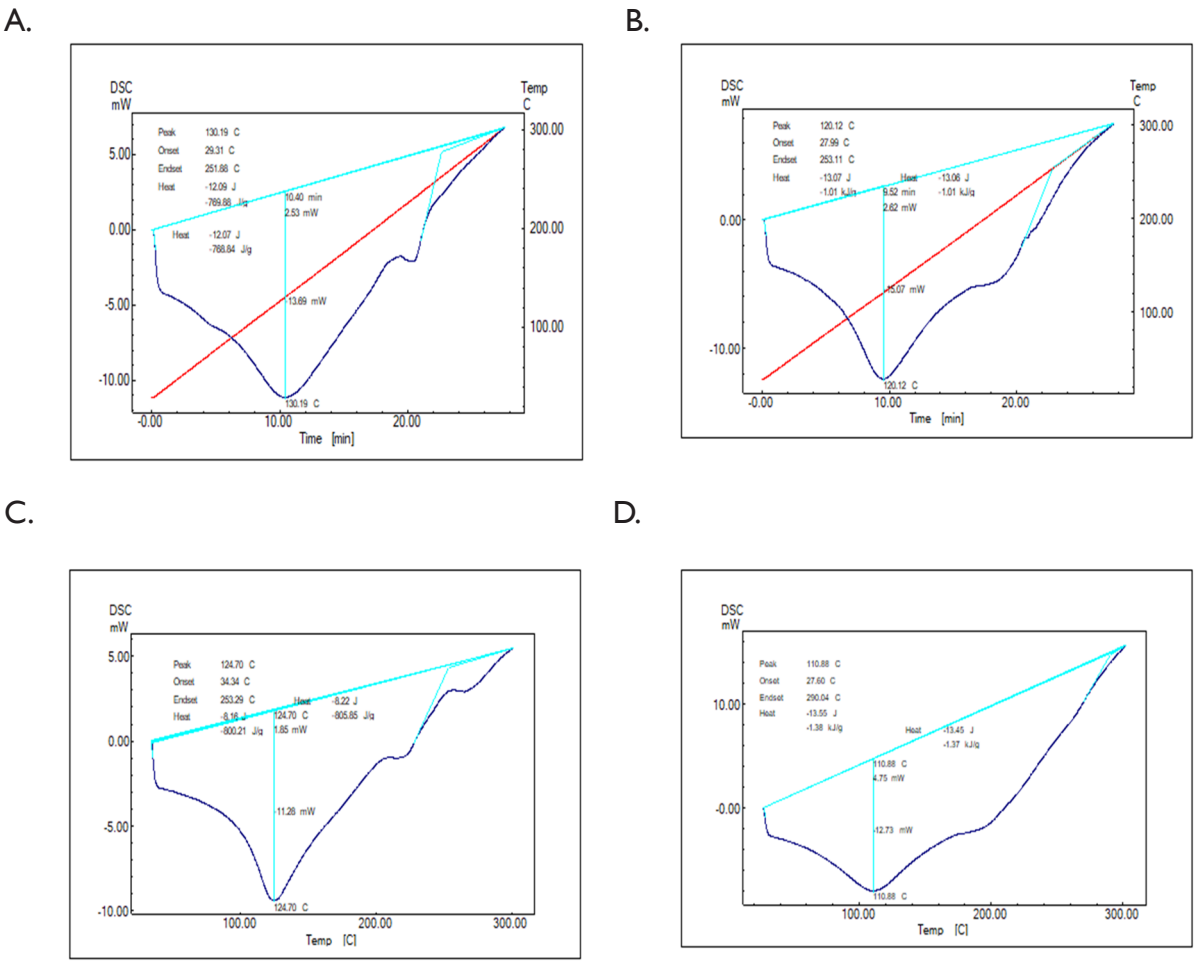

Fig. 5. DSC of (A) CTG, (B) CTG-B, (C) CTG-ECH, and (D) CTG-EDC.

Flocculation efficiency

All the cross-linked CTGs showed significant improvement in flocculation efficiency at 1.00 ppm as compared to the raw CTG.The reason for the improved efficiency of cross-linked polymer over linear polymer can be attributed to their increased hydrodynamic volume and better polymer bridging for the adsorption of different particles [26]. From the data in Figure 4, CTG-B at 1.0 ppm (pH 7.0) was found to be the most optimal (% turbidity removal of 62.43), with the potential to act as a suitable flocculating agent. CTG-ECH at pH 2.0 (% turbidity removal of 18.42) exhibited the potential to be used as a suspending agent at an optimal concentration of 1.5 ppm, as is also supported by its viscosity data.

Retrogradation

The obtained results show improved retrogradation values in cross-linked CTGs. When compared with the data of CTG, it could be concluded that the cross-linked CTG powder exhibited stronger retrogradation, which indicates higher cross-linking [8].

Thermal analysis

Thermogravimetric analysis (TGA) and Differen-tial Scanning Calorimetry (DSC)

The DSC spectra of the CTG (Figure 5A) and the crosslinked CTG-EDC (Figure 5D) show a broad peak, suggesting that moisture or bound solvent was released over a wide temperature range. In the case of crosslinked CTG-B, a comparatively narrower peak is observed, which may be due to reduced size and controlled water absorption. The DSC profiles of CTG, CTG-B, CTG-ECH, and CTG-EDC show an endothermic peak at melting temperature (Tm) of 130.19℃, 120.12℃, 124.70℃, and 110.88℃, respectively. The glass transition temperature (Tg) of all the cross-linked gums was observed in the range of 55-65℃. The peak around 200℃-300℃ represents thermal degradation process. This is usually found in case of carbohydrates, probably due to cleavage of the glycosidic bonds, and charring of the polysaccharide backbone, which releases energy. The event can also be confirmed by the TGA graph, which shows a significant weight loss around 200℃.

The thermogravimetric analysis of raw CTG and the cross-linked gums revealed two distinct zones of mass

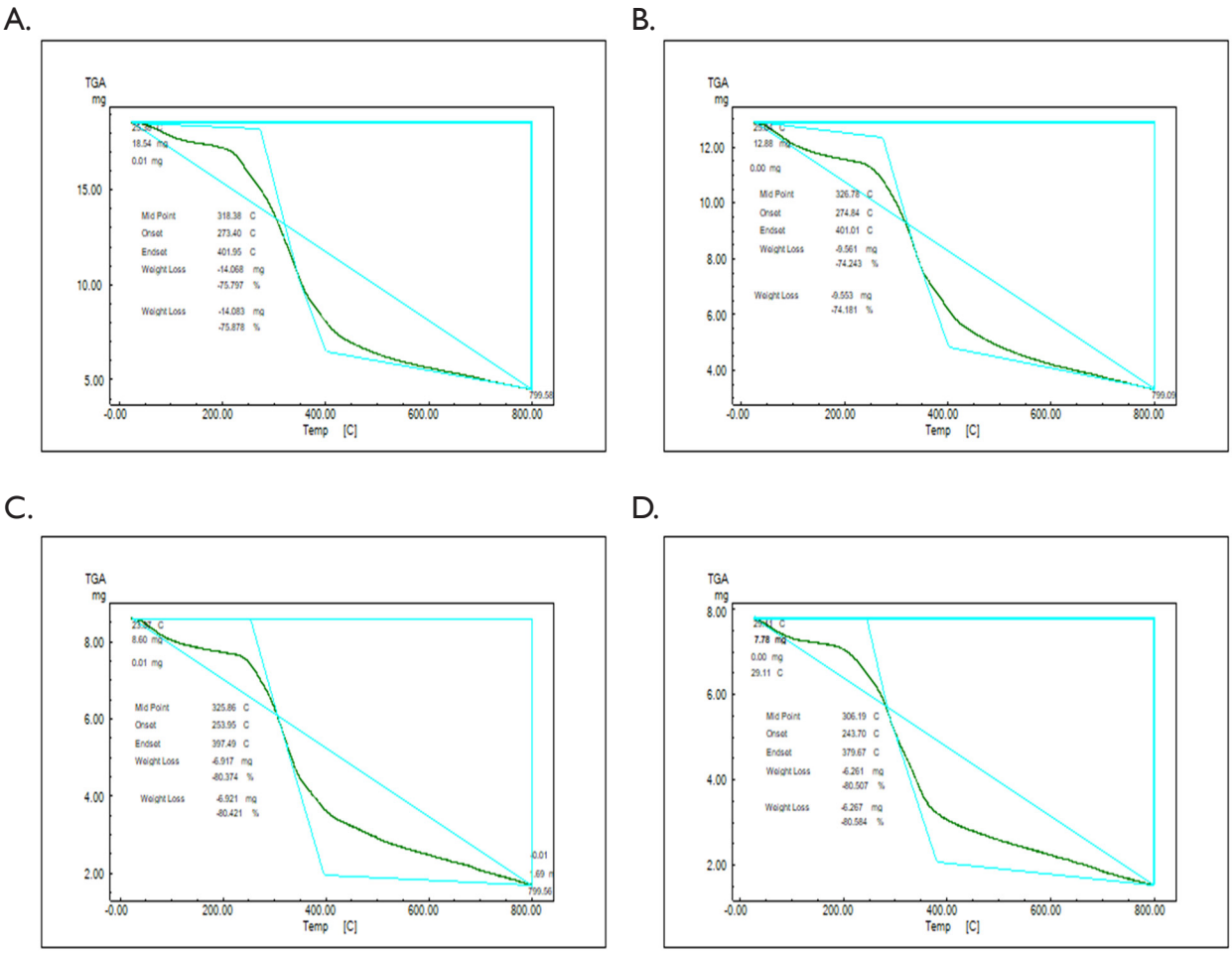

Fig. 6. TGA of (a) CTG, (b) CTG-B, (c) CTG-ECH, and (d) CTG-EDC. Thermal degradation of CTG and cross-linked CTGs was measured using TGA curves, as shown in Fig. 6.

loss. The initial mass loss is observed due to the presence of moisture in the samples,[33] and the melting of the gum. Although the observed maximum rate decomposition temperature and % weight loss in the second stage is due to depolymerization and complete decomposition [23]. As given in Table 3, the onset decomposition temperature, the end decomposition temperature, and the mass loss (%) of crosslinked CTG gum were modified upon crosslinking. The thermal stability is measured primarily by the onset decomposition temperature, which was slightly higher in the CTG-B than in the raw CTG, and the lower mass loss (%) indicated that cross-linking improved the thermal stability of the borax-crosslinked CTG (CTG-B).

Conclusion

In the present study, the CTG was cross-linked in a single-step process under standard laboratory conditions, without the use of any auxiliary. The free hydroxyl groups in the gum's pyranomannose backbone form covalent bonds with the cross-linking agents. Two of the three cross-linking agents, specifically the Borax and the ECH, produced intermolecular cross-linking of the polymeric chains, whereas the cross-linking agent EDC facilitated intramolecular cross-linking.

The structural analysis of chemically modified carbohydrate polymers revealed the potential influence of the size of the cross-linking reagent. The smaller crosslinker exhibited enhanced cross-link density in the modified gum, as evidenced by the findings of the swelling index and cross-linking density. The density of the cross-linkage subsequently improved the stability of the gums, as is evidenced by TGA and DSC analyses. The modified gums exhibited superior characteristics including swelling index, viscosity, flocculation efficiency, retrogradation, and freeze-thaw stability. The CTG-B is supposed to be the most suitably modified biopolymer produced by cross-linking, according to the observations.

The study concludes that the strategy may be useful for design and synthesis of tailor-made natural polymeric materials.

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RESEARCH ARTICLE

Phytochemical-mediated silver nanoparticles synthesized from *Vachellia sieberiana* suppress redox-inflammatory signaling in 1,2-dimethylhydrazine-induced organ toxicity

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Abstract

Background: Oxidative stress and chronic inflammation are central drivers of chemically induced organ injury and colorectal carcinogenesis, particularly following exposure to 1,2-dimethylhydrazine (DMH), a potent pro-oxidant and pro-inflammatory carcinogen. Plant-mediated nanotechnology has emerged as a promising strategy to enhance the bioactivity and systemic efficacy of phytochemicals against oxido-inflammatory damage. This study investigated the protective effects of *Vachellia sieberiana*–functionalized silver nanoparticles (AgNPs) on DMH-induced oxidative stress and inflammation in male Wistar rats.

Methods: Silver nanoparticles were synthesized using an aqueous leaf extract of *V. sieberiana* and characterized by UV–visible spectroscopy, confirming nanoparticle formation with a surface plasmon resonance peak at 437 nm. Thirty-five male Wistar rats were assigned to 7 groups of n=5. Group 1 served as the normal control, Group 2 DMH only (25 mg/kg). Groups 3, 4, and 5 received DMH + 100, 200, 400 mg/kg b.w of *V. sieberiana*-AgNPs. Group 6; DMH + Dox, and Group 7; 400 mg/kg of *V. sieberiana*-AgNPs only. Nanoparticles were administered daily via oral gavage for six weeks, concurrent with weekly DMH administration. Oxidative and inflammatory stress was induced by weekly subcutaneous administration of DMH (25 mg/kg) for six weeks, alongside oral treatment with *V. sieberiana*–AgNPs (100, 200, and 400 mg/kg). Antioxidant enzymes (GPx, GST, SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were quantified in the colon, liver, and kidney, while colonic IL-6, IL-1β, and TNF-α were measured using ELISA. Data were analyzed using one-way ANOVA with Tukey's post hoc test.

Results: DMH markedly suppressed colonic GPx (61.43%, p=0.0017), GST (68.72%, p=0.0009), GSH (57.07%, p=0.0012), and SOD (42.45%,), with a concomitant increase in MDA levels (39.55%). Hepatic and renal tissues showed similar antioxidant depletion, including reductions in hepatic GPx (73.53%, p=0.0003) and renal GSH (65.51%, p<0.0001), alongside significant elevations in MDA (up to 73.25%). *V. sieberiana*–AgNPs dose-dependently restored antioxidant defenses and reduced lipid peroxidation, achieving MDA reductions of 54.38% (p=0.0016) in the colon and 47.15% (p=0.0002) in the kidney at 400 mg/kg. DMH-induced increases in colonic IL-6 (23.42%, p=0.0110), IL-1β (43.30%, p<0.0001), and TNF-α (18.37%, p=0.0278). These were significantly attenuated following AgNPs treatment.

Conclusion: *Vachellia sieberiana*–functionalized silver nanoparticles confer significant protection against DMH-induced oxidative stress and inflammatory injury, highlighting their potential as phytochemical-based nanotherapeutics for managing chemically induced tissue damage.

Keywords: 1,2-Dimethylhydrazine; antioxidant defense; inflammation; oxidative stress; silver nanoparticles; *Vachellia sieberiana*.

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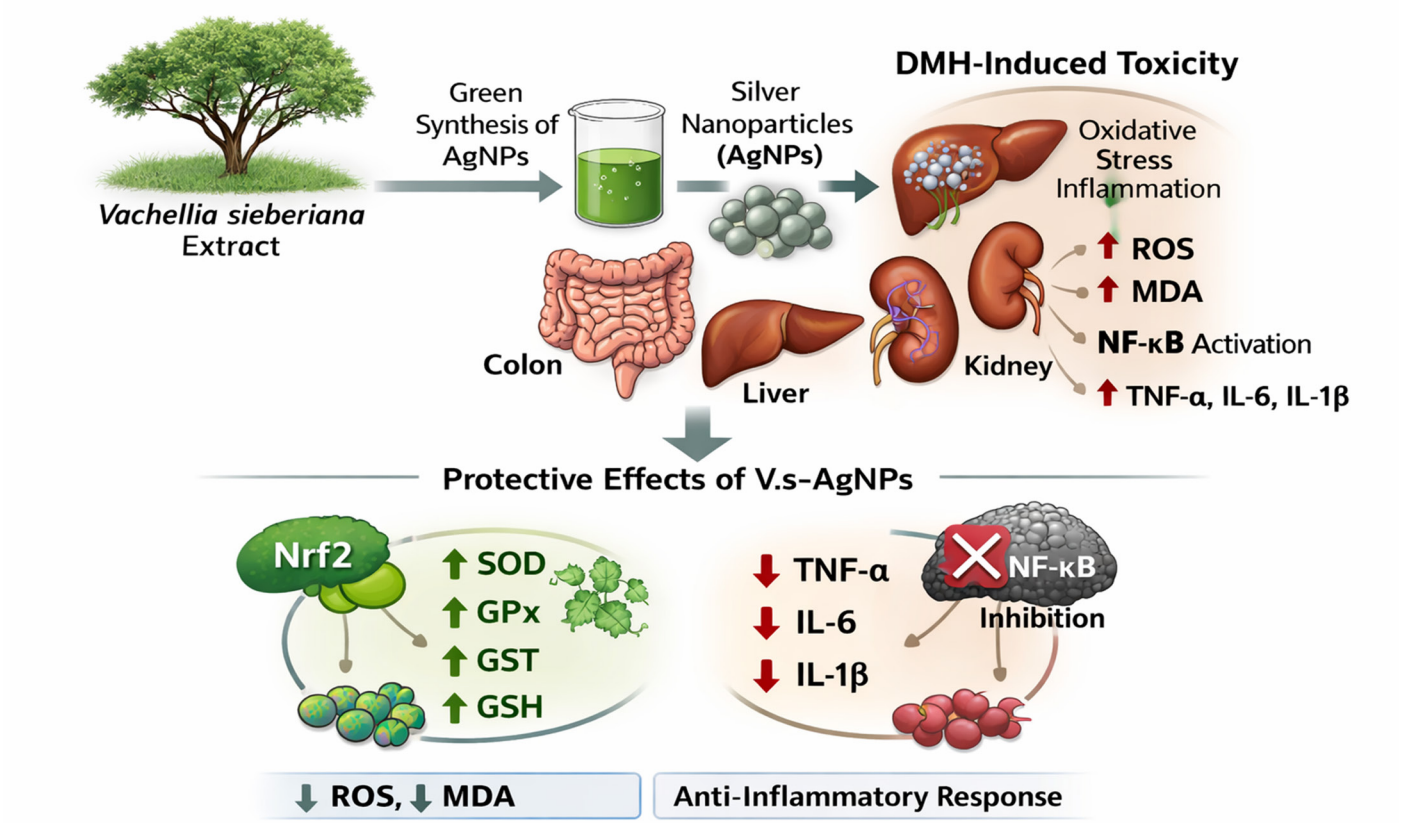

Graphical Abstract

Introduction

Oxidative stress and inflammation are closely interconnected biological processes that contribute to tissue injury and disease progression in chemically induced toxicity models. An imbalance favoring reactive oxygen species (ROS) over endogenous antioxidant defences such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST) promotes lipid peroxidation, macromolecular damage, and activation of pro-inflammatory signaling pathways [1]. Persistent oxidative stress stimulates nuclear factor-κB (NF-κB), a transcription factor that regulates the expression of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), thereby sustaining inflammatory injury [2].

The chemical carcinogen 1,2-dimethylhydrazine (DMH) is widely employed to model oxidative stress– and inflammation-mediated colon toxicity, reproducing early molecular events associated with colorectal carcinogenesis. Following metabolic activation, DMH generates reactive intermediates that elevate ROS production, suppress antioxidant enzyme activities, increase malondialdehyde (MDA) levels, and trigger inflammatory responses in colonic and hepatic tissues [3,4]. Experimental studies consistently demonstrate depletion of SOD, GPx, GST, and reduced glutathione (GSH) alongside increased lipid peroxidation and pro-inflammatory cytokines, indicating disruption of redox homeostasis [3].

Phytochemical-based interventions highlight the pivotal role of oxidative stress and inflammation in DMH toxicity. Flavonoids such as hesperetin restore antioxidant enzyme activities and suppress inflammatory mediators in DMH-treated animals, while lycoperoside H and tannic acid attenuate ROS generation and cytokine expression across affected tissues, underscoring the therapeutic relevance of plant-derived compounds [3,4,5]. The nuclear factor erythroid 2–related factor 2 (Nrf2) pathway regulates antioxidant defence by inducing phase II detoxifying enzymes, while NF-κB governs inflammatory gene expression in response to oxidative cues [1,2]. Evidence of functional cross-talk between Nrf2 and NF-κB suggests that enhancing antioxidant signaling while suppressing inflammatory activation may offer protection against chemical-induced organ injury [6,7].

Medicinal plants are rich in redox-active phytochemicals, but limitations in bioavailability and stability restrict their in vivo efficacy. Green nanotechnology addresses these challenges by integrating phytochemicals into nanostructures with improved biological activity[8]. Plant-mediated synthesis of silver nanoparticles (AgNPs) utilizes phytochemicals as reducing and stabilizing agents, producing biocompatible nanoparticles capable of

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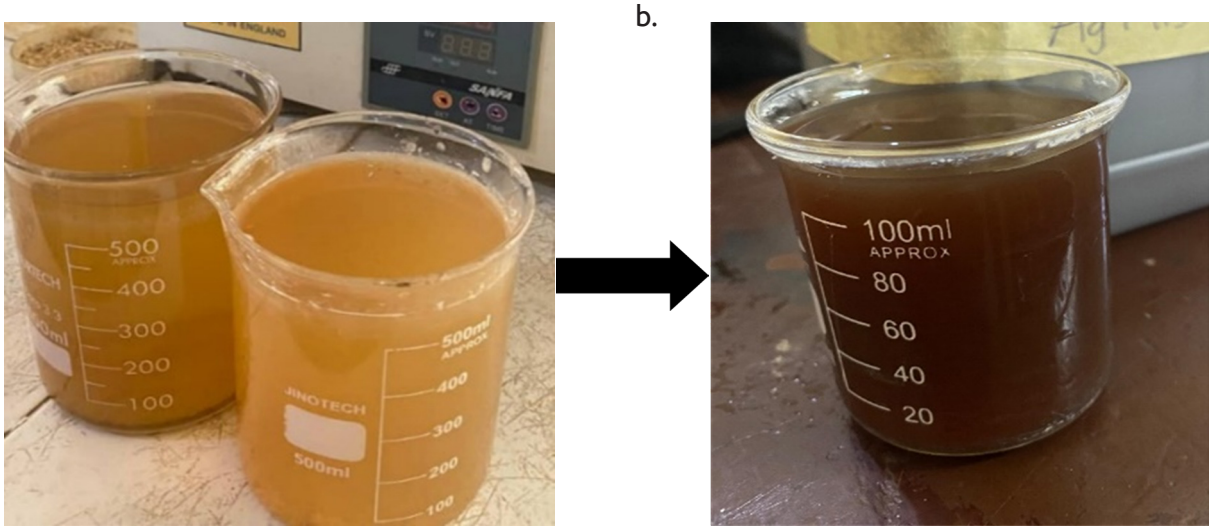

Figure 1: Synthesis of *Vachellia sieberiana*-AgNPs. (A) *Vachellia sieberiana* aqueous extract. (B) Synthesised *Vachellia sieberiana*-AgNPs

Table 1: Experimental protocol using *Vachellia sieberiana*-AgNPs.

S/N	Groups	Treatments
1	GROUP 1	Normal Control
2	GROUP 2	Positive Control (DMH; 25 mg/kg)
3	GROUP 3	DMH + 100 mg/kg *Vachellia sieberiana*-AgNPs
4	GROUP 4	DMH + 200 mg/kg *Vachellia sieberiana*-AgNPs
5	GROUP 5	DMH + 400 mg/kg *Vachellia sieberiana*-AgNPs
6	GROUP 6	DMH + Doxorubicin (2 mg/kg)
7	GROUP 7	400 mg/kg *Vachellia sieberiana*-AgNPs

modulating oxidative and inflammatory pathways [8,9,10]. Green AgNPs exhibit antioxidant and anti-inflammatory properties, including suppression of NF-κB activation and cytokine production, though their role in systemic chemical-induced toxicity remains underexplored [11,12,13].

The therapeutic efficacy of herbal medicines is often attributed to the synergistic interactions of their bioactive constituents; however, their practical application is limited by poor solubility, low bioavailability, rapid systemic clearance, and instability under physiological conditions, which can reduce their overall effectiveness [3,4]. To overcome these challenges, nanotechnology-based delivery systems have been developed to enhance the pharmacological performance of plant-derived compounds by improving solubility, stability, controlled release, and tissue targeting [9,11]. In particular, green synthesis of metallic nanoparticles, especially silver nanoparticles (AgNPs), has emerged as a promising strategy that integrates the advantages of nanotechnology with the intrinsic bioactivity of phytochemicals [9,10,11]. This approach employs plant metabolites as both reducing and stabilizing agents, resulting in biocompatible nanoparticles with enhanced antioxidant and anti-inflammatory properties and improved interaction with cellular redox systems [10,12]. Despite these advances, there remains limited evidence regarding the effectiveness of plant-mediated nanoparticles in models of chemically induced systemic toxicity, especially in relation to their ability to modulate interconnected oxidative stress and inflammatory pathways across multiple organs and regulate key signalling mechanisms such as Nrf2 and NF-κB [13].

Vachellia sieberiana is traditionally valued for its antioxidant and anti-inflammatory properties due to its rich phytochemical composition. The selection of *Vachellia sieberiana*-functionalized silver nanoparticles is based on both biological relevance and functional nanomaterial design. *Vachellia sieberiana* is widely used in African traditional medicine and has demonstrated antioxidant and anti-inflammatory activities through modulation of oxidative stress and inflammatory pathways. These properties are directly relevant to colorectal carcinogenesis, which is driven by chronic inflammation and redox imbalance. Incorporating these bioactive compounds into AgNPs may enhance their therapeutic potential as they have been established to modulate oxidative stress and inflammatory.

Despite increasing reports on plant-mediated silver nanoparticles, most studies have focused on single-organ models or isolated antioxidant effects, with limited evaluation of systemic redox–inflammatory interactions across multiple tissues in chemically induced carcinogenesis. In addition, there remains insufficient understanding of how phytochemical-functionalized nanoparticles influence the interplay between oxidative stress and inflammatory signaling in vivo.

Therefore, this study provides a multi-organ assessment (colon, liver, and kidney) of oxidative stress and inflammatory responses in a DMH-induced model, offering

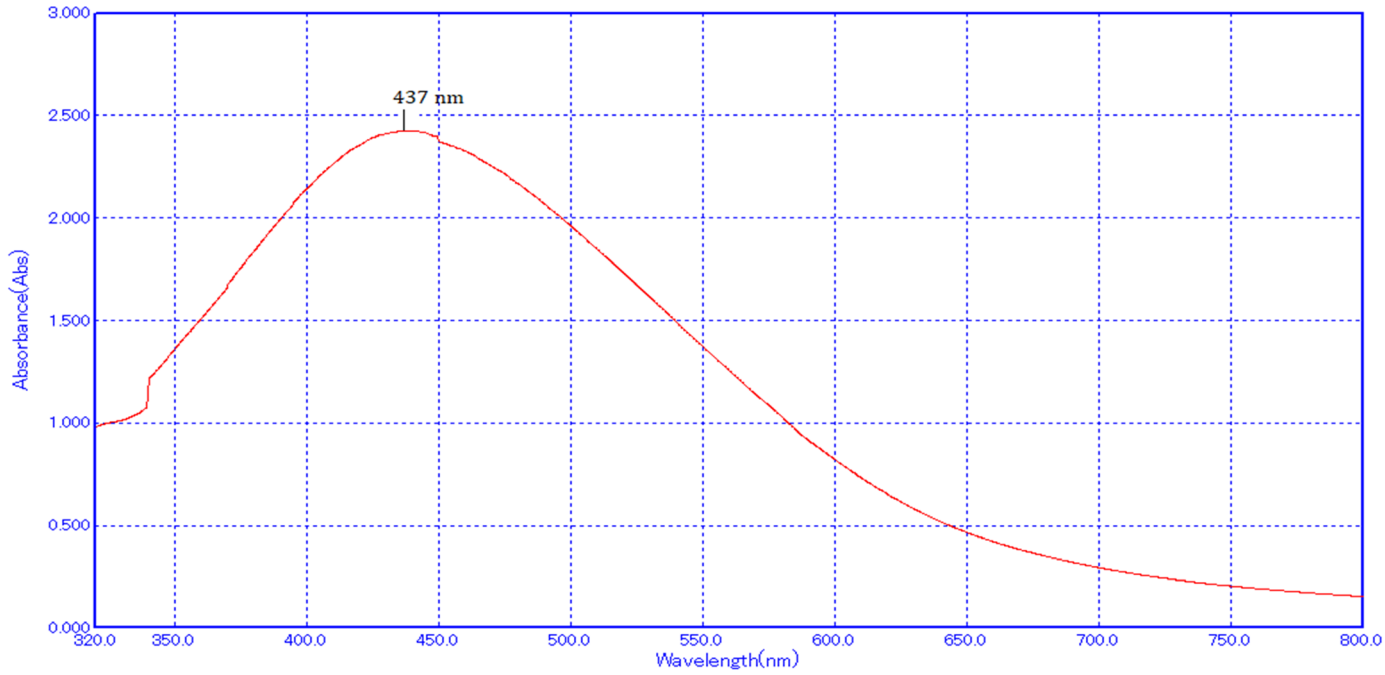

Figure 2: UV-vis Characterization of *Vachellia sieberiana*-AgNPs with an absorbance peak of 437 nm.

integrated insight into the redox–inflammation axis and the potential systemic effects of *Vachellia sieberiana*-functionalized AgNPs.

Methods

Plant-Mediated Nanoparticles Synthesis Strategy

Plant-mediated nanoparticles synthesis offers a biologically relevant approach for generating redox-active nanomaterials through the intrinsic reducing and stabilizing capacity of plant phytochemicals. *Vachellia sieberiana*, a medicinal tree species of the *Fabaceae* family, was selected as the biogenic reducer for silver nanoparticles (AgNPs) synthesis due to its abundance of redox-active constituents, including flavonoids, tannins, saponins, and phenolic compounds. These phytochemicals possess hydrogen- and electron-donating properties that enable effective reduction of metal ions while conferring antioxidant functionality to the resulting nanoparticles.

Aqueous extraction was deliberately employed to preserve polar antioxidant compounds and minimize chemical modification of phytoconstituents, thereby favouring biological redox activity and compatibility. This strategy enables the generation of phytochemical-functionalized silver nanoparticles with enhanced relevance for oxidative stress and inflammation modulation.

Collection, Authentication, and Processing of *Vachellia sieberiana*

Fresh leaves of *Vachellia sieberiana* were collected from Ogbomoso in Oyo state, Nigeria during the dry season (July, 2025). Botanical authentication was performed at the Herbarium Unit, Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, and a voucher specimen was deposited under the number LHO 806. To preserve phytochemical integrity and redox activity, the leaves were air-dried under ambient laboratory conditions away from direct sunlight and excessive heat prior to extraction.

Chemicals and Reagents-

All chemicals and reagents used were of analytical grade (≥99% purity). 1,2-Dimethylhydrazine (DMH) was procured from Shanghai Macklin Biochemical Co. Ltd., China. Doxorubicin was procured from a registered pharmaceutical store in Ogbomoso, Oyo State, Nigeria. The ELISA kits were acquired from Shanghai Ideal Medical Co. Ltd, China. All other chemicals and reagents used in this study were of analytical grade.

Other reagents include; methanol, Aluminum chloride, Sodium Acetate, phosphate buffer, Ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide (H2O2), Glacial acetic acid, Thiobarbituric acid (TBA), Eggyolk, sodium hydroxide, Hydrochloric acid (HCl), Sodium carbonate (Na2CO3), Folin, Trichloroacetic Acid (TCA), Tris Buffer, Sodium hydrogen phosphate (Na2HPO4), Potassium's dihydrogen phosphate (KH2PO4),

Preparation of Aqueous Extract for Nanoparticle Functionalization

The air-dried leaves were pulverized into a fine powder using an electric blender. Aqueous extraction was conducted at a concentration of 0.1 g/mL using double-

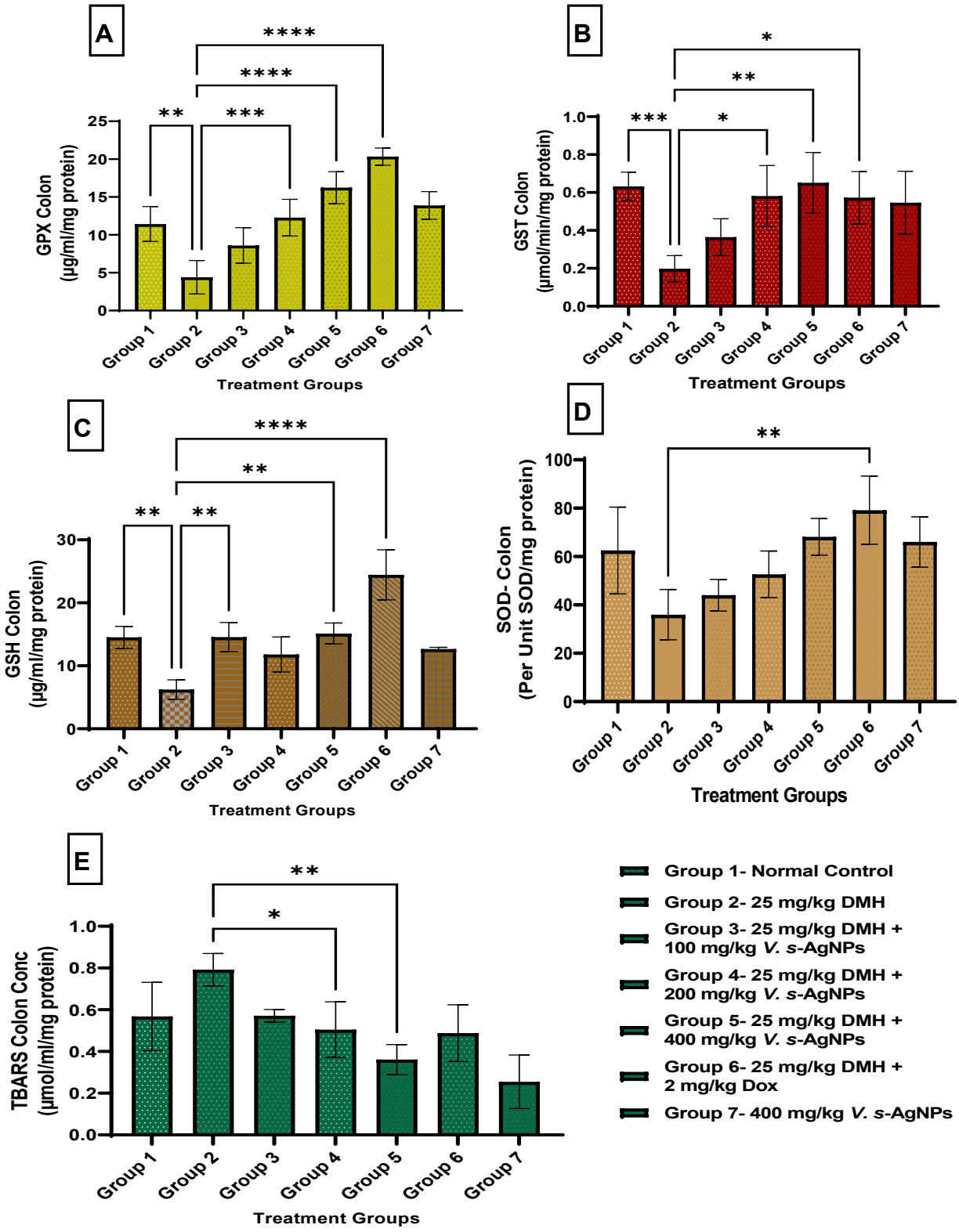

Figure 3: Oxidative stress markers in the colon of male Wistar rats with DMH-induced toxicity. (A) Colonic GPx activities. (B) Colonic GST activities. (C) Colonic reduced GSH levels. (D) Colonic SOD activities. (E) Colonic MDA concentrations.

distilled water at 60 °C for 2 h. Harsh extraction conditions and organic solvents were deliberately avoided to prevent degradation of hydrogen-donating and redox-sensitive phytochemicals. The extract was allowed to cool, centrifuged at 3000 rpm for 10 min, and filtered through Whatman No. 1 filter paper to obtain a clear aqueous extract, which was subsequently used for nanoparticles synthesis.

Phytochemical-Guided Synthesis of Silver Nanoparticles (AgNPs)

Phytochemical-functionalized silver nanoparticles were

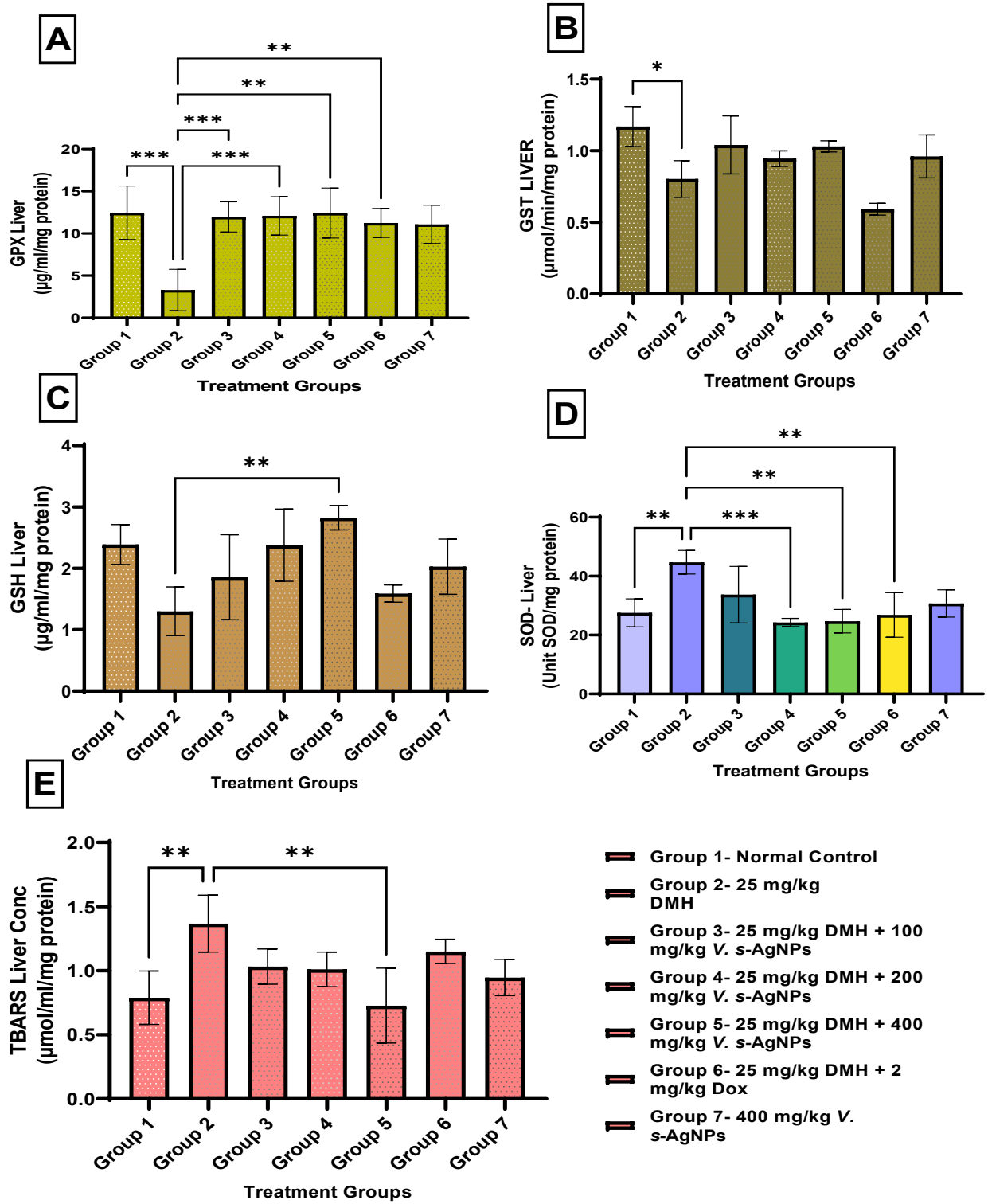

Figure 4: Oxidative stress markers in the liver of male Wistar rats with DMH-induced toxicity. (A) Hepatic GPx activities. (B) Hepatic GST activities. (C) Hepatic reduced GSH levels. (D) Hepatic SOD activities. (E) Hepatic MDA concentrations.

generated using *V. sieberiana* aqueous extract as a reducing, capping, and stabilizing agent. Briefly, 25 mL of the aqueous extract was added to 1000 mL of 1 mM silver nitrate ($AgNO_3$) solution and continuously stirred under light exposure for 30 min, as previously reported Badmus et al. [14].

Reduction of Ag^+ ions was visually monitored through the progressive color change from pale yellow to dark brown, attributable to surface plasmon resonance (SPR), indicating the formation of silver nanoparticles stabilized by plant-derived phytochemicals.

Nanoparticle Characterization Relevant to Biological Activity

The synthesized *V. sieberiana*-functionalized AgNPs were

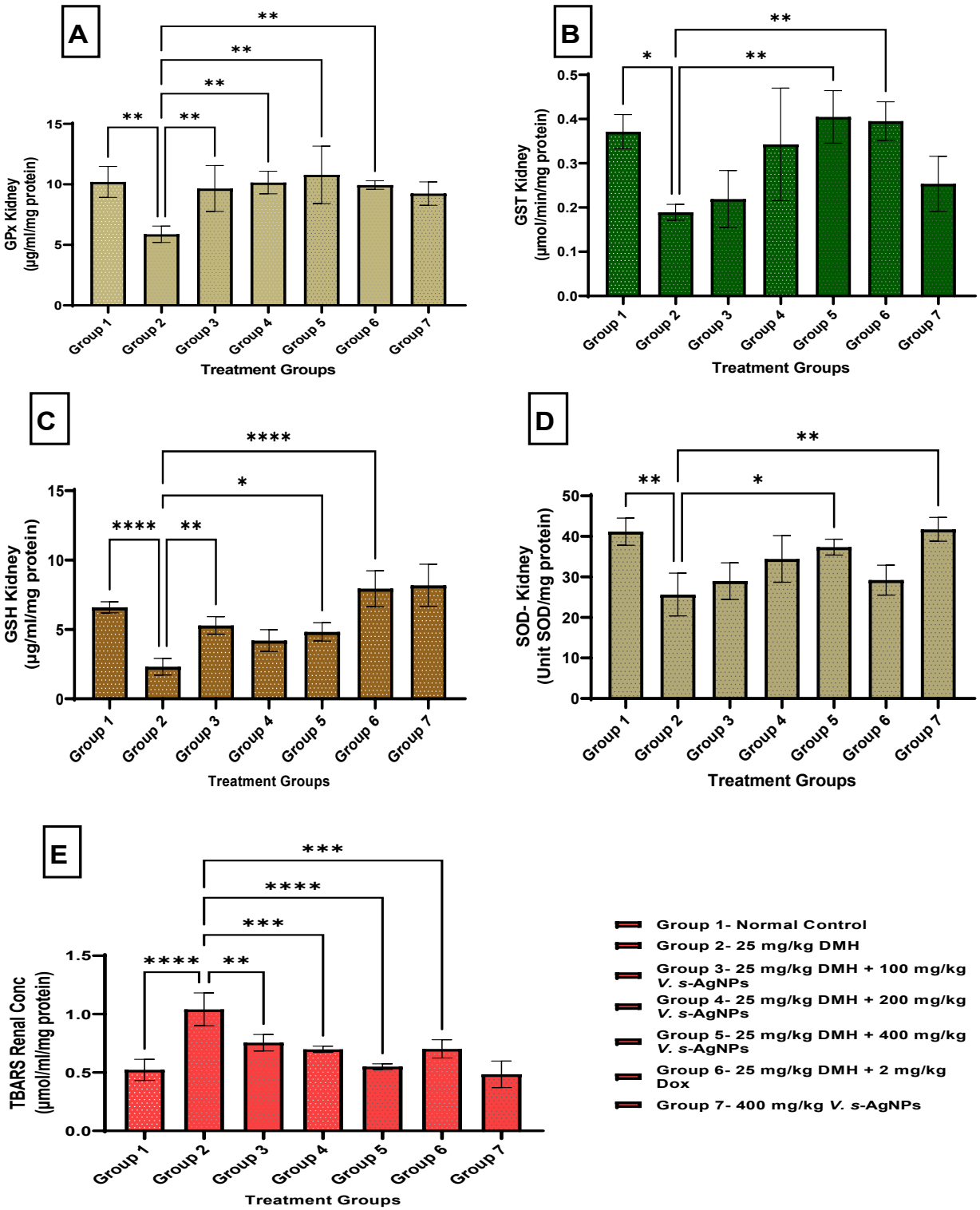

Figure 5: Oxidative stress markers in the kidney of male Wistar rats with DMH-induced toxicity. (A) Renal GPx activities. (B) Renal GST activities. (C) Renal reduced GSH levels. (D) Renal SOD activities. (E) Renal MDA concentrations.

characterized with emphasis on properties relevant to their biological activity. UV–visible spectroscopy was employed as an initial and widely accepted method to confirm nanoparticle formation based on the detection of the characteristic surface plasmon resonance (SPR) band of silver nanoparticles, typically observed within the 400–450 nm range, as reported in previous studies [15]. Absorption spectra were recorded within the 200–800 nm wavelength range using a UV–visible spectrophotometer and analyzed using UV-Vis Analyst software (version 5). The presence of the SPR peak was taken as preliminary confirmation of successful phytochemical-mediated nanoparticle synthesis.

Acute Toxicity Study

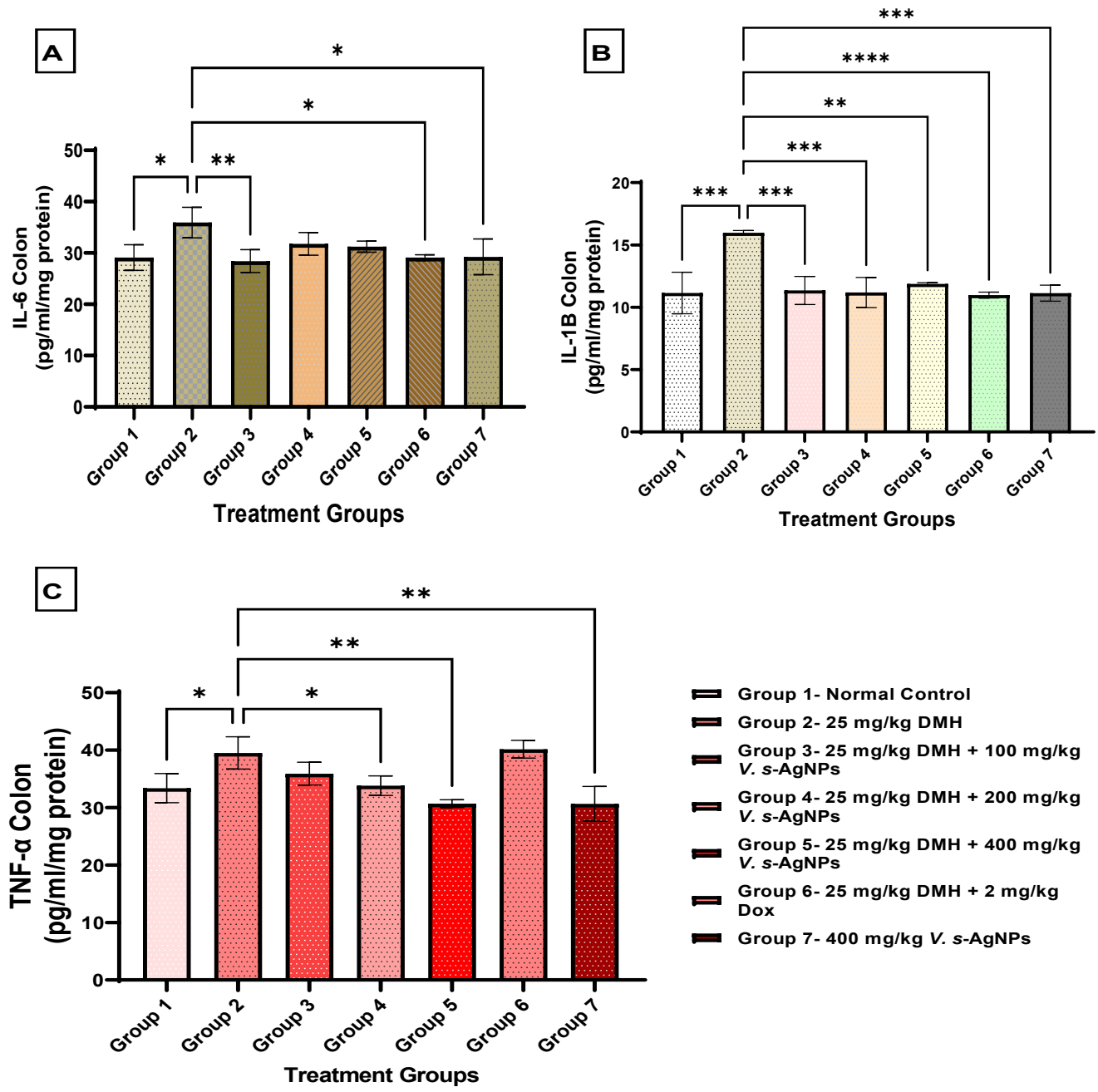

Figure 6: Colonic levels of pro-inflammatory cytokines in male Wistar rats with DMH-induced toxicity. (A) Interleukin-6 concentrations. (B) Interleukin-1β concentrations. (C) Tumor Necrosis Factor-α concentrations.

An acute oral toxicity study was conducted using Lorke's method to determine the safety profile of the synthesized silver nanoparticles. Animals were administered graded doses of the nanoparticles and observed for signs of toxicity and mortality over a 24-hour period, followed by continued monitoring. The median lethal dose (LD_{50}) was estimated to be 4000 mg/kg, indicating a relatively wide safety margin.

The selection of experimental doses (100, 200, and 400 mg/kg) was guided by the results of an acute toxicity study conducted using Lorke's method, which established the median lethal dose (LD_{50}) of the synthesized AgNPs to be 4000 mg/kg. The administered doses therefore represent 1/40, 1/20, and 1/10 of the LD_{50}, respectively, in line with standard toxicological practices for evaluating pharmacological activity within a safe margin. Notably, this LD_{50} value is consistent with previous reports indicating relatively low acute toxicity of silver nanoparticles, with values exceeding 2000 mg/kg and 5000 mg/kg in experimental models [16,17]. This consistency further supports the safety profile of the synthesized nanoparticles and justifies the selected dose range for the present study.

The selected doses (100, 200, and 400 mg/kg) represent conservative fractions (1/40, 1/20, and 1/10) of the LD_{50} (4000 mg/kg), ensuring a wide safety margin. Furthermore, no overt signs of toxicity or additional oxidative damage were observed in hepatic and renal tissues, supporting the tolerability of these doses within the experimental duration.

Nevertheless, long-term toxicity and nanoparticle accumulation remain important considerations for future investigation.

Experimental Animals and Ethical Considerations

Thirty-five apparently healthy male Wistar rats (average body weight ≈100 g) were obtained from the Animal House, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Animals were housed under standard laboratory conditions (12-h light/dark cycle, controlled temperature and humidity) and allowed to acclimatize for one week with free access to standard pellet diet and water. All experimental procedures complied with established guidelines for the care and use of laboratory animals as approved by the Research Ethical Committee of the Faculty of Basic Medical Sciences, LAUTECH (REC/FBMS No. 019/0257) that is in agreement with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

Induction of Oxidative and Inflammatory Stress Using DMH

Oxidative and inflammatory stress was induced using 1,2-dimethylhydrazine (DMH), a well-established pro-oxidant and pro-inflammatory agent known to generate reactive oxygen species (ROS) and trigger inflammatory signaling cascades. DMH was procured from Shanghai Macklin Biochemical Co. Ltd. and dissolved in 0.9% NaCl prior to administration. Rats received subcutaneous injections of DMH at a dose of 25 mg/kg body weight once weekly for six weeks. Administration of *V. sieberiana*-functionalized AgNPs was carried out concurrently during this period to evaluate their modulatory effects on DMH-induced oxidative and inflammatory responses.

Treatment Protocol with *V. sieberiana*-Functionalized AgNPs

The experimental design is presented in Table 2.1. Group 1 served as the normal control, while Group 2 received DMH only (25 mg/kg body weight). Groups 3, 4, and 5 received DMH alongside graded doses (100, 200, and 400 mg/kg body weight) of *V. sieberiana*-functionalized AgNPs to assess dose-dependent redox and anti-inflammatory modulation. Group 6 received DMH in combination with doxorubicin (2 mg/kg body weight) as a reference treatment, while Group 7 received *V. sieberiana*-AgNPs alone (400 mg/kg body weight). Nanoparticles were administered daily via oral gavage for six weeks, concurrent with weekly DMH administration.

Assessment of Oxidative Stress and Antioxidant Defense

Redox status was evaluated by assessing key antioxidant defense biomarkers. Colonic, hepatic and renal activities of GST[18], GPx [19], SOD [20], and levels of GSH [21] were evaluated using standard spectrophotometric method. Lipid peroxidation was quantified by measuring thiobarbituric reactive substances (TBARS) in the tissues, a measure of malondialdehyde (MDA), were evaluated according to Okhawa *et al.* [22] using a spectrophotometric (532 nm) method. All reagents utilized were of analytical grade.

Evaluation of Inflammatory Cytokines

Colonic concentrations of pro-inflammatory cytokines; Interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α), were quantified using enzyme-linked immunosorbent assay (ELISA) assay kit according to the manufacturer's protocols (Shanghai Ideal Medical Technology Co. Ltd., China).

Histological Evaluation

For the investigation of histological changes, colon and liver tissues were carefully excised and immediately preserved in 10% formalin and sectioned for H&E staining following the method described by Feldman and Wolfe. This staining technique highlights the cellular and tissue structures, enabling detailed examination under a microscope.

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical analyses were performed using GraphPad Prism software (version 9; GraphPad Software Inc., California, USA), with statistical significance set at $p < 0.05$.

Results

Characterization of *Vachellia sieberiana*-Silver Nanoparticles (AgNPs) by UV-Visible Spectroscopy.

The relatively sharp SPR peak observed at 437 nm further suggests the formation of stable and well-dispersed nanoparticles. This absorbance profile is consistent with previously reported plant-mediated silver nanoparticles, including *Annona muricata*-AgNPs with a reported peak at 420 nm (*Badmus et al.*[14]), supporting successful phytochemical-mediated nanoparticles synthesis.

Results of Oxidative Stress and Antioxidant

Defense Activities

Oxidative stress status was assessed by evaluating antioxidant defense systems;glutathione peroxidase (GPx), glutathione-S-transferase (GST), reduced glutathione (GSH), and superoxide dismutase (SOD), alongside lipid peroxidation indexed by malondialdehyde (MDA) levels in the colon, liver, and kidney.

Colonic Oxidative Stress Markers

Administration of DMH alone (Group 2) resulted in marked suppression of colonic antioxidant defense systems relative to the normal control (Group 1). Specifically, significant reductions were observed in GPx (61.43%; p = 0.0017), GST (68.72%; p = 0.0009), reduced GSH (57.07%; p = 0.0012), and SOD (42.45%; p = 0.1117).

Co-treatment with *V.s*-AgNPs produced a dose-dependent restoration of antioxidant enzyme activities, with the highest dose (400 mg/kg; Group 5) showing the most pronounced increases across all measured parameters.

Colonic lipid peroxidation was significantly elevated following DMH administration, with MDA levels increased by 39.55% relative to the normal control. Treatment with *V. sieberiana*-AgNPs significantly attenuated MDA accumulation in a concentration-dependent manner, with significant reductions observed at 200 mg/kg (36.33%; p = 0.0296) and 400 mg/kg (54.38%; p = 0.0016).

Hepatic Oxidative Stress Markers

In the liver, DMH administration significantly depleted antioxidant defenses, as evidenced by reductions in GPx (73.53%; p = 0.0003), GST (31.35%; p = 0.0155), and reduced GSH (45.56%; p = 0.0829) relative to the normal control. Co-treatment with *V. sieberiana*-AgNPs (Groups 3–5) markedly restored hepatic antioxidant enzyme activities, returning most parameters toward normal levels.

Notably, rats treated with *V. sieberiana*-AgNPs exhibited

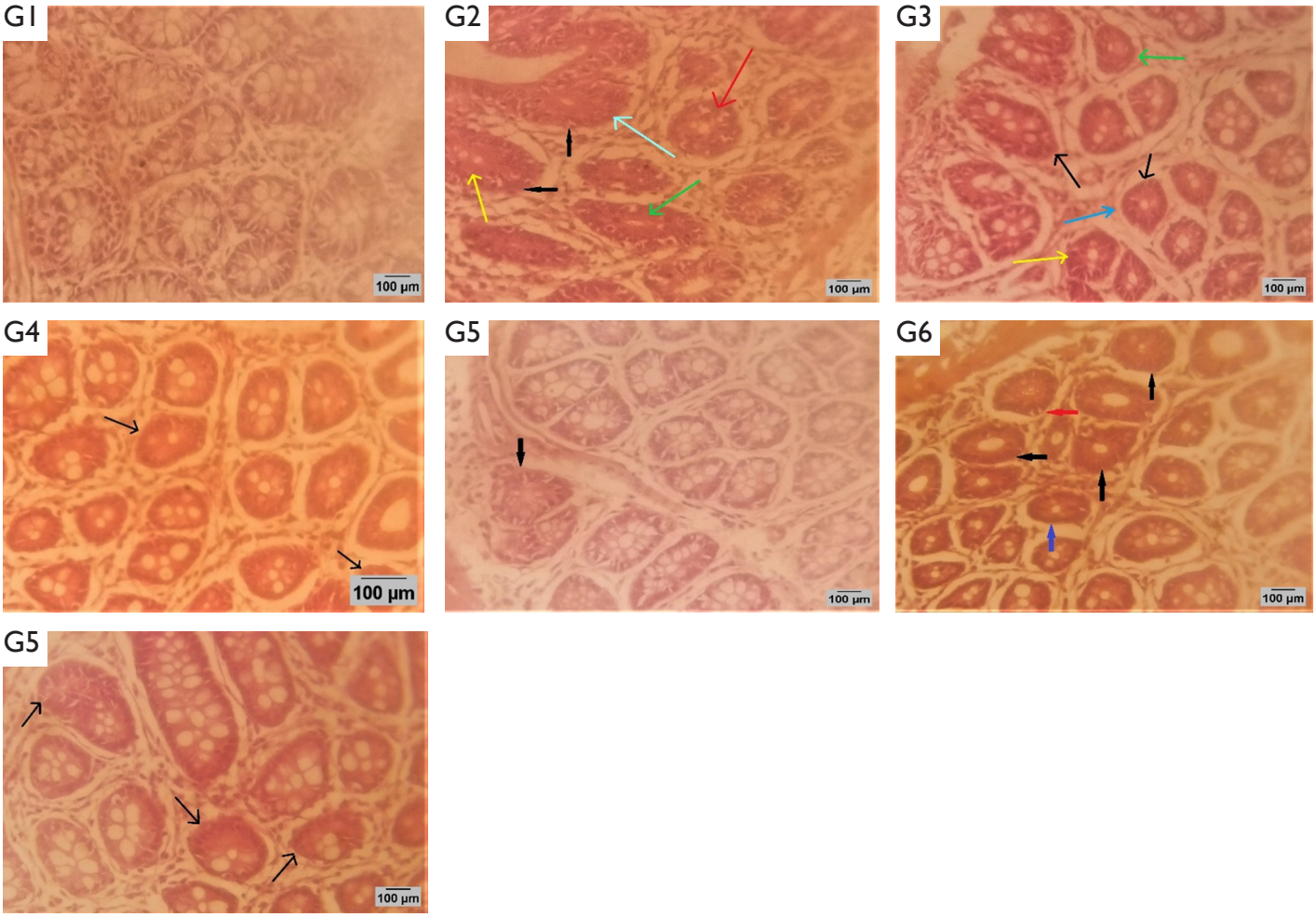

Plate 1: Photomicrographs of *V. sieberiana*-AgNPs effects on colon tissue architecture in DMH induced premalignant colon cancer in male Wistar rats. G1 (Normal control). G2 (Positive control; 25 mg/kg DMH). G3 (DMH plus 100 mg/kg AgNPs). G4 (DMH plus 200 mg/kg AgNPs). G5 (DMH plus 400 mg/kg AgNPs). G6 (DMH plus 2 mg/kg Dox). G7 (400 mg/kg AgNPs only). (H &E) x400. Black arrow indicates dysplastic cells, red arrow indicate inflammation, blue arrow indicates hyper-chromatic nucleus, yellow arrow indicates pseudo-stratified nucleus, and green arrow indicate loss of nucleus polarity.

higher preservation of antioxidant enzymes compared with the doxorubicin-treated group (Group 6).

In contrast to other antioxidant enzymes, hepatic SOD activity was significantly elevated following DMH exposure (62.46%; p = 0.0033). Co-administration of *V. sieberiana*-AgNPs at 200 and 400 mg/kg normalized SOD activity toward control values. Similarly, doxorubicin- and nanoparticles-only–treated groups showed no significant deviation from normal control levels.

Hepatic MDA concentrations were significantly increased by DMH administration (73.25% increase vs. Group 1). Treatment with *V. sieberiana*-AgNPs significantly reduced lipid peroxidation, with the 400 mg/kg dose producing the greatest reduction (46.78%; p = 0.0041).

Renal Oxidative Stress Markers

DMH exposure significantly impaired renal antioxidant defense, resulting in decreases in GPx (42.36%; p = 0.0012), GST (49.00%; p = 0.0117), reduced GSH (65.51%; p < 0.0001), and SOD (37.69%; p = 0.0019).

Co-treatment with *V. sieberiana*-AgNPs significantly ameliorated these alterations, restoring antioxidant enzyme activities toward normal levels. GST and SOD activities demonstrated a clear dose-dependent response to nanoparticles treatment.

Renal lipid peroxidation was significantly elevated following DMH administration, with a 49.74% increase in MDA levels (p < 0.0001). *V. sieberiana*-AgNPs significantly reduced renal MDA concentrations in a concentration-dependent manner, with the most pronounced effect observed at 400 mg/kg (47.15% reduction; p < 0.0001).

Effects of *V. sieberiana*–AgNPs on Colonic Pro-inflammatory Cytokines

Colonic Interleukin-6 (IL-6)

DMH administration significantly increased colonic IL-6 concentrations by 23.42% (p = 0.0110) compared with the normal control. Co-treatment with *V. sieberiana*-AgNPs significantly attenuated this elevation, with the most pronounced reduction observed at 100 mg/kg (20.90%; p = 0.0047). Doxorubicin treatment also resulted in a significant reduction in IL-6 levels (18.99%; p = 0.0201).

Colonic Interleukin-1β (IL-1β)

Colonic IL-1β concentrations were markedly elevated following DMH exposure (43.30%; p = 0.0001). Treatment with *V. sieberiana*-AgNPs at all administered doses significantly suppressed IL-1β expression, restoring cytokine levels toward normal control values. A comparable reduction was observed in the doxorubicin-treated group.

Colonic Tumor Necrosis Factor-α (TNF-α)

DMH administration resulted in a significant increase in colonic TNF-α concentrations (18.37%; p = 0.0278). Co-treatment with *V. sieberiana*-AgNPs significantly suppressed TNF-α expression in a dose-dependent manner, with reductions of 14.43% and 22.33% observed at 200 and 400 mg/kg, respectively, compared with the positive control.

Histopathological Evaluation

Histopathological examination (H&E staining) of colon tissues revealed marked mucosal damage, inflammatory cell infiltration, and epithelial disruption in the DMH-treated group. Treatment with *V. sieberiana*-AgNPs ameliorated these alterations, as evidenced by improved mucosal architecture and reduced inflammatory infiltration.

In contrast, liver sections across experimental groups did not exhibit pronounced histopathological alterations, with general preservation of hepatic architecture observed.

Discussion

The present study demonstrates that *Vachellia sieberiana*-functionalized silver nanoparticles (*V.s*-AgNPs) significantly attenuate 1,2-dimethylhydrazine (DMH)-induced oxidative stress and inflammatory signaling in male Wistar rats. DMH is a well-established colonotropic chemical that induces reactive oxygen species (ROS) overproduction, lipid peroxidation, and depletion of endogenous antioxidant defenses (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and reduced glutathione (GSH)), culminating in inflammation and molecular damage in gastrointestinal tissues [23,24]. These perturbations mimic early events in colon carcinogenesis and oxidative injury, making DMH a valuable toxicology model for studying redox–inflammation interactions. It is important to note that the present study focuses on the biological evaluation of phytochemical-mediated nanoparticles, and therefore UV–visible spectroscopy was employed as a preliminary confirmation method. While additional physicochemical characterization (e.g., DLS, TEM, zeta potential) would provide more detailed structural insights, the observed biological responses across multiple organs serve as functional validation of the synthesized nanostructures.

In this study, DMH administration produced significant reductions in GPx, GST, GSH, and SOD activities across colonic, hepatic, and renal tissues, accompanied by elevated malondialdehyde (MDA), a marker of lipid peroxidation. This pattern of antioxidant depletion and oxidative injury aligns with reports that oxidative stress

markers are elevated in DMH models and that several phytochemical interventions (e.g., hesperetin, tannic acid, luteolin) can reverse these biochemical disruptions [24,25,26]. For example, hesperetin treatment restored GPx and SOD and reduced expression of pro-inflammatory proteins such as TNF-α, IL-6, iNOS, and NF-κB in the colon of DMH rats, suggesting that antioxidant supplementation can ameliorate both oxidative and inflammatory insults [24,25]. Similarly, tannic acid diminished DMH-induced TNF-α release and attenuated oxidative enzymes, further demonstrating the interdependence of redox status and inflammation [25]. These prior studies establish a strong backdrop for interpreting the mechanistic effects observed with *V.s*-AgNPs.

Co-treatment with *V.s*-AgNPs in this study significantly restored antioxidant enzyme activities and reduced MDA accumulation in a dose-dependent fashion. These findings suggest that *V.s*-AgNPs not only scavenge ROS but may also participate in upregulating endogenous antioxidant defense systems. Plant-mediated green synthesis confers nanoparticles with a phytochemical corona possessing flavonoids, phenols, tannins, and other bioactive compounds that function as reducing, capping, and stabilizing agents during synthesis [27]. These phytochemicals are well known for their ability to donate electrons, neutralize free radicals, and regenerate intracellular antioxidants, enhancing GPx and GST activity and stabilizing GSH pools [27]. In this context, *V.s*-AgNPs combine the physicochemical properties of silver with the biological functionality of plant phytochemicals, yielding enhanced antioxidant activity relative to unmodified nanoparticles.

Mechanistically, restoration of redox balance by *V.s*-AgNPs likely involves modulation of the Nrf2/ARE signaling pathway, a master regulator of antioxidant gene expression. Under oxidative stress, Nrf2 translocates to the nucleus to activate genes encoding detoxifying enzymes, including GPx, GST, and heme oxygenase-1 (HO-1), thereby strengthening the cell's capacity to counteract ROS [28,29]. Importantly, crosstalk between Nrf2 and the pro-inflammatory transcription factor NF-κB provides a pivotal regulatory node linking oxidative stress to inflammatory responses, where ROS-mediated activation of NF-□B promotes the expression of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), while Nrf2 activation enhances antioxidant defenses and concurrently suppresses NF-κB signaling [30]. Beyond this regulatory axis, the protective effects of *V.s*-AgNPs may also extend to the preservation of mitochondrial function, a key source and target of ROS during chemical-induced toxicity. Excessive ROS generation by DMH can compromise mitochondrial membrane integrity and disrupt electron transport chain activity, thereby amplifying oxidative stress and inflammatory signaling. By attenuating ROS accumulation and restoring antioxidant capacity, *V.s*-AgNPs may help maintain mitochondrial stability, limit ROS propagation, and prevent downstream activation of inflammatory pathways. Furthermore, phytochemical constituents associated with the nanoparticles may contribute to maintaining mitochondrial redox homeostasis and protecting cellular components from oxidative damage, collectively reinforcing their protective role.

Although the observed effects are consistent with modulation of Nrf2 and NF-κB signaling pathways, these pathways were not directly measured in the present study and are therefore proposed based on established literature. Importantly, the biological activity observed is likely attributable to a synergistic interaction between the silver nanoparticle core and the phytochemical constituents acting as capping and stabilizing agents. These phytochemicals contribute intrinsic antioxidant and anti-inflammatory properties, while the nanoparticle structure may enhance cellular uptake and bioavailability, collectively amplifying the overall biological effect.

Consistent with this mechanistic model, *V.s*-AgNPs significantly suppressed colonic IL-6, IL-1β, and TNF-α levels compared with DMH controls. This anti-inflammatory effect mirrors findings from plant-derived nanoparticles studies reporting reduced pro-inflammatory mediator expression and improved tissue antioxidant status. For instance, green-synthesized silver nanoparticles using diverse plant extracts have demonstrated anti-inflammatory activities, including inhibition of NF-κB activation and downregulation of inflammatory cytokines, by virtue of their phytochemical constituents and small size facilitating cellular uptake [11,27]. These findings are congruent with *V.s*-AgNPs attenuating not only oxidative damage but also inflammatory signaling, reinforcing the critical link between redox homeostasis and inflammation in DMH toxicity [31].

Although aqueous extracts of *Vachellia sieberiana* are known to exhibit antioxidant and anti-inflammatory effects, their efficacy is often limited by poor bioavailability and instability of active constituents [27]. The nanoformulation used in this study appears to overcome some of these limitations. The small particle size and larger surface area of the *V.s*-AgNPs likely improve cellular uptake and interaction with redox-sensitive pathways, while the phytochemical coating may help preserve the activity of bioactive compounds. This could explain the more marked improvements observed in antioxidant enzymes (SOD, GPx, GST, and GSH), along with the reductions in MDA and pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α). Overall, these findings suggest that formulating *Vachellia sieberiana* into silver nanoparticles enhances its biological activity compared to the conventional aqueous extract.

Organ-specific observations provide further nuance. The

colon, being the primary site of DMH-induced insult, responded robustly to *V.s*-AgNPs with normalization of antioxidant enzymes and cytokines, reflecting restoration of epithelial resilience and mucosal integrity. In contrast, hepatic SOD activity in DMH-only animals exhibited an initial compensatory increase, a phenomenon noted in other toxicant models where early antioxidant responses may transiently upregulate to counter acute ROS surges, but are insufficient to prevent damage [32]. *V.s*-AgNPs appeared to normalize this response, suggesting improved ROS handling that precludes excessive compensatory upregulation. The kidneys, similarly affected by DMH, demonstrated restored antioxidant activity with *V.s*-AgNPs, indicating that these nanoparticles reach systemically relevant tissues and exert protective effects beyond the colon.

Given the well-documented concerns regarding the potential toxicity of silver nanoparticles, particularly in the liver and kidneys, the present findings provide important insight into their biocompatibility. In this study, *V.s*-AgNPs did not induce further oxidative damage in hepatic and renal tissues; rather, they restored antioxidant enzyme activities and reduced lipid peroxidation, suggesting a protective rather than toxic effect at the administered doses. This may be attributed to the phytochemical-mediated green synthesis approach, which enhances nanoparticle stability and reduces the likelihood of adverse biological interactions. The histopathological improvements observed in colon tissues further support the anti-inflammatory and antioxidant effects of the synthesized nanoparticles. These observations support the potential biocompatibility of *V.s*-AgNPs and highlight their promise for safe therapeutic application, although further detailed toxicological and histopathological evaluations are warranted.

Comparing *V.s*-AgNPs with conventional agents underscores their translational potential. Some chemotherapeutic or synthetic antioxidant drugs possess inherent oxidative side effects that limit their utility; plant-functionalized nanoparticles may provide a dual modality that both enhances antioxidant capacity and suppresses inflammation without the same adverse profiles. Indeed, green synthesized AgNPs from garlic peel and other botanical sources have shown liver-protective and anti-inflammatory effects by enhancing antioxidant enzymes and reducing pro-inflammatory cytokines in hepatotoxic models, suggesting broad applicability of plant-mediated nanoparticles in redox-related diseases [33].

The choice of *Vachellia sieberiana* relates critically to phytochemical composition. Rich in flavonoids, tannins, saponins, and phenolics, this plant species provides abundant hydrogen-donating and redox-active constituents that improve nanoparticles biosynthesis and biological functionality. Preserving these labile compounds through aqueous extraction likely maximized their contribution to nanoparticles capping and bioactivity, consistent with best practices in green nanotechnology [27].

Despite these promising results, certain subtleties warrant discussion. For example, maximal suppression of IL-6 occurred at intermediate *V.s*-AgNPs doses rather than strictly increasing with dose, suggesting potential hormetic or biphasic effects, a phenomenon observed in other phytochemical and nanoparticles studies wherein moderate doses elicit optimal signaling responses [27]. Such dynamics emphasize the need for comprehensive dose–response analysis in future studies.

Despite these promising findings, certain limitations should be acknowledged. While UV–visible spectroscopy provided preliminary evidence for nanoparticle formation, further physicochemical characterization, including particle size distribution, zeta potential, and morphological analysis, would be necessary to comprehensively establish nanoparticle properties, stability, and reproducibility. Future studies should incorporate these techniques to strengthen the translational applicability of the present findings.

Conclusion

In summary, this study provides compelling evidence that *V.s*-AgNPs effectively disrupt the DMH-induced oxidative stress–inflammation axis by both enhancing endogenous antioxidant defenses through Nrf2/ARE modulation and suppressing inflammatory signaling via NF-κB attenuation. The synergistic integration of plant phytochemicals with nanostructured silver confers a mechanistically distinct advantage that merits further translational exploration in oxidative stress and inflammation-driven disorders.

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RESEARCH ARTICLE

Mapping International and Regional Collaboration Networks in African Traditional Medicine Research: A Bibliometric Analysis (2000 – 2024)

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Abstract

Background: African Traditional Medicine (ATM) plays a central role in healthcare across Africa and contributes to global drug discovery through its long-standing use of herbal, ritual, and spiritual practices. Although research output in ATM has increased, evidence on international and regional collaboration patterns remains limited. Understanding these partnerships is critical for strengthening research productivity, knowledge exchange, and the integration of traditional medicine into formal healthcare systems.

Methods: A descriptive bibliometric analysis was conducted using peer-reviewed ATM-related publications indexed in the Scopus database from 2000 to 2024. Publication trends, authorship patterns, institutional productivity, and country-level collaborations were analysed. Co-authorship and collaboration networks were visualised using VOSviewer and Bibliometrix (R package).

Results: A total of 2,392 publications were identified, representing an approximately 85-fold increase over the study period. South Africa led research output (37.4%), followed by Nigeria (4.8%), while the United States and the United Kingdom were the main non-African collaborators. Van Staden J., Afolayan A. J., and Maroyi A. were the most prolific authors. South African institutions, particularly the University of KwaZulu-Natal, University of Pretoria, and University of Fort Hare, dominated institutional output. Strong collaborative links were observed between South Africa and Nigeria, the USA, and the UK, with additional contributions from France, Germany, India, and Australia.

Conclusions: ATM research has expanded substantially, with South Africa serving as a key collaboration hub. However, intra-African collaboration remains limited. Strengthening regional partnerships, institutional capacity, and funding is essential for advancing evidence-based traditional medicine in Africa.

Keywords: African Traditional Medicine, Bibliometric analysis, Collaboration networks, Co-authorship, Research productivity, Africa

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Introduction

African Traditional Medicine (ATM) remains a cornerstone of healthcare in Africa, serving as the first line of treatment for over 80% of the population [1–3]. It encompasses a long-standing system of healing that integrates the use of medicinal plants, spiritual practices, rituals, and ceremonies to diagnose, treat and prevent diseases [4,5]. Although herbal medicine constitutes the major component of ATM, the spiritual and ritual dimensions remain integral to its holistic approach to health. Rooted in indigenous knowledge systems, ATM plays a crucial role in disease prevention, management and the discovery of new drugs

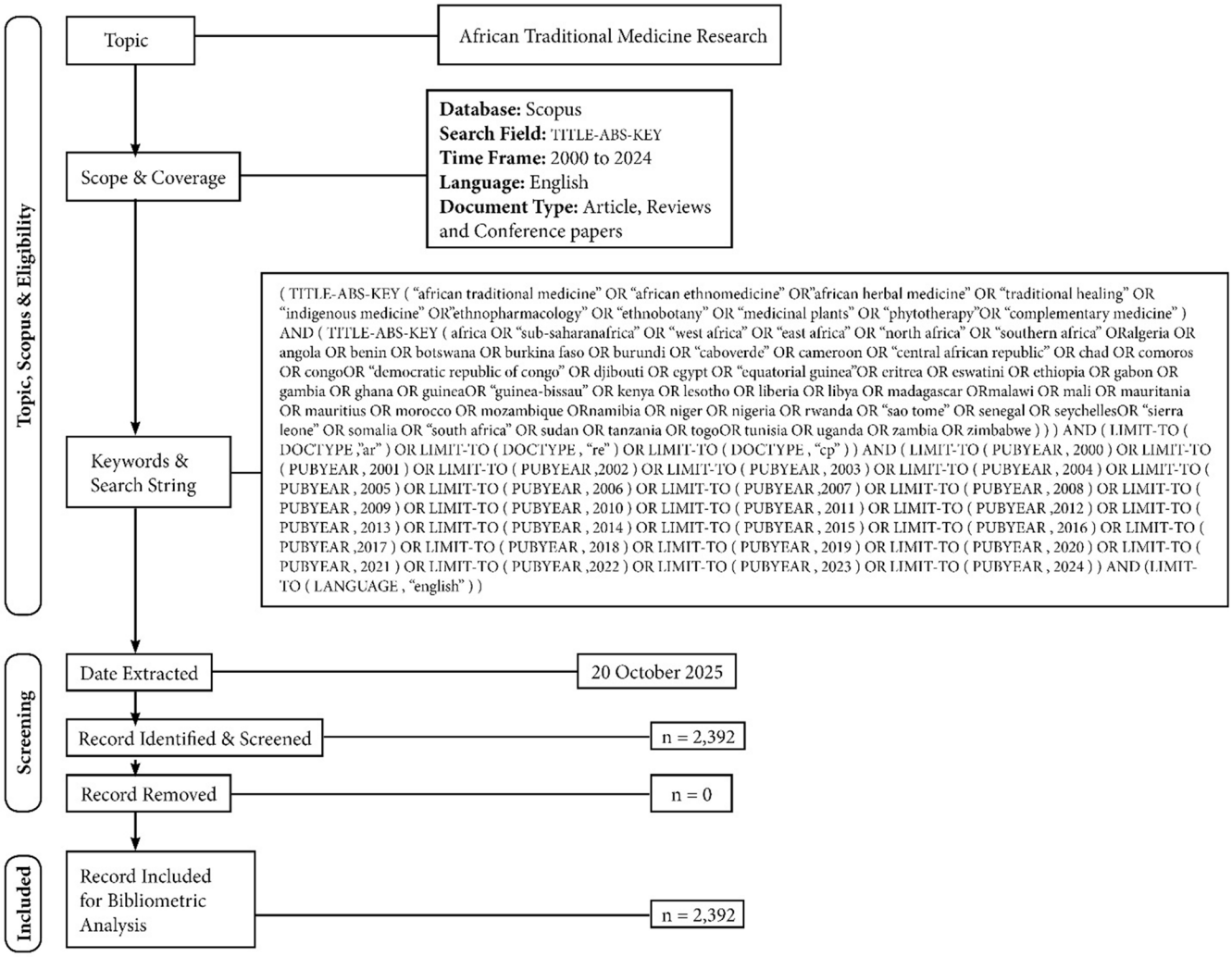

Figure 1: Flow diagram of the search strategy

[6,7]. Studies have identified numerous bioactive compounds from African medicinal plants with therapeutic potential, particularly in treating malaria, inflammation and pain [8,9]. These highlight the importance of ATM as both a cultural heritage and a scientific resource for modern drug development.

Global attention to traditional and complementary medicine has grown rapidly, motivated by the demand for affordable, accessible, and culturally relevant healthcare solutions [10,11]. The World Health Organization (WHO) recognises traditional medicine as a vital pathway to achieving universal health coverage and has supported African countries to strengthen research, regulation, and integration into national health systems [12–14]. Significant progress has been made in areas such as policy development, collaboration between traditional and conventional health practitioners, and quality assurance [12]. However, evidence-based integration of ATM remains limited, primarily due to a lack of rigorous clinical validation and standardised methodologies [9,15]. Researchers have emphasised the need for robust, evidence-based studies, including clinical trials, to confirm the safety and efficacy of traditional medicines and support their inclusion in formal healthcare systems [9].

Research collaboration is central to advancing ATM knowledge production and innovation. Partnerships among traditional healers, scientists, policymakers, and global health stakeholders enhance the documentation and validation of traditional medical knowledge [1,2]. Such collaborations foster interdisciplinary research, resource sharing, and capacity building, thereby enhancing the visibility and impact of African scholarship within global scientific communities. Strengthened collaborative networks can also address challenges related to fragmented data, duplication of research efforts, and limited research infrastructure, thereby facilitating the development of evidence-based traditional medicine practices [12,16,17].

Despite growing research outputs in ATM, no bibliometric study has systematically examined the patterns and dynamics of international and regional collaboration in this field. Existing bibliometric efforts have focused mainly

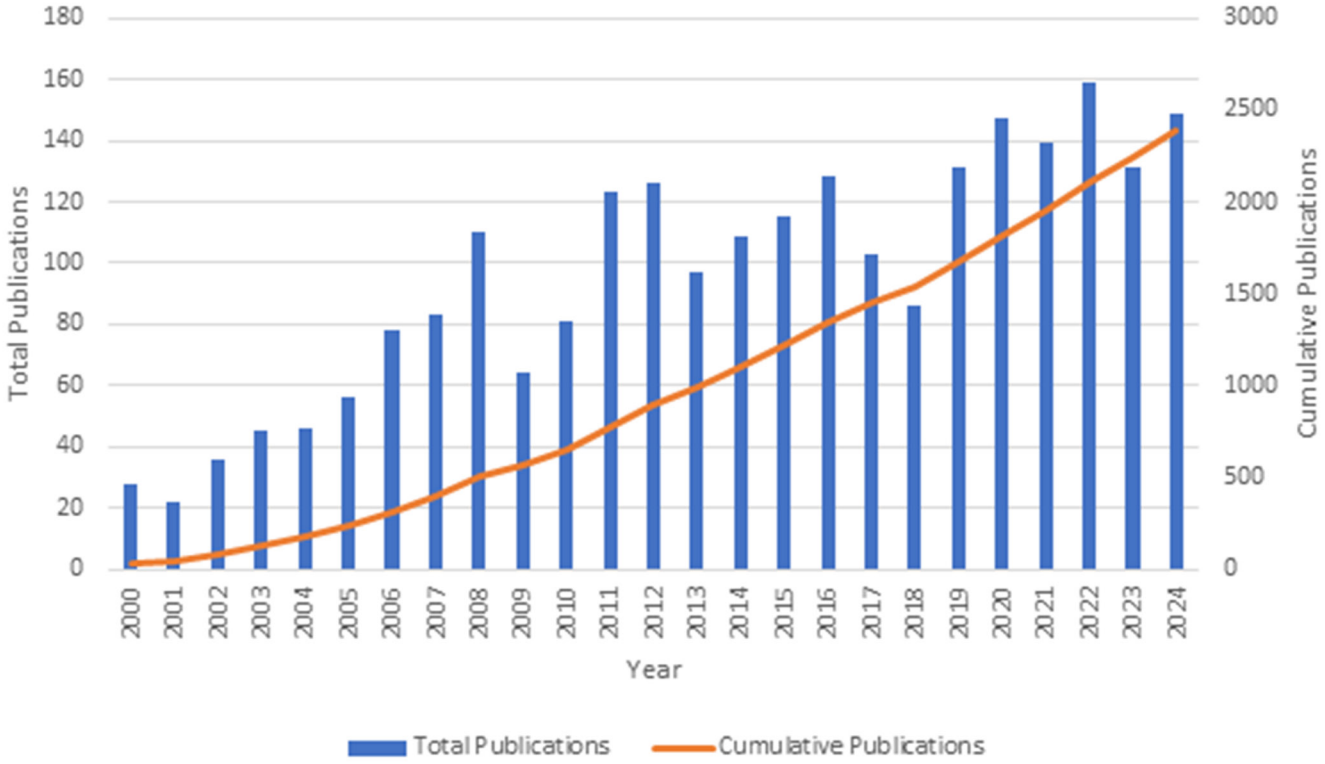

Figure 2: Publication Trends of African Traditional Medicine Research.

on thematic trends or species-specific analyses rather than mapping collaborative structures. For instance, Rafiu et al. (2025) conducted a bibliometric and ethnobotanical analysis of plant utilisation in Nigeria, revealing regional variations and limited collaboration among researchers. Similarly, Reddy et al. (2024) used bibliometric mapping to visualise research trends on *Sceletium* species in South Africa, focusing on phytochemical and pharmacological aspects rather than co-authorship networks. Chelghoum et al. (2021) explored the use of medicinal plants among Algerian diabetic patients using ethnopharmacological and bibliometric approaches, but did not analyse research partnerships or institutional linkages. While these studies provide valuable insights into national and species-level research developments, they do not capture the broader network of collaboration that drives ATM research across Africa and beyond.

This lack of systematic investigation into collaborative networks represents a major gap in the literature. Understanding how researchers, institutions, and countries interact can reveal the structure, intensity, and evolution of partnerships supporting ATM research. Mapping these networks can identify leading contributors, cross-border collaborations, and emerging hubs of innovation that influence research visibility, knowledge exchange, and policy development [17,21].

Through bibliometric analysis of co-authorship patterns, institutional linkages, and country-level partnerships, the study sought to uncover the key actors, trends, and collaborative structures shaping the advancement and global integration of ATM research.

The specific objectives were to assess publication and authorship trends in ATM research (2000–2024), identify leading authors, institutions, and countries, examine patterns of international and regional co-authorship, highlight gaps and provide recommendations to strengthen collaboration.

Methods

Study Design

This study employed a descriptive, retrospective bibliometric design to analyse research output and collaboration networks in African Traditional Medicine (ATM) between 2000 and 2024. Bibliometrics is a quantitative research approach that applies mathematical and statistical techniques to measure and analyse scientific publications, their relationships, and their influence within specific fields of knowledge. This definition is well established across numerous authoritative sources, including highly cited works by Durieux and Gevenois (2010) and Ninkov et al. (2021), which emphasise

Table 1. The Top 20 Countries contributed to African Traditional Medicine Research

Country	Total Publications (N=3545)	Percentage (%)
1. South Africa	1325	37.38
2. Nigeria	170	4.80
3. United States of America	158	4.46
4. United Kingdom	123	3.47
5. Burkina Faso	111	3.13
6. Germany	94	2.65
7. France	89	2.51
8. Cameroon	81	2.28
9. Kenya	66	1.86
10. Uganda	64	1.81
11. India	63	1.78
12. Ghana	48	1.35
13. Belgium	45	1.27
14. Denmark	44	1.24
15. Italy	42	1.18
16. Morocco	39	1.10
17. Netherlands	39	1.10
18. Ethiopia	38	1.07
19. Canada	36	1.02
20. Tanzania	36	1.02

Data source: Scopus (Elsevier), 2025

bibliometrics as a systematic and objective means of evaluating scientific communication [24] .

The methodology serves three primary purposes: to measure research productivity, to assess quality and impact using citation-based indicators, and to map structural relationships among publications, authors, and research areas [24] . By applying these principles, bibliometric analysis enables the identification of patterns, trends, and the evolution of knowledge across disciplines [24].

Data Source

Data for this study were obtained from the Scopus database (Elsevier), which was selected for its comprehensive coverage of peer-reviewed literature and detailed bibliographic information on authors, affiliations, and citations. Scopus is widely recognised as a reliable source for bibliometric analysis due to its multidisciplinary scope and consistent citation tracking [22,23].

Search Strategy

A systematic search was conducted in Scopus to retrieve publications related to African Traditional Medicine. The search incorporated a combination of keywords and their alternative terms, such as "African traditional medicine", "ethnomedicine", "herbal medicine", (details in the keyword string of Figure 1). The search was conducted on 20 October 2025 and covered the period from 1 January 2000 to 31 December 2024, allowing for a 25-year overview of developments in the field. Only documents published in English were included to maintain consistency in analysis. The Flow diagram of the search strategy is shown below.

Inclusion and Exclusion Criteria

The inclusion criteria comprised peer-reviewed journal articles, reviews, and conference papers explicitly focused on ATM or its applications in healthcare. Studies were included if they fell within the defined time frame and geographic scope. Publications were excluded if they focused on non-African traditional medicine systems such as Chinese or Indian medicine, were unrelated to health or use of medicinal plants, spiritual practices, rituals, and ceremonies, or were non-peer-reviewed materials such as editorials, theses, or book chapters.

Data Extraction and Cleaning

All bibliographic records were exported from Scopus in CSV format, including information on authors, titles, affiliations, countries, publication years, document types, and citation counts. The dataset was cleaned and standardised through a structured disambiguation process. Author names were harmonised using unique identifiers such as Scopus Author IDs and ORCID IDs to minimise ambiguity and ensure consistency. Institutional affiliations were standardised by consolidating name variants into uniform formats, while country names were aligned with ISO standards. No duplicate records were identified. Consistency checks and manual verification were conducted to confirm the accuracy, reliability, and overall quality of the dataset before analysis.

Bibliometric Indicators and Network Analysis

A range of bibliometric indicators was used to assess research productivity and collaboration patterns. Productivity indicators included annual publication counts and document types, while collaboration was measured through the international collaboration rate and co-authorship distribution across regions. The study also identified the most prolific authors, institutions, and countries based on publication.

Co-authorship network analysis was conducted at the author, institutional, and country levels to examine patterns and structures of collaboration.

Table 2. Most Productive Authors of African Traditional Medicine Research

Author's Name	No. of Publications (N=1934)	Percentage (%)
1. Van Staden, J.	122	6.31
2. Afolayan, A.J.	86	4.45
3. Maroyi, A.	48	2.48
4. McGaw, L.J.	45	2.33
5. Aremu, A.O.	41	2.12
6. Eloff, J.N.	41	2.12
7. Viljoen, A.M.	40	2.07
8. Finnie, J.F.	36	1.86
9. Grierson, D.S.	32	1.65
10. JÃ¤ger, A.K.	30	1.55
11. Van Wyk, B.E.	29	1.50
12. Lall, N.	27	1.40
13. Witkowski, E.T.F.	25	1.29
14. Ndhlala, A.R.	23	1.19
15. Van Vuuren, S.F.	23	1.19
16. Semenya, S.S.	22	1.14
17. Williams, V.L.	22	1.14
18. Diallo, D.	20	1.03
19. Stafford, G.I.	19	0.98
20. Makunga, N.P.	18	0.93

Data source: Scopus (Elsevier), 2025

Tools and Software

Data analysis and visualisation were carried out using VOSviewer (version 1.6.20) and Bibliometrix/Biblioshiny in R.VOSviewer was employed to generate co-authorship maps while Biblioshiny supported the computation of bibliometric indicators and collaboration indices. These software tools are widely accepted and validated for bibliometric studies [25].

Ethical Considerations

This study relied entirely on publicly available bibliographic data obtained from Scopus. No human participants or confidential data were involved. Ethical approval was therefore not required. All information was used solely for academic and research purposes, in compliance with Scopus' data usage policy and ethical standards for bibliometric research.

Results

Publication Trends of African Traditional Medicine Research

Figure 2 presents the annual publication trends of African Traditional Medicine (ATM) research from 2000 to 2024. Over the past 24 years, a total of 2,392 publications were recorded, indicating a consistent increase in scholarly output.The findings reveal a modest beginning in the early

Figure 3: Leading International and Regional Collaboration patterns of African Traditional Medicine research.

Table 3. Most productive institutions of African Traditional Medicine Research

Institution	Total Publications (n=3331)	Percentage (%)
1. University of KwaZulu-Natal	302	9.07
2. University of Pretoria	166	4.98
3. University of Fort Hare	163	4.89
4. University of the Witwatersrand, Johannesburg	137	4.11
5. University of Johannesburg	107	3.21
6. University of Limpopo	105	3.15
7. Tshwane University of Technology	83	2.49
8. North-West University	82	2.46
9. Université Joseph Ki-Zerbo	64	1.92
10. Stellenbosch University	59	1.77
11. University of Ouagadougou	59	1.77
12. University of the Western Cape	57	1.71
13. Agricultural Research Council, Pretoria	55	1.65
14. University of the Witwatersrand Faculty of Health Sciences	49	1.47
15. University of South Africa	48	1.44
16. University of Zululand	45	1.35
17. University of Venda	44	1.32
18. Rhodes University	42	1.26
19. South African Medical Research Council	42	1.26
20. Makerere University	41	1.23

Data source: Scopus (Elsevier), 2025

2000s, followed by steady growth in subsequent years, as seen in Figure 1.

Between 2000 and 2004, annual publications ranged from n = 22 (0.92%) to n = 46 (1.92%), representing less than 2% of the total output per year. A gradual increase was observed between 2005 and 2008, with annual publications rising from n = 56 (2.34%) to n = 110 (4.60%).

From 2011 to 2016, annual outputs consistently exceeded 100 publications, reaching a peak of n = 128 (5.35%) in 2016. The highest publication counts were recorded in recent years, particularly in 2020 (n = 147; 6.15%), 2022 (n = 159; 6.65%), and 2024 (n = 149; 6.23%).

Overall, cumulative publications increased from n = 28 in 2000 to n = 2,392 in 2024, representing approximately an 85-fold growth in scholarly productivity.

Geographical Distribution of African Traditional Medicine Research

Table 1 presents the geographical distribution of ATM. A total of 3,545 publications were identified, reflecting both regional and international engagement in this field. The distribution indicates that African countries dominate ATM research output, though substantial contributions also come from non-African countries, underscoring growing global interest in African medicinal knowledge systems.

South Africa is the leading contributor with n = 1,325 (37.38%), followed by Nigeria (n = 170; 4.80%). Among non-African countries, the United States (n = 158; 4.46%) and the United Kingdom (n = 123; 3.47%) stand out, illustrating significant international collaboration. Other major contributors include Burkina Faso (n = 111; 3.13%), Germany (n = 94; 2.65%), and France (n = 89; 2.51%).

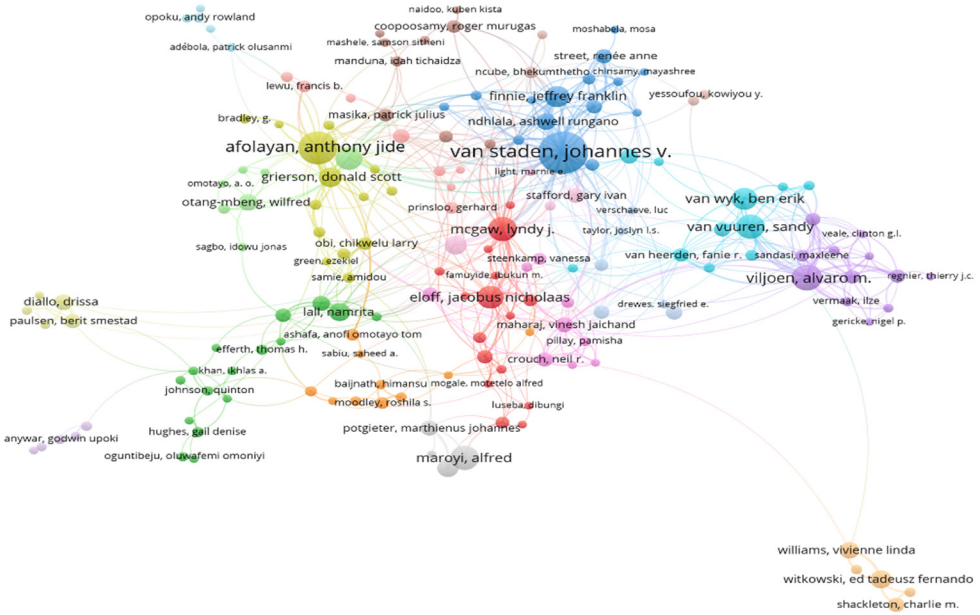

Figure 4: International and Regional Collaborative Network by Authors of African Traditional Medicine Research. Counting method: Full counting; Minimum number of documents of an author = 5; Minimum number of citations of an author = 0; Data source: Scopus (Elsevier), 2025

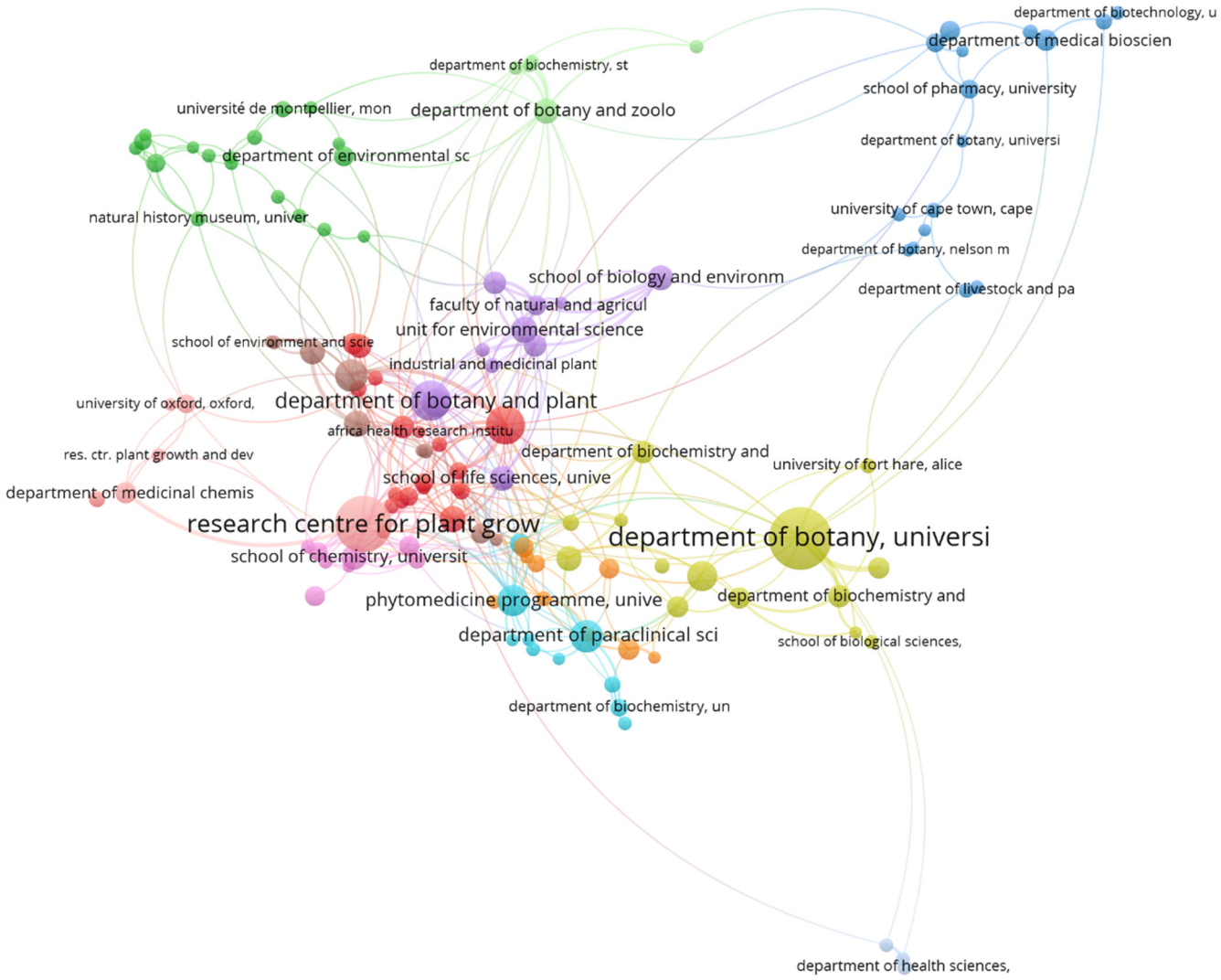

Figure 5: International and Regional Collaborative Network by Institutions of African Traditional Medicine Research Counting method: Full counting; Minimum number of documents of an author = 5; Minimum number of citations of an author = 0; Data source: Scopus (Elsevier), 2025.

Additional African contributors include Cameroon (n = 81; 2.28%), Kenya (n = 66; 1.86%), Uganda (n = 64; 1.81%), Ghana (n = 48; 1.35%), Ethiopia (n = 38; 1.07%), and Tanzania (n = 36; 1.02%). Other non-African countries such as India (n = 63; 1.78%), Belgium (n = 45; 1.27%), Denmark (n = 44; 1.24%), Italy (n = 42; 1.18%), Morocco (n = 39; 1.10%), the Netherlands (n = 39; 1.10%), and Canada (n = 36; 1.02%) further highlight the globalised and multidisciplinary nature of ATM research.

Overall, the findings confirm that while South Africa leads the field by a substantial margin, ATM research is geographically diverse and increasingly international in scope.

Authorship of African Traditional Medicine Research

Table 2 identifies the most productive authors in ATM over the period. A total of 1,934 publications were attributed to the 20 leading scholars, indicating concentrated productivity among a small number of highly active contributors.

Van Staden, J. is the most prolific author with n = 122 (6.31%), followed by Afolayan, A. J. (n = 86; 4.45%), and Maroyi, A. (n = 48; 2.48%). Other major contributors include McGaw, L. J. (n = 45; 2.33%), Aremu, A. O. (n = 41; 2.12%), Eloff, J. N. (n = 41; 2.12%), and Viljoen, A. M. (n = 40; 2.07%).

Several additional authors, such as Finnie, J. F. (n = 36; 1.86%), Grierson, D. S. (n = 32; 1.65%), and Jäger, A. K. (n = 30; 1.55%), have made significant contributions through interdisciplinary research collaborations. The remaining leading contributors, including Van Wyk, B. E., Lall, N., Ndhlala, A. R., Van Vuuren, S. F., Semenya, S. S., Williams, V. L., Diallo, D., Stafford, G. I., and Makunga, N. P., each produced between n = 18 (0.93%) and n = 29 (1.50%).

Overall, the authorship trends indicate that ATM research

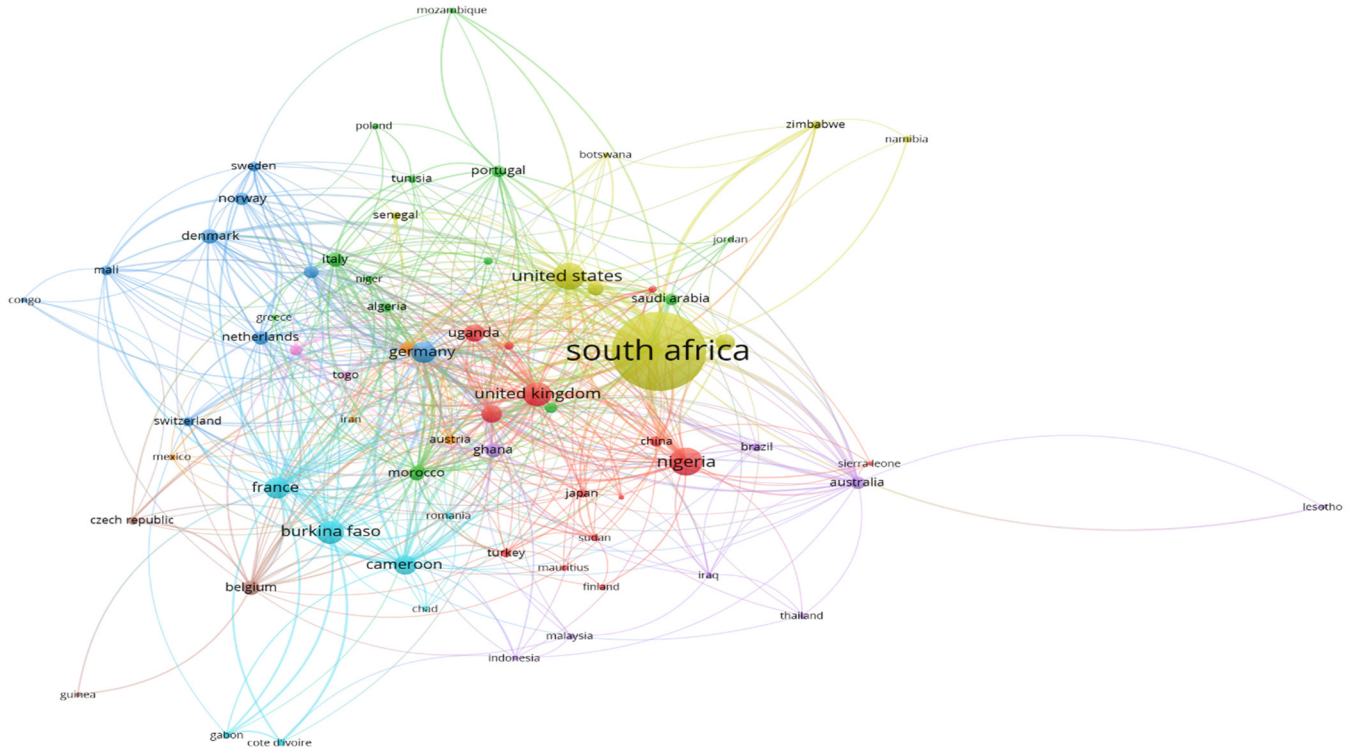

Figure 6: International and Regional Collaborative Network by Countries of African Traditional Medicine Research; Counting method: Full counting; Minimum number of documents of an author = 5; Minimum number of citations of an author = 0; Data source: Scopus (Elsevier), 2025.

is driven by a core group of prolific scholars whose sustained productivity and collaboration have shaped the field's intellectual development and global visibility.

Most Productive Institutions of African Traditional Medicine Research

Table 3 summarises the most productive institutions. A total of 3,331 publications were produced by the 20 leading universities and research organisations, demonstrating the institutional concentration of research output, particularly in South Africa.

The University of KwaZulu-Natal recorded the highest output (n = 302; 9.07%), followed by the University of Pretoria (n = 166; 4.98%) and the University of Fort Hare (n = 163; 4.89%). Other major contributors include the University of the Witwatersrand, Johannesburg (n = 137; 4.11%), University of Johannesburg (n = 107; 3.21%), and University of Limpopo (n = 105; 3.15%).

Additional contributors were Tshwane University of Technology (n = 83; 2.49%), North-West University (n = 82; 2.46%), and Stellenbosch University (n = 59; 1.77%). Outside South Africa, Université Joseph Ki-Zerbo (n = 64; 1.92%) and the University of Ouagadougou (n = 59; 1.77%) in Burkina Faso, as well as Makerere University (n = 41; 1.23%) in Uganda, represent key regional research hubs in West and East Africa.

Overall, the findings show that ATM research is institutionally concentrated, with South African universities leading continental output while other African institutions demonstrate increasing regional engagement and diversification.

International and regional collaboration patterns of African Traditional Medicine Research

Figure 3 illustrates the leading international and regional collaboration patterns. Based on 1,553 co-authorship links, the data reveal South Africa as the central hub of collaborative activity both within Africa and globally.

The most frequent collaborations occurred between South Africa and Nigeria (n = 44; 2.83%), South Africa and the USA (n = 42; 2.70%), and South Africa and the United Kingdom (n = 37; 2.38%). Other notable partnerships include Burkina Faso and France (n = 29; 1.87%), South Africa and Australia (n = 21; 1.35%), and South Africa and Denmark (n = 16; 1.03%).

Emerging intra-African collaborations include South Africa–Cameroon (n = 13; 0.84%) and South Africa–Zimbabwe (n = 11; 0.71%), while transcontinental partnerships such as France–Cameroon (n = 10; 0.64%), United Kingdom–Denmark (n = 9; 0.58%), and USA–Australia (n = 8; 0.52%) underscore the increasingly global nature of ATM research.

ATM research collaboration is dominated by South Africa's networks with Europe and North America, while intra-

African partnerships, though growing, remain relatively limited and present opportunities for strengthening continental research integration.

International and Regional Collaborative Network by Authors of African Traditional Medicine Research

Figure 4 summarises the international and regional collaborative networks of leading authors based on 1,170 co-authorship links.

Johannes Van Staden recorded the highest number of links (n = 36; 3.08%), followed by Lyndy J. McGaw (n = 34; 2.91%) and Anthony Jide Afolayan (n = 23; 1.97%). Other leading collaborators include Adeyemi Oladapo Aremu and Alvaro M. Viljoen (each n = 22; 1.88%), Jacobus Nicholaas Eloff (n = 21; 1.79%), and Jeffrey Franklin Finnie (n = 20; 1.71%).

Additional active collaborators include Esam E. Elgorashi, Vinesh Jaichand Maharaj, and Sandy Van Vuuren (each n = 19; 1.62%).

International and regional Collaborative network by institutions of African Traditional Medicine Research

Figure 5 presents institutional collaboration in African Traditional Medicine (ATM) research from 2000 to 2024, based on 716 co-authorship links. Collaboration is concentrated among South African universities and research institutes, reflecting their leadership in advancing ethnomedicine and related fields.

The Department of Botany and Plant Biotechnology, University of Johannesburg, recorded the highest number of collaborative links (n = 19; 2.65%), followed by the Department of Pharmaceutical Sciences, Tshwane University of Technology (n = 18; 2.51%). Three institutions shared equal collaboration strength with n = 16 (2.23%) each: the Agricultural Research Council, Pretoria, the Department of Botany, University of Fort Hare, and the Phytomedicine Programme, University of Pretoria.

Other key collaborators include the Department of Paraclinical Sciences, University of Pretoria (n = 15; 2.09%), and the Department of Botany and Zoology, Stellenbosch University (n = 14; 1.96%).

International and regional Collaborative network by Countries of African Traditional Medicine Research

Figure 6 presents the international and regional collaborative networks by countries based on 2,362 co-authorship links. The data reveal an extensive web of cross-border partnerships, with South Africa emerging as the central hub of both intra-African and global collaboration.

South Africa recorded the highest number of collaborative links (n = 348; 14.73%), underscoring its pivotal role in driving ATM research across the continent. It was followed by Nigeria (n = 143; 6.06%), the United States (n = 124; 5.25%), and the United Kingdom (n = 117; 4.95%). Other countries with significant collaborative activity include Burkina Faso (n = 109; 4.61%), Cameroon (n = 91; 3.85%), Ghana (n = 78; 3.30%), and Kenya (n = 74; 3.13%).

Among non-African nations, France (n = 82; 3.47%), Germany (n = 68; 2.88%), India (n = 61; 2.58%), and Australia (n = 57; 2.41%) represent major contributors. Additional collaborative participation is evident from Denmark (n = 49; 2.07%), Belgium (n = 44; 1.86%), Canada (n = 41; 1.74%), and Italy (n = 39; 1.65%).

Several African countries, including Uganda (n = 36; 1.52%), Ethiopia (n = 34; 1.44%), Tanzania (n = 32; 1.35%), Zimbabwe (n = 28; 1.19%), and Mozambique (n = 25; 1.06%), also maintain moderate collaboration intensity, mainly through regional research partnerships and South–South networks.

Discussion

This study provides a comprehensive overview of publication output, authorship patterns, institutional contributions, and international and regional collaboration networks in African Traditional Medicine (ATM) research from 2000 to 2024. The findings demonstrate sustained growth in research productivity, alongside increasing participation from both African and non-African institutions, underscoring the growing recognition of ATM as both a scientific domain and a socio-cultural resource.

Growth of Research Output

The steady rise in ATM publications over the past 25 years reflects expanding scholarly and policy interest in traditional medicine across Africa and globally. This trend aligns with international efforts to recognise traditional and complementary medicine as integral to healthcare systems, particularly within the context of universal health coverage [12,14]. Comparable growth trajectories have been reported in bibliometric studies of herbal and ethnomedicine research in other developing regions [18,19], reinforcing the global expansion of traditional medicine scholarship.

Geographical Concentration and Global Engagement

The dominance of South Africa and Nigeria among African contributors highlights the role of established research infrastructure, sustained funding mechanisms, and strong institutional networks in shaping scientific productivity. South Africa's leading position, accounting for over one-third of total output, is consistent with broader patterns in health-related research across sub-Saharan Africa, where the country frequently serves as a regional research hub. This leadership is supported by national policies promoting indigenous knowledge systems and well-developed university–industry collaborations.

Beyond the continent, substantial contributions from the United States, the United Kingdom, France, and Germany illustrate strong global engagement with ATM research, particularly in ethnopharmacology and medicinal plant studies. These patterns reflect the increasing globalisation of traditional medicine research and the growing interdependence of knowledge systems across the Global North and Global South.

Authorship Patterns and Scholarly Leadership

Authorship analysis reveals the presence of a core group of highly productive scholars, including J. Van Staden, A. J. Afolayan, and A. Maroyi, whose sustained contributions have significantly shaped the intellectual landscape of ATM research. Their prominence reflects the maturation of research traditions in fields such as ethnobotany, phytochemistry, and pharmacology. More broadly, the concentration of scholarly output among a relatively small number of authors suggests a "core–periphery" structure within the research community [26,27].

While such patterns are common in scientific networks, they also point to structural inequalities in participation. Expanding opportunities for emerging researchers through mentorship, targeted funding, and capacity-building initiatives will be essential to diversify authorship and strengthen the long-term sustainability of ATM research across Africa.

Institutional Contributions and Network Centrality

Institutional analysis further reinforces South Africa's central role, with universities such as KwaZulu-Natal, Pretoria, and Fort Hare emerging as leading contributors with extensive collaborative linkages. These institutions host well-established research programmes in pharmacognosy, ethnobotany, and plant biotechnology, positioning them as regional centres of excellence [28–31]. The presence of institutions from Burkina Faso and Uganda among notable contributors also signals the gradual expansion of research capacity in other parts of the continent.

However, institutional collaboration networks remain unevenly distributed, with a strong concentration around South African institutions and comparatively limited interconnections among other African universities. Such centralisation may constrain the broader diffusion of knowledge and capacity development. Strengthening cross-institutional partnerships through continental initiatives, including programmes under the African Union, could facilitate more balanced research leadership and regional integration.

Patterns of International and Regional Collaboration

Co-authorship and network analyses reveal a centralised collaboration structure characterised by strong North–South linkages, particularly between South Africa and partners in Europe and North America. Frequent collaborations with countries such as the United States and the United Kingdom highlight the role of international partnerships in supporting resource access, technological exchange, and research visibility.

Despite these benefits, intra-African collaboration remains comparatively limited. This imbalance is consistent with earlier findings that identify structural barriers such as uneven funding distribution, infrastructural disparities, and limited regional coordination [1,2]. Enhancing South–South collaboration within Africa is therefore critical for fostering knowledge exchange, strengthening regional research ecosystems, and addressing shared health challenges through contextually relevant traditional medicine research (Kasprowicz et al., 2020, 2023) [32,33].

Limitations

This study has several limitations. First, the analysis was restricted to the Scopus database, which, although comprehensive, may not fully capture all relevant African Traditional Medicine (ATM) publications, particularly those published in regional or non-indexed journals. This may result in the underrepresentation of locally produced research. Second, the search strategy, despite being carefully developed, may have excluded relevant studies due to variations in terminology, indexing practices, or language restrictions, potentially introducing selection bias.

Third, bibliometric indicators primarily assess patterns of publication and collaboration but do not evaluate the quality, methodological rigour, or clinical relevance of the included studies. Fourth, co-authorship was used as a proxy for collaboration, which may not fully reflect informal, interdisciplinary, or non-publishing partnerships.

Finally, the study provides a descriptive and retrospective analysis and does not account for contextual factors such as funding disparities, policy environments, or socio-cultural influences that may shape research collaboration in ATM. Future studies could incorporate multiple databases and qualitative approaches to provide a more comprehensive understanding.

Conclusions

The study reveals a remarkable and steady increase in ATM research output, driven largely by South Africa and supported by emerging contributions from other African and non-African countries. South Africa remains the core hub of scholarly productivity, institutional strength, and international collaboration, while intra-African research partnerships remain relatively underdeveloped.

The dominance of a small group of prolific authors and a concentration of research activity in a few institutions suggest both leadership and dependency structures within the field. Although global collaborations with Europe and North America have enhanced research visibility and quality, regional integration within Africa still lags. Addressing these disparities is essential for building a more balanced and sustainable research ecosystem that supports knowledge exchange, innovation, and the integration of traditional medicine into formal healthcare systems.

Recommendations

To enhance the development and visibility of African Traditional Medicine (ATM) research, stronger intra-African collaboration is essential. Governments, universities and funding agencies should prioritise South-South cooperation through multi-country projects, research exchanges and regional research consortia. Such initiatives will foster knowledge sharing, resource pooling and the creation of a sustainable research culture across the continent, helping to address the current imbalance where research activity is concentrated in a few countries, particularly South Africa.

Institutional capacity building remains critical. Many African universities and research centres face constraints in infrastructure, funding and human resources. Targeted investments in laboratory facilities, training and mentorship programmes are needed to empower emerging scholars and establish local centres of excellence capable of producing high-impact research.

Policy and funding support should be strengthened by integrating traditional medicine into national science, technology and innovation agendas. Dedicated funding streams and long-term grants for interdisciplinary projects will sustain research continuity and facilitate the validation and standardisation of traditional medicines for wider healthcare use.

Promoting knowledge sharing and data integration through open-access repositories, regional bibliographic databases and collaborative digital platforms will improve research accessibility, reduce duplication and enhance global visibility. These platforms can also help track research impact and inform evidence-based policies.

Lastly, fostering interdisciplinary collaboration and community engagement is vital. Partnerships among traditional healers, biomedical scientists, pharmacists and policymakers can ensure quality assurance, safety and the effective integration of traditional medicines into formal healthcare systems. Future bibliometric and scientometric studies should examine citation impact, thematic evolution, funding trends and equity dimensions, particularly gender representation, to better understand the inclusivity and sustainability of ATM research networks.

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REVIEW ARTICLE

Antiviral Activity of Indigenous Medicinal Plants in Kenya and Their Potential Role in Managing Viral Infections: A Systematic Review

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Abstract

Background: The use of indigenous medicinal plants to manage viral diseases such as human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), Herpes, Hepatitis, and measles is a global practice, especially in sub-Saharan Africa and southern Asia. During the COVID-19 pandemic, reliance on traditional remedies increased significantly. However, concerns remain regarding the scientific validation of efficacy, dosage and safety of these remedies.

Objective: To systematically summarise the available scientific evidence on the antiviral properties of Kenyan medicinal plants and highlight those suitable for further pharmacological research and development.

Methods: A systematic search was conducted in Google Scholar and PubMed using Boolean combinations of keywords: "antiviral," "activity," "herbal," "plant," and "Kenya." Eligible sources included original research articles, conference papers, and abstracts that assessed antiviral activity through in vitro, in-vivo, or clinical methods.

Results: Eighteen studies met the inclusion criteria. Of the 54 plant species evaluated, 28 exhibited antiviral activity against six viruses: human immunodeficiency virus (HIV) (14 plants), herpes simlex virus (HSV) (9), measles virus (MV) (5), human cytomegalovirus (HCMV) (4), hepatitis B virus (HBV) (3), and dengue virus (DV) (1).

Conclusion: Several Kenyan medicinal plants show promising antiviral properties. Further research is needed to investigate their mechanisms of action, toxicity/safety, and dosaging to support their integration into evidence-based healthcare.

Keywords: Medicinal Plant, Antiviral Activity, Cytotoxicity, Mechanism of Action , Herpes Simplex Virus, Hepatitis B Virus, Human Cytomegalovirus, Dengue Virus, Measles Virus, Human Immuno-defficiency Virus.

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Introduction

Herbal medicine has played a central role in healthcare in Kenya since the precolonial era, when traditional practices were the primary method for treating human and animal ailments [1]. Today, more than 1,200 plant species are used by various Kenyan communities for their perceived therapeutic effects [1,2]. The continued reliance on these remedies is driven by accessibility, affordability, cultural acceptance, and the belief in their safety, efficacy, and low

tendency to promote drug resistance [3,4].

The World Health Organisation (WHO) recognised the importance of traditional medicine in global health during the 1978 Alma-Ata Declaration and has since encouraged its integration into national healthcare systems to support universal health coverage [5]. However, WHO emphasises the importance of validating these products through rigorous scientific assessment of their safety, efficacy, and quality before integration into routine care.

Despite their widespread use, many traditional medicines in Kenya are prepared under unhygienic conditions, often without standardised dosages or quality controls. As a result, the potential for contamination, toxicity, adverse reactions, or treatment failure remains a significant concern [1,6-8]. With increased reliance on herbal medicine during viral outbreaks (e.g COVID-19), there is a need to evaluate the antiviral properties, safety margins, and dosage parameters of Kenyan medicinal plants consumed to treat viruses through robust laboratory research.

Viral agents such as human immunodeficiency virus (HIV), herpes simlex virus (HSV), measles virus (MV), human cytomegalovirus (HCMV), hepatitis B virus (HBV), and Dengue virus (DV) are associated with diseases that cause significant morbidity and mortality globally which is disproportionately higher within the WHO African region. According to WHO, an estimated 40.8 million people globally are living with HIV of which 65% are in Africa [1,9,10].

The infection severity of HIV is only managed through administering antiretroviral therapy (ART). However, the virus develops resistance to even the most potent ART[11]. ART-resistance rapidly renders conventional treatment for HIV ineffective thereby increasing disease burden. This creates the need for new homegrown treatment options that will be effective, accessible, and affordable. Isolation of natural compounds from medicinal plants and testing them for antiviral properties against the virus could lead to a breakthrough in new HIV drug discoveries.

The prevalence of HSV-1 is higher in Africa compared to other regions. In Africa, over 90% of the population acquire HSV-1 infections orally by the age of 15 years [12]. A steep increase in prevalence of HSV-2 was observed by age, with figures ranging from 10% in 13- to 14-year-olds, 28% in 15- to 19-year-olds, to 70% among the 20- to 24-year-olds in Kenya. The HSV infections are managed by antiviral drugs of which acyclovir nucleoside analog is the drug of choice. The resistance of HSV to acyclovir is an emerging concern in clinical management of HSV-associated diseases [13,14]. Human-cytomegalovirus (HCMV) belongs to the same family of herpesviruses with HSV. HCMV has a significant global prevalence at 60-90% sero-prevalence [15] and is one of the leading causes of congenital infections posing high risk of health complications, morbidity, and mortality to immunocompromised populations such as people with HIV/AIDS, organ transplant recipients, and those with developing fetuses. A Kenyan study involving pregnant women reported a CMV seropositivity of 77.3% and 28% IgG and IgM, respectively10 Just like HSV, HCMV infections have no cure but antiviral drugs such Ganciclovir, Valganciclovir, Foscarnet, and Cidofovir are administered to manage symptoms. The virus has however shown resistance to these drugs thereby hampering its clinical management efforts [15]. To overcome the clinical impacts of herpesviruses' drug resistance, medicinal plants form a promising source for bioactive compounds that could be useful in new drug development.

Hepatitis B virus (HBV) is an endemic disease in Africa causing complications like liver cirrhosis and hepatocellular carcinoma. In 2019 alone, 80,000 new HBV infections and over 40,000 HBV-associated mortalities were experienced in the WHO African region [16]. The prevalence of HBV in Kenya is generally high (6.025%) [17]. There is no cure for HBV. The available antiviral agents for HBV including Tenofovir and Entecavir only manage disease severity and prevent liver damage by suppressing viral replication. However, HBV has been reported to exhibit resistance to these medications. Drug resistance in addition to lack of an effective cure creates the need for new drug development.

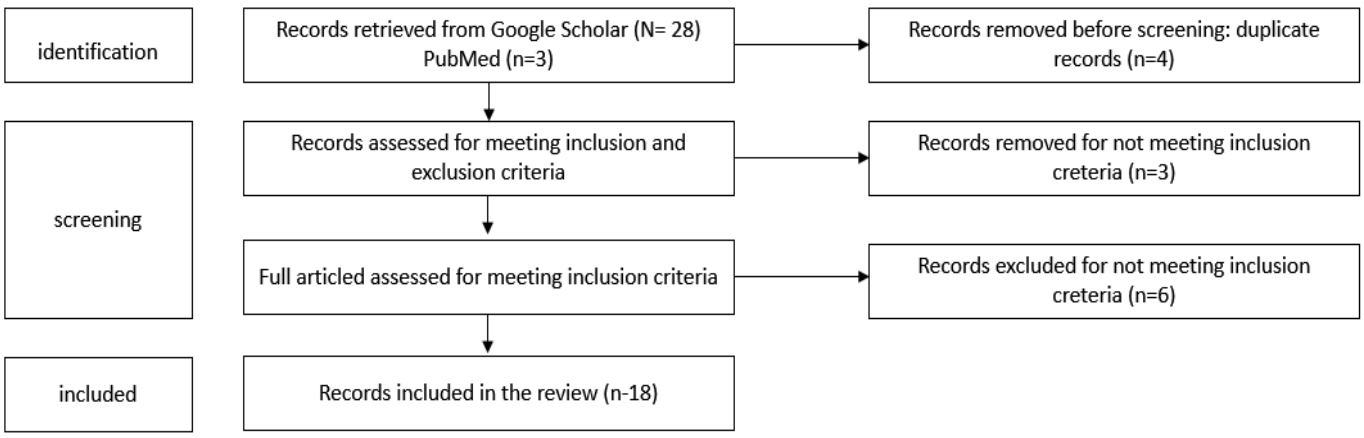

Figure 1: Flow diagram of included studies

Table 1: Risk of bias for in-vitro studies

Study	Sequence Generation	Baseline Characteristics	Allocation Concealment	Random Housing	Researcher Blinding	Random Outcome Assessment	Outcome Assessor Blinding	Incomplete Outcome Data	Selective Reporting	Other Bias
[32]	Negative	Positive	Positive	Positive	Unclear	Positive	Negative	Positive	Positive	Positive
[31]	Negative	Positive	Positive	Positive	Unclear	Positive	Negative	Positive	Positive	Positive
[27]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Positive	Positive
[35]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Unclear	Unclear
[23]	Negative	Positive	Positive	Positive	Unclear	Positive	Negative	Unclear	Unclear	Unclear
[24]	Negative	Positive	Positive	Positive	Unclear	Positive	Negative	Negative	Unclear	Unclear
[25]	Negative	Positive	Positive	Positive	Unclear	Positive	Negative	Positive	Positive	Unclear
[29]	Negative	Positive	Positive	Positive	Unclear	Positive	Negative	Positive	Positive	Unclear
[26]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Unclear	Negative	Unclear
[39]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Unclear	Negative	Negative
[22]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Unclear	Negative	Negative
[30]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Unclear	Negative	Negative
[33]	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Unclear	Negative
[38]	Negative	Positive	Positive	Positive	Negative	Unclear	Negative	Positive	Positive	Unclear
[35]	Negative	Positive	Negative	Unclear	Negative	Negative	Negative	Unclear	Unclear	Unclear
[26]	Negative	Positive	Negative	Unclear	Negative	Negative	Negative	Unclear	Negative	Negative
[22]	Negative	Positive	Negative	Positive	Negative	Unclear	Negative	Unclear	Negative	Negative
[37]	Negative	Positive	Positive	Positive	Negative	Unclear	Negative	Unclear	Negative	Positive

Table 2: Risk of bias for in-vivo studies

Study	Sequence Generation	Baseline Characteristics	Allocation Concealment	Random Housing	Researcher Blinding	Random Outcome Assessment	Outcome Assessor Blinding	Incomplete Outcome Data	Selective Reporting	Other Bias
[35]	Negative	Positive	Negative	Unclear	Negative	Negative	Negative	Unclear	Unclear	Unclear
[26]	Negative	Positive	Negative	Unclear	Negative	Negative	Negative	Unclear	Negative	Negative
[22]	Negative	Positive	Negative	Positive	Negative	Unclear	Negative	Unclear	Negative	Negative
[37]	Negative	Positive	Positive	Positive	Negative	Unclear	Negative	Unclear	Negative	Positive

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Table 3: Cytotoxicity Profiles of Antiviral Active Medicinal Plants

Plant Species	Plant Parts	Extract Type and Safety Data		Cell Culture/Animal Model	Comments	Reference
		Solvent	Cytotoxicity/Toxicity			
Chrysanthemum cinerariaefolium (Pyrethrum)	Flower	Methanol	CC50= 42.23 ± 0.32(µg/ml)	Vero E6 Cells	Methanol extract more cytotoxic	31
		Water	CC50= 249 ± 8.4(µg/ml)			
	Flower	Both Water and Methanol extracts	LD50=2000mg/Kg	Mice	Non-cytotoxic in in-vivo	36
Dichrocephala integrifolia (Kuntze)	Leaves	Water	CC50 >100 (µg/ml)	Vero E6 Cells; Female Swiss albino mice	Methanol extract of flower moderately cytotoxic. All extracts non-toxic to mice at 300-2000mg/kg dose	35
		Methanol	CC50 >100 (µg/ml)			
	Flower	Water	CC50 >100 (µg/ml)			
		Methanol	CC50 = 71.31± 2.65 (µg/ml)			
	Stem Bark	Water	CC50 >100 (µg/ml)			
		Methanol	CC50 >100 (µg/ml)			
	Roots	Water	CC50 >100 (µg/ml)			
		Methanol	CC50 >100 (µg/ml)			
Acacia mellifera (Vahl Benth)	Roots	Methanol	CC50 >100 (µg/ml)	SNU-182 Cell line	Non-cytotoxic	24
Moringa oleifera	Leaves	Water	CC50= 1965.23 ± 10.26µg/mL	Vero cells	Non-cytotoxic	28
	Leaves	Methanol	CC50= 1622.10±11.98µg /mL			
Garcinia buchanii	Stembark		CC50>500(µg/ml)	Vero Cells	Non-cytotoxic	22
Caesalpinia decepetala	Whole root	Water	CC50>500(µg/ml)	Vero Cells	Non-cytotoxic	22
Prunus Africana	Roots	Water	CC50 <100 (µg/ml)	SNU-182 Cell line	Non-cytotoxic	24
	Stembark	Water	CC50 >100 (µg/ml)	Human embryonic lung fibroblast cells (HEL)	Non-cytotoxic	33
Plumeria alba	Latex		50% of latex cause 100% reduction of cells	Vero E6 Cells	More than 2.5% concentration is cytotoxic. 1.5mg/ml concentration cause cell lysis	26
Melia azedarach	Leaves	DCM/MEOH	Concentration above 0.5mg/ml cause cell lysis	Vero E6 cells	Moderately Cytotoxic at concentration>0.5mg/ml	26
	Stembark	Water	CC50 >80 (µg/ml)	Human embryonic lung fibroblast cells (HEL)	Non-cytotoxic	33
Croton dichogamus	Aerial parts	DCM:MEOH	CC50= 4.70 ± 0.26 µg/mL	Human T-lymphocytic MT-4 cells (ARP-120)	Cytotoxic	34
Croton megalocarpus	Leaves	DCM:MEOH	CC50=27.7 ± 0.65 µg/mL	Human T-lymphocytic MT-4 cells (ARP-120)	Cytotoxic	34
	Stembark	DCM:MEOH	CC50= 3.27 ± 0.12 µg/mL	Human T-lymphocytic MT-4 cells (ARP-120)	Cytotoxic	
Croton macrostachyus	Stembark	Water	CC50>500(µg/ml)	Vero Cells	Non-cytotoxic	22
	Leaves	DCM:MEOH	CC50= 0.008 ± 0.00 µg/mL	Human T-lymphocytic MT-4 cells (ARP-120)	Leaf extract Most cytotoxic among the 3 Croton genus reported to have anti-HIV activity	34
	Stembark	DCM:MEOH	CC50= 45.9 ± 0.12 µg/mL	Human T-lymphocytic MT-4 cells (ARP-120)		
Rhus natalensis	Stembark	Methanol	No toxicity at concentration≤1µg/ml	Vero cells; U937 cells	Non-cytotoxic	39

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Table 3: Cytotoxicity Profiles of Antiviral Active Medicinal Plants (cont'd)

Plant Species	Plant Parts	Extract Type and Safety Data		Cell Culture/Animal Model	Comments	Reference
		Solvent	Cytotoxicity/Toxicity			
Carissa edulis	Rootbark	Water	CC50>100 µg/ml	Human embryonic lung fibroblast cells (HEL)	Non-cytotoxic	33
Olinia rochetiana	Stembark	Methanol	No toxicity at concentration≤1 µg/ml	Vero cells; U937 cells	Non-cytotoxic	39
Scutia myrtina	Stembark	Methanol	No toxicity at concentration≤1 µg/ml	Vero cells; U937 cells	Non-cytotoxic	39
Warbugia ugandensis	Leaves	DCM/MEOH	Concentration above 0.5mg/ml cause cell lysis	Vero E6 cells	Cytotoxic at concentration>0.5mg/ml	26
	Stembark	Methanol	No toxicity at concentration≤1 µg/ml	Vero cells; U937 cells	Toxic when the concentration increases.	39
Albizia amara	Stembark	Methanol	No toxicity at concentration≤1 µg/ml	Vero cells; U937 cells	Toxic when the concentration increases.	39
Rhamnus prinoides	Stembark	Methanol	No toxicity at concentration≤1 µg/ml	Vero cells; U937 cells	Non-cytotoxic	39

The measles virus infects over 30 million children causing 1 million deaths a year in developing countries. Kenya reported 597 cases in 2020, an increase of 158 cases from those reported in 2019. The infection is a leading cause of blindness at 15,000-60,000 cases per year globally with Africa experiencing a disproportionately higher disease burden [18]. Simillarly, the dengue viral infection is currently putting half of the global population at risk of dengue-associated disease. It is estimated that about 100-400 million dengue infections occur globally every year. Kenya has had sporadic outbreaks of dengue the latest reported in Wajir, Malindi, Kilifi, and Mombasa, on the Kenyan Coast in 2017 [19]. The disease is endemic in over 125 countries where it continues to cause a significant burden of dengue fever and dengue hemorrhagic fever[20]. There is no pharmacological cure for both dengue and measles viruses. As the search for effective treatments for these viruses continue, medicinal plants can be considered as a good source for phyto-compounds that could be investigated as potential anti-measles and anti-dengue drugs.

This systematic review is aimed at synthesising evidence of antiviral efficacy, safety, and dosaging of Kenyan medicinal plants to support their safe, effective and evidence-based use in healthcare.

Methodology

We conducted this review in accordance with PRISMA 2020 guidelines [21]. We conducted a systematic search of PubMed and Google Scholar using predefined Boolean search strategies. The search terms were structured into three main concept groups: antiviral activity, medicinal plants, and geographic focus (Kenya), and were combined using appropriate Boolean operators as follows:

("antiviral" OR "antiviral activity" OR "antiviral efficacy" OR "antiviral effect")

AND ("medicinal plant" OR "herbal medicine" OR "traditional medicine" OR "phytotherapy" OR "herbs")

AND ("Kenya" OR "Kenyan")

Full database-specific search strategies are provided in Supplementary File 1. No date or language restrictions were applied. The final search was conducted on 23rd July 2024.

Eligibility Criteria

We included primary studies (in vitro, in vivo, or clinical) evaluating antiviral activity of indigenous Kenyan plant species as extracts or isolated compounds. Studies conducted outside Kenya were eligible if they investigated Kenyan indigenous species. We excluded reviews, ethnobotanical surveys without pharmacological data, modelling/in-silico-only studies (unless paired with laboratory data), and studies lacking antiviral outcomes. Exclusion of ethnobotanical surveys and reviews was decided following a unanimous decision by authors that their inclusion could introduce biases and redundancy in

data reporting.

Study Selection

Two reviewers independently screened titles/abstracts and then full texts against the criteria. Discrepancies were resolved by discussion or third-reviewer adjudication. Non-English articles were machine-translated (Google Translate) and verified by a second reviewer for data items; uncertainties were flagged during extraction.

Study Selection Results

The search identified 31 records. After deduplication, 30 records were screened by title/abstract; 25 full-text articles were assessed for eligibility; 18 studies met inclusion criteria and were included in the qualitative synthesis[22-39]. Reasons for full-text exclusion are detailed in Supplement 2.A PRISMA 2020 flow diagram is provided in Figure 1.

Risk of Bias (RoB) Assessment

Two reviewers (John Kirema and Raphael Lwembe) independently assessed risk of bias. We used SYRCLE's RoB tool for in vivo studies and a modified SYRCLE-based checklist for in vitro studies, adapted from previous studies[40]. Both tools assess 10 domains in the following order: sequence generation, baseline characteristics, allocation concealment, random housing, researcher blinding, random outcome assessment, outcome assessor blinding, incomplete outcome data, selective reporting, and other sources of bias. These domains were used to evaluate potential risks of selection, performance, attrition, detection, reporting, and other biases across both in vivo and in vitro studies. The RoB assessments were tabulated, and each domain was classified as low risk ("positive"), high risk ("negative"), or unclear risk where insufficient methodological information was available to permit judgment. Any disagreements between the two reviewers were resolved through discussion and consensus or, where necessary, through consultation with two additional investigators (B.I. and S.N.). All authors reviewed the final RoB judgments to ensure consistency and accuracy. The results of the RoB assessment were considered during interpretation of the overall strength and reliability of the evidence.

Data Extraction

Data were extracted using a Cochrane-compliant form developed collaboratively by all investigators. The form was created in Microsoft Word and adapted to include relevant components of the Cochrane template specific to this review. Extracted items included study identifiers (title, authors, and references), study design and eligibility, methodological characteristics, risk of bias (RoB), intervention details, outcome assessment methods, key findings, and authors' main conclusions.

In addition, plant-specific data were extracted, including species, plant part used, extract type (aqueous or organic solvent), study model (*in vitro*, *in vivo*, or *in silico*), virus type, measures of antiviral activity, and reported toxicity outcomes.

Two reviewers (R.L. and J.K.) independently performed data extraction to ensure accuracy and consistency. Discrepancies were resolved through discussion and consensus or, when necessary, by consultation with two additional investigators (B.I. and S.N.).

The findings were synthesized narratively and organized according to plant species and viral targets. A narrative synthesis approach was adopted due to substantial heterogeneity across the included studies in terms of study design, plant species, extraction methods, viral targets, and outcome measures, which precluded meaningful quantitative analysis.

Results

Risk of Bias Assessment

The RoB assessments for every publication included in our current review are included in Tables 1 and 2 below. For all publications related to in-vitro studies, the Modified SYRCLE RoB tool revealed a high risk of three categories of bias including selection, performance and detection biases. These results are consistent with findings by Sanchez-Fernandez study[41] which reported significant bias risks associated with selection and detection in in-vitro studies. Selection bias in these studies is contributed by the overall lack of sequence generation methods. The baseline characteristics of the control versus intervention groups (Culture cells) are similar since they are prepared in same laboratory environment, a factor that buffers selection bias impact. Performance and detection bias in our included in-vitro studies are caused by poor blinding when introducing the intervention in the experimental phase and when selecting the cells to examine first between the control and intervention groups. Blinding can be improved by ensuring the assessor is unaware of which group is the control or intervention thereby giving the two groups equal chance for selection during outcome assessment. Simillarly, the SYRCLE RoB for in-vivo studies indicated high risks of performance, detection, and selection biases influenced by poor randomization of research animals, compromised animal grouping and selection methods, and poor blinding of the whole experiment process.

Risk of Bias Assessment

The risk of bias (RoB) assessments for all included studies are presented in Tables 1 and 2. Overall, the included studies exhibited several methodological limitations, with

Table 4: Antiviral activity of Indigenous medicinal plants against HSV-1 in Kenya.

Plant Species, Local name	Parts Assessed	Solvent used for extraction	Study type	Activity	Ref
Chrysanthemum cinerariaefolium (Pyrethrum)	Flower	Methanol	In-vitro	Active with (IC50= 1.69 µg/ml)	31
Chrysanthemum cinerariaefolium (Pyrethrum)	Flower	Water	In-vitro	Active with (IC50= 23.21 µg/ml)	36
Chrysanthemum cinerariaefolium (Pyrethrum)	Flower	Methanol	Invivo	Active at (10 mg/kg, 25 mg/kg)	36
Chrysanthemum cinerariaefolium (Pyrethrum)	Flower	Water	Invivo	Active at (50 mg/kg)	36
Dichrocephala integrifolia (Kuntze)	Leaves	Methanol	In-vitro	Active with (IC50=63.95±5.36) in Pre-treatment method.	35
Dichrocephala integrifolia (Kuntze)	Flower	Methanol	In-vitro	Active with (IC50= 86.20±7.56) using Post-Treatment Strategy	35
Dichrocephala integrifolia (Kuntze)	Stem Bark	Methanol	In-vitro	Active with (IC50=54.45±3.45) using Pre-treatment method	35
Dichrocephala integrifolia (Kuntze)	Leaves	Water	In-vitro	Active with (IC50=86.20±7.56) using Pre-treatment Method	35
Dichrocephala integrifolia (Kuntze)	Leaves	Water	In-vitro	Active with (IC50=82.44±7.92) using Post-treatment strategy	35
Dichrocephala integrifolia (Kuntze)	Flower	Methanol	In-vitro	Showed Virucidal activity with (IC50=45.27±2.41)	35
Dichrocephala integrifolia (Kuntze)	Leaves	Methanol	In-vitro	Showed Virucidal activity with (IC50=30.53±4.51)	35
Dichrocephala integrifolia (Kuntze)	Roots	Water	In-vitro	Showed virucidal activity with (IC50= 0.333±1.23)	35
Acacia mellifera (Vahl) Benth)	Stembark	DCM and methanol	Invitro	Betulin Compound highly active at 50 µg/ml. Low activities at concentration between 1-10 µg/ml.	29
Moringa oleifera	Leaves	Water	In-vitro	Active with (IC50= 627.29± 0.33) using Post-infection treatment strategy	28
Moringa oleifera	Leaves	Water	In-vitro	Active with (IC50 of 695.10±0.28) using pre-infection treatment strategy	28
Moringa oleifera	Leaves	Methanol	In-vitro	Active with (IC50=1350.61) using post-infection treatment strategy	28
Moringa oleifera	Leaves	Methanol	In-vitro	Active with (IC50=2427.83 ± 0.23) using pre-infection treatment strategy.	28
Garcinia buchanii	Stembark	Water	In-vitro, in-vivo	Active (IC50) at 20µg/mL.	22
Caesalpinia decepetala	Root	Water	In-vitro	Active IC50 at 80µg/mL.	22
Carissa edulis (Forssk.) Vahl	Rootbark	Diethyether and pure methanol	In-vitro	Lupeol showed activity with (EC50 at 2.98 µg/ml-4.2 µg/ml).	38
Carissa edulis (Forssk.) Vahl)	Rootbark	Diethyether and pure methanol	In-vivo	Lupeol showed activity at 20.0 µg/ml with a delayed onset of infections at ($p \leq 0.05$ test vs. control)	38
Prunus Africana	Stembark	Water	In-vivo	Active at a dose of 250 mg/kg.	37
Acacia mellifera	Stembark	Water	In-vivo	Active at a lower dose of 250 mg/kg.	37
Plumeria alba	Plumeria alba latex	Hexane	In-vitro	Active at 1mg/ml with 0.5 log units viral yield reduction.	26
Plumeria alba	Plumeria alba latex	DCM	In-vitro	Active at 1mg/ml with 0.75 log units viral yield reduction.	26
Plumeria alba	Plumeria alba latex	Ethyl-acetate	In-vitro	Active at 1mg/ml with 1.5 log units viral yield reduction	26

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the most common concerns observed in domains related to selection bias, performance bias, and detection bias.

Among the in vitro studies, the modified SYRCLE-based assessment indicated a predominantly high or unclear risk of bias in domains related to sequence generation, researcher blinding, and outcome assessment. In particular, most studies did not report methods of sequence generation, contributing to potential selection bias. Although baseline characteristics of control and intervention cell cultures were generally comparable due to standardized laboratory conditions, lack of randomization procedures remained a concern. Additionally, blinding during intervention administration and outcome assessment was often absent or insufficiently reported, increasing the likelihood of performance and detection bias.

Similarly, the SYRCLE assessment of in vivo studies revealed frequent concerns related to sequence generation, allocation procedures, blinding, and outcome assessment. Many studies did not clearly describe randomization of experimental animals or blinding of investigators, which may have introduced bias in group allocation and outcome measurement.

Overall, these findings indicate that although several studies reported promising antiviral activity, the reliability of the evidence is limited by methodological weaknesses. Therefore, the findings of this review should be interpreted with caution, particularly where conclusions are based on studies with high or unclear risk of bias.

Summary of Included Studies

Approximately half of the included studies focused on *Herpes simplex virus type 1* (HSV-1), likely reflecting its biosafety level 2 (BSL-2) classification, which facilitates research in resource-limited settings. Overall, 18 studies evaluated 54 Kenyan medicinal plant species, of which 28 demonstrated antiviral activity against six viruses: *Human immunodeficiency virus* (HIV), HSV, measles virus, *Human cytomegalovirus* (HCMV), *Hepatitis B virus* (HBV), and dengue virus, as summarized in Tables 1–3.

Several plant species exhibited broad-spectrum antiviral activity. Notably, *Carissa edulis*, *Prunus africana*, *Melia azedarach*, *Rhus natalensis*, and *Acacia mellifera* demonstrated activity against multiple viral targets, including HBV, HCMV, HIV-1, HSV-1, and measles virus (Table 4). Most studies were conducted *in vitro*, with only five incorporating *in vivo* models [22,26,36-38]. This predominance of preclinical evidence, together with the generally high or unclear risk of bias identified across several methodological domains, limits confidence in the overall strength of the evidence.

The observed antiviral activity may be partly attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, alkaloids, and terpenoids, although detailed phytochemical characterization was inconsistently reported across studies.

Overall, the strength of evidence was judged to be low to moderate, reflecting the predominance of *in vitro* studies, limited *in vivo* validation, and methodological limitations identified in the risk of bias assessment.

A structured comparison of antiviral activity across included studies indicates that several plant extracts demonstrated strong *in vitro* activity, with reported IC_{50} or EC_{50} values generally ranging from less than 5 µg/mL for anti-HIV activity to approximately 1.69–23.21 µg/mL for anti-HSV activity, depending on plant species and extraction method. However, differences in experimental models, extract preparation, and outcome reporting limited direct quantitative comparison across studies [22,26,36-38].

Toxicity Profiles of the Active Plants

Toxicity or cytotoxicity data were reported for 19 of the 28 plant species identified as having antiviral activity (Table 3). Overall, while the majority of active plants had some form of safety evaluation, nine species lacked any reported toxicity data. These included *Maytenus buchananii*, *Maytenus senegalensis*, *Maytenus heterophylla*, *Erythrina abyssinica*, *Azadirachta indica*, *Leptotrichilia* spp., *Carica papaya*, *Myrica salicifolia*, and *Grewia mollis*. The absence of safety data for these plants limits interpretation of their potential therapeutic relevance.

Among the studies that reported toxicity, assessments were primarily conducted using cell culture models or animal studies. However, in some cases, antiviral activity was evaluated without accompanying cytotoxicity testing, particularly in studies employing *in silico* approaches or enzyme-based assays such as reverse transcriptase inhibition [27,30]. Additionally, some studies focused on isolated compounds rather than crude extracts, which may reduce the presence of toxic impurities [25,29].

Variability in cytotoxicity profiles across plant extracts may be influenced by differences in phytochemical composition, extraction methods, and experimental models. This highlights the importance of standardized extraction procedures and comprehensive toxicity evaluation in future studies.

Overall, although several plants demonstrated promising antiviral activity, incomplete toxicity data for some species necessitates cautious interpretation of their potential for therapeutic application.

The included studies exhibited substantial heterogeneity in terms of plant species investigated, plant parts used, extraction methods, experimental models (*in vitro* vs. *in vivo*), viral targets, and outcome measures. This variability limited the ability to directly compare findings across studies and precluded quantitative synthesis, such as meta-analysis. Consequently, the findings were synthesized narratively, and comparisons of antiviral activity across studies should be interpreted with caution.

Discussion

This review highlights the antiviral potential of Kenyan medicinal plants. Notably, *Croton dichogamus*, *Croton megalocarpus*, *Croton macrostachyus*, and *Rhus natalensis* demonstrated strong activity against *Human immunodeficiency virus type 1* (HIV-1), with reported IC_{50} values below 5 µg/mL. Several other species also exhibited notable activity against *Herpes simplex virus* (HSV) and measles virus. However, the observed findings should be interpreted in the context of potential publication bias, methodological variability, and uneven research focus across plant species.

Despite these promising findings, confidence in the overall evidence remains limited. The risk of bias assessment indicated that many studies had high or unclear risk across key methodological domains, particularly in sequence generation, blinding, and outcome assessment. Furthermore, the majority of the evidence was derived from *in vitro* studies, with only a few *in vivo* investigations and no clinical trials. These limitations reduce the certainty of the evidence and increase the likelihood that reported antiviral effects may be overestimated. Accordingly, the findings of this review should be interpreted as preliminary and hypothesis-generating rather than definitive evidence of therapeutic efficacy.

a. Indigenous Medicinal Plants for Herpes Viruses Treatment in Kenya

Acyclovir remains the first-line treatment, increasing reports of resistance pose a growing challenge to effective HSV management [42]. In this context, medicinal plants represent a promising source of novel antiviral agents. For instance, extracts of Chrysanthemum cinerariaefolium demonstrated potent anti-HSV-1 activity: methanol extracts inhibited 50% of plaques at 1.69 µg/mL in vitro, while aqueous extracts achieved comparable inhibition at 23.21 µg/mL[31]. In vivo, both extracts delayed HSV-1 progression in mice at doses of 10 mg/kg and 50 mg/kg, respectively[36]. Several other Kenyan medicinal plants including *Dichrocephala integrifolia*, *Acacia mellifera*, *Garcinia buchananii*, *Caesalpinia decapetala*, *Prunus africana*, and *Plumeria alba* have also demonstrated strong anti-HSV-1 activity [22,26,29,35,37]. Notably, *D. integrifolia* exhibited direct virucidal properties [35], while Chrysanthemum cinerariaefolium exhibited remarkable potency through both pre-and-post treatment infection methods. Pure compound, Lupeol obtained from *Carissa edulis* showed activity against both wild type and acyclovir resistance HSV-1 (EC50 at 2.98 µg/ml for 7401H HSV-1, 3.66 µg/ml for APr 7401H HSV-1 and 4.2 µg/ml for the TK–B2006 HSV-1) [38].

The findings on *Carissa edulis* potency against acyclovir-resistance HSV-1 suggest that Lupeol could be exhibiting a different mechanism of action from acyclovir. The compound could be acting by blocking adsorption receptors thereby preventing viral attachment or by preventing synthesis of viral structural components once the virus has entered the cells. Virucidal properties of *D. integrifolia* suggest a similar mechanism of action while the findings on *Chrysanthemum cinerariaefolium* efficacy in both pre-and-post treatment experiments suggest the potential for a multi-action mechanisms of some extracts. Besides blocking cell receptors to prevent viral attachment, the other mechanisms of action could include hindering some steps in replication cycle once the virus has entered the cell. The multi-directional action mechanisms could be associated by the presence of polyphenols such as tannins and tannic acids previously reported to act against HSV-2 by preventing viral attachment and inducing production of cytokines and chemokines that interfere with replication[43]

b. Indigenous Medicinal Plants for Hepatitis B Virus Treatment in Kenya

Several Kenyan medicinal plants including *Carissa edulis*, *Prunus africana*, and *Acacia mellifera* have demonstrated in vitro anti-HBV activity [24]. Among them, aqueous extracts of *C. edulis* demonstrated the highest level of viral inhibition (12.15%), whereas *P. africana* and *A. mellifera* exhibited lower inhibition rates of 5% and 2.15%, respectively [24]. These findings were supported by real-time PCR, which confirmed sustained antiviral effects at concentrations ranging from 31.25 to 125 µg/mL. Reported EC_{50} values of 331.6 and 295.0 µg/mL suggest potential antiviral efficacy. Phytochemical screening of *Carissa edulis* have shown the presence of polyphenols such as tannins, flavonoids, steroids, glycosides, lignins, coumarins, and terpenoids [44]. Screening of *Prunus africana* reveals its richness in flavonoids, phenols, quinones, steroids, coumarins, saponins, alkaloids, tannins, and terpenoids [45]. Simmilar phyto-compounds including phenolics, cardiac glycosides, alkaloids, steroids, flavonoids, sapponins, tannins ,and terpenoids are found in *Acacia mellifera* [46].

The presence of flavonoids from these plants could be responsible for their anti-HBV activity. Previous studies have shown that certain types of plant-derived flavonoids such as epigallocatechin and epigallocatechin-3-gallate (EGCG) that is abundant in green tea exhibits antiviral action against HBV by blocking attachment receptors, hindering DNA synthesis and replication, and suppressing gene expression[47]. Other flavonoids such as betulinic acid and baicalin have been shown to act against HBV in a similar manner of inhibiting HBV RNAs and cutting viral replication pathway thereby limiting chances of producing new virions [47]. The flavonoids present in *Carissa edulis*, Prunus africana, and *Acacia mellifera* could be responsible for their antiviral efficacy. The extracts probably have similar mechanisms of action of blocking entry and interfering with pathways for development of viral structural components like other flavonoids.

c. Indigenous Medicinal Plants for Measles Virus Treatment in Kenya

The measles virus, a highly contagious RNA virus belonging to the Paramyxoviridae family, remains a major cause of childhood morbidity and mortality worldwide [48]. An in vitro evaluation of 13 Kenyan medicinal plants identified four species - *Rhus natalensis*, *Albizia amara*, *Olinia rochetiana*, and *Warburgia ugandensis;* that exhibited significant measles virus neutralization activity [39]. Among them, *O. rochetiana* and *W. ugandensis* demonstrated the highest potency, achieving 107.3 and 98.0 neutralization units, respectively, relative to 293.5 observed in human serum. Both extracts achieved 50% neutralization of measles viral particles at concentrations as low as 0.1 µg/mL. In addition, *R. natalensis* and *A. amara* demonstrated notable 17-fold increases in neutralization capacity. Furthermore, *Rhamnus prinoides* and *Scutia myrtina* were found to reduce viral yield in U937 cell cultures. The mechanisms of action of these extracts could be through blocking viral entry into the cell. This could be due to the presence of saponins and amyrin-acetate which have previously demonstrated significant binding affinity with the 5e4v-receptor proteins of the measles virus in an in-sillico model[49]. Saponins and amyrin-acetate are key phyto-compounds present in *Warbugia ugandensis* and *Olinia rochetiana* [50,51].

These plants are traditionally used by the Maasai community in Kenya as dietary additives for children, a practice that may contribute to measles protection. The convergence of their traditional use with demonstrated antiviral activity underscores the need for further research to isolate and characterize active phyto-compounds for potential therapeutic development.

d. Indigenous Medicinal Plants for Human Cytomegalovirus Treatment in Kenya

Human cytomegalovirus (HCMV), a β-herpesvirus, is globally prevalent with seropositivity rates ranging from 60% to 90%, and poses serious health risks to immunocompromised individuals, neonates, and transplant recipients [52]. Given the absence of a licensed vaccine, the exploration of plant-derived antivirals has gained considerable attention. Root bark extracts of *Maytenus heterophylla* yielded pristimerin, a compound that demonstrated potent anti-HCMV activity (IC_{50} = 0.53 µg/mL), effectively inhibiting viral replication without compromising cell viability [25]. Mechanistic studies using Western blot analysis confirmed reduced amount of immediate early (IE) antigen including the expression of immediate-early (IE2) viral antigens [25]. In addition, aqueous extracts of *Carissa edulis*, *Prunus africana*, and *Melia azedarach* displayed in vitro anti-HCMV activity, with EC_{50} values ranging from 40 to 80 µg/mL [33]. The findings from Western blot show that Pristimerin could be acting by hindering the synthesis of vital viral components (IE2 antigens) thereby blocking infectivity and replication.

Two similarities can be identified in the treatment of HCMV versus HSV-1. First, two of the plants, *Carissa edulis* and *Prunus africana* found to be active against HCMV are also active against HSV-1. Secondly, both HCMV and HSV-1 belong to the same class of herpes viruses. Their similarities in pathogenesis and replication cycles suggests that phytochemicals present in *Carissa edulis* and *Prunus africana* could be exhibiting similar mechanisms of action on HCMV and HSV-1.

Collectively, these findings underscore the potential of Kenyan medicinal plants as promising sources for developing alternative antiviral therapies against HCMV, particularly for high-risk populations.

e. Indigenous Medicinal Plants for Dengue Virus Treatment in Kenya

Among Kenyan medicinal plants, *Carica papaya* leaf methanol extracts have demonstrated notable anti-dengue potential in a silico docking study which identified 5,7-dimethoxycoumarin and quercetin as key bioactive compounds with strong binding affinity to the DENV-2 NS5 protein [27]. Subsequent in vitro experiments demonstrated that silver nanoparticle formulations containing these compounds effectively inhibited DENV-2 replication, with an IC_{50} of 9.20 µg/mL [27]. By binding with the NS5 protein, the compounds 5,7-dimethoxycoumarin and quercetin blocks the ability of the virus to attach to the host receptor proteins thereby stoping the replication of the viral RNA.

These findings suggest that *Carica papaya*, a plant widely cultivated and traditionally used in Kenya, holds a significant promise as a source of novel plant-based dengue therapeutics.

f. Indigenous Medicinal Plants against HIV-1 in Kenya

Kenyan medicinal plants have demonstrated promising anti-HIV-1 activity, particularly through inhibition of the viral reverse transcriptase enzyme, which is essential for viral replication. Several species including *Maytenus buchananii*, *Maytenus senegalensis*, *Acacia mellifera*, *Erythrina abyssinica*, *Azadirachta indica*, *Leptotrichilia sp.*, *Melia azedarach*, *Myrica salicifolia*, *Prunus africana*, *Grewia mollis*, and *Rhus natalensis* have shown significant reverse transcriptase inhibition capacity in vitro [23,30]. The Croton plant genus seems to be a promising source for anti-HIV drugs. From *Croton macrostachyus*, compounds such as lupenone, lupeol acetate, and betulin have exhibited potent anti-HIV-1 effects, with IC_{50} values below 5 µg/mL, possibly through interactions with viral envelope proteins [32]. Dichloromethane (DCM)-methanol extracts from *Croton dichogamus* and *Croton megalocarpus* inhibited more than 70% of HIV-induced cytopathic effects at low IC_{50} concentrations (0.05 + 0.03 µg/mL) [34].

The antiviral activity observed in several Kenyan medicinal

plants may be partly attributed to their phytochemical composition. For example, *Carissa edulis* has been reported to contain a wide range of bioactive compounds, including polyphenols such as tannins, flavonoids, glycosides, coumarins, lignins, steroids, and terpenoids[38]. Similarly, *Prunus africana* contains flavonoids, phenols, quinones, alkaloids, saponins, tannins, and terpenoids, while *Acacia mellifera* has been shown to contain phenolics, flavonoids, alkaloids, cardiac glycosides, steroids, saponins, tannins, and terpenoids [24]. These classes of compounds are well known for their antiviral, antioxidant, and immunomodulatory properties, and may contribute to the observed inhibition of viral replication through mechanisms such as interference with viral entry, replication, or protein synthesis.

The overall strength of evidence in this review was assessed qualitatively based on study design, consistency of findings, availability of toxicity data, and risk of bias. Most of the included evidence was derived from *in vitro* studies, with only a limited number of *in vivo* investigations and no clinical studies. In addition, several studies demonstrated high or unclear risk of bias across key methodological domains.

Based on these factors, the overall confidence in the evidence was judged to be low to moderate for most plant–virus combinations. Higher confidence was observed where findings were supported by both *in vitro* and *in vivo* data with reported toxicity assessments. However, in many cases, the absence of toxicity data and methodological limitations reduced the reliability of the findings. Therefore, the evidence should be interpreted cautiously and considered preliminary.

Although structured summaries of antiviral activity were considered, the substantial heterogeneity across included studies—including differences in plant species, plant parts used, extraction methods, viral targets, experimental models, and outcome measures—limited the feasibility of detailed quantitative comparison or tabulation. Consequently, a narrative synthesis approach was retained as the most appropriate method for summarizing the available evidence. This heterogeneity also limits direct comparability across studies and reduces confidence in drawing generalized conclusions.

Furthermore, variability in reported antiviral activity may be influenced by differences in extraction methods, as solvent polarity determines the types of phytochemicals extracted. Aqueous extracts are more likely to yield polar compounds such as phenolics and flavonoids, whereas organic solvents, including methanol or dichloromethane:methanol mixtures, may extract a broader range of bioactive constituents such as terpenoids and other lipophilic compounds. However, because most included studies did not perform detailed phytochemical characterization or standardization, the relationship between chemical composition, extraction method, and antiviral activity remains suggestive rather than definitive. Despite these encouraging findings, the precise mechanisms of action and pharmacodynamic properties of these medicinal plants remain poorly understood. Current evidence is largely limited to *in vitro* studies, underscoring the need for well-designed *in vivo* and clinical investigations to validate their therapeutic potential. In addition, the generally high or unclear risk of bias across several included studies further limits confidence in the reliability of the findings.

The study selection approach, which excluded publications with substantially overlapping findings to reduce redundancy, may also have introduced selection bias and potentially limited the comprehensiveness of the evidence base.

Finally, although several medicinal plants demonstrated antiviral activity, the absence of toxicity data for a number of species limits their translational potential. Safety evaluation is a critical component of drug development, and the lack of cytotoxicity or *in vivo* toxicity data makes it difficult to assess therapeutic windows and potential adverse effects. Therefore, plants without toxicity data should be interpreted cautiously, and further studies are required to establish their safety profiles before any consideration of clinical application.

Conclusion

Several indigenous Kenyan medicinal plants demonstrate promising antiviral activity against viruses such as *Human immunodeficiency virus* (HIV), *Herpes simplex virus* (HSV), *Hepatitis B virus* (HBV), *Human cytomegalovirus* (HCMV), measles virus, and dengue virus. However, the current evidence base is predominantly preclinical, with most findings derived from *in vitro* studies, limited *in vivo* validation, and no clinical trials.

The overall strength of evidence is low to moderate and is further constrained by methodological heterogeneity, high or unclear risk of bias, and incomplete toxicity data for several plant species. Consequently, these findings should be interpreted cautiously and regarded as preliminary.

Future research should prioritize well-designed pharmacological, toxicological, and clinical studies to validate these findings, establish safety profiles, and identify bioactive compounds responsible for antiviral activity. In particular, standardization of extraction methods, detailed phytochemical characterization, and comprehensive toxicity evaluation will be essential to advance these plants toward therapeutic application.

Overall, while Kenyan medicinal plants represent a valuable resource for antiviral drug discovery, further rigorous investigation is required before their potential clinical utility can be established.

Recommendations

Further research should expand screening of Kenyan medicinal plants for antiviral properties, safety and cytotoxicity, isolate active compounds, and clarify their mechanisms of action. Emphasis on multi-virus testing and

toxicity assessment is needed. Strengthening partnerships among researchers, traditional practitioners, and pharmaceutical developers will support drug discovery and sustainable use of indigenous resources.

Future Perspectives

Future research should prioritise in-vivo testing of antiviral plant compounds, integrating ethnobotany, molecular biology, and pharmacology. Collaboration between academia, traditional healers, and industry is essential to develop and clinically validate novel antiviral drugs from indigenous plants for global health impact.

Author contributions

RLM, JK, BI, JWL, SN, AM and JKN designed the study, interpreted the data, and wrote the main manuscript text. All authors reviewed the final manuscript.

Conflict of interest

The authors state no conflict of interest.

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REVIEW ARTICLE

Plant-Derived Bioactive Proteins and Peptides as Emerging Therapeutics: Current Evidence and Potential Translational Challenges

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Abstract

Medicinal plants have long served as a cornerstone of traditional therapies, primarily recognized for their rich repertoire of secondary metabolites. Beyond these small molecules, plants produce a diverse array of proteins endowed with potent bioactive properties that remain comparatively underexplored in modern drug discovery. This review aims to consolidate and highlight the current knowledge on major families of plant-derived proteins and peptides with medicinal value, with particular emphasis on their mechanisms of action, therapeutic limitations, mitigation strategies, and translational potential. A narrative literature survey was conducted using peer-reviewed studies to feature plant proteins/peptides, such as lectins, pathogen-related proteins, RIPs, proteases, etc., with reported medicinal values. The review highlights how plant proteins exert therapeutic effects through defined molecular interactions, including enzymatic catalysis, selective membrane disruption, receptor binding, immune modulation, and interference with pathogen or cancer-associated cellular processes, resulting in antiviral, antifungal, anticancer, anti-inflammatory, and immunomodulatory activities. It is believed that the evolutionary diversification and lineage-specific expansion of these protein families have generated extensive functional variability, increasing the likelihood of identifying molecules with novel or enhanced bioactivity. Advances in genomics, proteomics, and recombinant expression technologies have further accelerated protein discovery, functional characterization, and bioengineering, enabling improved specificity, stability, and delivery. Collectively, the evidence supports plant-derived proteins as a versatile and multifunctional class of biomolecules that complement conventional small-molecule therapeutics, while underscoring the need for systematic characterization, optimized production strategies, and well-designed preclinical and clinical studies to support their future application in disease prevention, management, and biomedical innovation.

Keywords: Plant proteins; Bioactive molecules; Therapeutic potential; Immunomodulation; Anticancer; Antiviral

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Introduction

Medicinal plants have been used as therapeutic resources across human civilizations, forming the foundation of traditional medical systems such as Ayurveda, Traditional Chinese Medicine, Unani, African traditional medicine, and ethnomedicine worldwide [1]. Historically, the pharmacological value of medicinal plants has been attributed predominantly to low-molecular-weight secondary metabolites, including alkaloids, flavonoids, terpenoids, phenolics, and glycosides. These compounds have yielded numerous modern drugs and lead molecules [2,3]. Consequently, this metabolite-centric view has overshadowed another biologically powerful class of plant-derived molecules: proteins and peptides. In recent decades, accumulating biochemical, molecular, and pharmacological evidence has demonstrated that plants produce a wide array of proteins with potent and

highly specific bioactivities relevant to human health [4]. Unlike secondary metabolites, proteins often act through well-defined molecular mechanisms, such as enzymatic catalysis, receptor binding, membrane disruption, or direct modulation of immune and signaling pathways. These characteristics confer high target specificity and biological efficacy, frequently at nanomolar concentrations [5].

Most medicinally relevant plant proteins originate from conserved gene families involved in innate plant defense, stress responses, or nutrient storage. These include lectins, ribosome-inactivating proteins, pathogenesis-related (PR) proteins [6], antimicrobial peptides 7, proteases, protease inhibitors, and seed storage proteins 8. From an evolutionary perspective, many of these protein families have undergone lineage-specific expansion and diversification, particularly in plants exposed to strong biotic pressures such as pathogens and herbivores. This evolutionary plasticity has generated extensive functional diversity, increasing the likelihood of discovering proteins with novel or enhanced bioactivities [6,8]. Technological advances in genomics, transcriptomics, proteomics, and recombinant protein expression have dramatically accelerated the discovery and functional characterization of plant protein families. High-quality plant genome assemblies have revealed extensive expansion of bioactive protein families, particularly in medicinal and stress-adapted species. At the same time, protein engineering, molecular docking, and targeted delivery systems are overcoming historical limitations related to toxicity, immunogenicity, and stability [9,10].

Although numerous reviews have examined individual classes of plant-derived bioactive proteins in detail, the literature remains fragmented across protein families. This review consolidates the major bioactive plant protein groups within a single structured framework, enabling cross-family comparison of mechanisms and translational challenges. By emphasizing shared principles and common development constraints, it provides an integrated reference to guide future therapeutic and industrial applications. In this review, we highlight the major types and classes of proteins and peptides with potential medicinal value, explore their possible mechanisms of action, and discuss the challenges and limitations associated with their biotechnological and medicinal applications.

Materials and Methods

Literature search strategy

This review was conducted as a narrative, non-systematic literature review focusing on protein-based molecules from plant sources with reported or proposed medicinal value, as the available data remained fragmented and hard to harmonize under a systematic review framework. The literature survey aimed to capture foundational mechanistic studies, representative experimental evidence, and recent advances relevant to therapeutic applications rather than to exhaustively quantify outcomes. Primary literature searches were performed using major scientific databases, including PubMed, Web of Science, Scopus, and Google Scholar, and refined via Boolean operators. The search was conducted using combinations of controlled vocabulary and free-text terms, such as: "plant proteins, plant-derived antimicrobial proteins, plant defensins, pathogenesis-related proteins, ribosome-inactivating proteins, lectins, mechanism of action, antifungal, antiviral, anticancer, mode of action, therapeutic application, clinical potential." To ensure mechanistic depth, priority (inclusion criteria) was given to (i) peer-reviewed original research articles describing biochemical, structural, molecular, or cellular mechanisms; (ii) studies reporting experimentally validated bioactivities in vitro, in vivo, or in early-phase clinical contexts; and (iii) review articles used to contextualize protein families and identify foundational references. Exclusion criteria included: (i) studies that were agricultural-based only with no relevance to biomedical or therapeutic applications, or (ii) obtained from non-peer-reviewed sources. Furthermore, no strict publication date limits were imposed; however, recent studies were given particular emphasis.

Figure development and visualization

The figures presented in this review were generated using a text-to-figure visualization tool of Figurelabs.ai (Available from: https://figurelabs.ai, Last accessed Jan, 2026), which converts structured textual descriptions into schematic illustrations. The textual inputs provided to the tool were derived exclusively from detailed mechanistic information curated from peer-reviewed research articles. The tool was used solely to facilitate the visual representation of established or experimentally supported pathways, while all biological interpretations, pathway definitions, molecular interactions, and mechanistic frameworks were manually curated, validated, and summarized by the authors. No mechanistic conclusions or biological inferences were generated by the visualization tool itself.

Plant-Derived Proteins and Peptides

Lectin Protein Families

Plant lectins are non-enzymatic, carbohydrate-binding proteins that recognize and reversibly bind specific mono- or oligosaccharide moieties without altering their covalent structure. They are ubiquitous in the plant kingdom and are particularly abundant in seeds, bulbs, rhizomes, bark, latex, and leaves. Molecular weights of plant lectins typically range from ~10 kDa to >120 kDa, depending on oligomeric state and domain composition. Structurally, most lectins are oligomeric proteins composed of identical or closely related subunits, which enhances multivalent carbohydrate binding and biological potency [11]. From a physiological perspective, plant lectins function primarily in defense against pathogens, herbivores, and insects, as well as in symbiotic recognition and intracellular signaling [12]. Their high stability, resistance to proteolysis, and strong affinity for glycoconjugates underpin both their ecological function and their biomedical relevance. The medicinal

potency of plant lectins is closely linked to their tissue localization and expression level. Seed lectins are often present at high concentrations, enabling strong bioactivity upon ingestion or extraction [13]. Lectins localized in latex or epidermal tissues tend to exhibit higher toxicity and antimicrobial potency, reflecting their role as immediate defense molecules [14].

Plant lectins have been extensively investigated for their diverse medicinal potential. This encompasses anticancer activities such as the induction of apoptosis, cell-cycle arrest, and inhibition of angiogenesis, as well as antiviral effects through the blockade of viral entry and membrane fusion [15]. In addition, lectins exhibit immunomodulatory properties [16], enabling them to either activate or suppress immune responses, and display broad antimicrobial and antifungal activities [14]. They were also studied for their potential effects as antiulcer agents and antinociceptives [17-20]. Owing to their high specificity for carbohydrate moieties, plant lectins are also widely employed as diagnostic and targeting tools in glycobiology and oncology. Several lectins are already established as research reagents, and several candidates are currently undergoing preclinical or clinical evaluation for therapeutic applications [21]. Plant lectins are classified based on sequence homology, three-dimensional fold, and carbohydrate specificity. Structural aspects of major lectin families with established or emerging medicinal relevance are summarized in Table 1.

Mechanism of action

Plant lectin families exhibit diverse yet convergent mechanisms of action that underpin their therapeutic potential (Figure 1). Legume lectins, such as concanavalin A (*Canavalia ensiformis*) and phytohemagglutinin (*Phaseolus vulgaris*), selectively bind aberrant surface glycans overexpressed on cancer cells, including the Thomsen–Friedenreich antigen, triggering mitochondrial apoptosis via cytochrome c release and caspase activation, while also inducing autophagy through BNIP3 upregulation and suppression of PI3K/Akt/mTOR signaling [30]. In parallel, their strong mitogenic activity promotes T-lymphocyte proliferation and cytokine secretion (e.g., IL-2, IFN-γ), thereby amplifying antitumor immune responses. Hevein (Hevea brasiliensis) and related small chitin-binding lectins exert potent antimicrobial effects by binding chitin in fungal cell walls, disrupting cell wall biosynthesis, and causing hyphal rupture. Additionally, hevein domains can activate innate immune cells, inducing oxidative bursts in neutrophils, and have been exploited as targeting motifs in nanoparticle-based drug delivery systems to enhance chemotherapeutic uptake [31,32]. Hevein-like lectins identified in spike moss (*Selaginella moellendorffii*) have been investigated as potential inhibitors of SARS-CoV-2 and several early viral variants. These lectins are proposed to impede viral entry by interacting with terminal N-acetylgalactosamine residues on the spike glycoprotein (S1), thereby interfering with ACE2-mediated host cell attachment [28]. Jacalin (*Artocarpus integra*) exerts therapeutic effects by binding O-glycosylated CD45, which triggers the ERK/p38 MAPK pathways to induce IL-2 production and T-cell proliferation. It simultaneously inhibits HIV-1 infection by sterically blocking the gp120–CD4 interaction and, in oncological models, promotes apoptosis by suppressing Lyn kinase activity. Furthermore, it stimulates macrophages to release cytotoxic TNF-α and reactive oxygen species via NF-κB activation, enhancing anti-tumor immunity [33-36]. Finally, GNA (*Galanthus nivalis*) functions as a potent antiviral agent by binding to high-mannose glycans on viral envelope proteins, such as gp120 in HIV-1, effectively neutralizing the virus and blocking entry/fusion into host cells. In oncology, GNA induces apoptosis and autophagy by binding to cell-surface receptors and internalizing to the mitochondria, where it triggers ROS production, cytochrome c release, and the activation of p38/p53 signaling pathways. It also serves as a specialized carrier molecule in transgenic medicine, facilitating the oral delivery and absorption of fused therapeutic proteins across biological barriers like the gut epithelium [37,38].

Limitations, mitigation strategies, and therapeutic relevance

Plant lectins share common translational limitations, primarily off-target glycan binding, mitogenicity or immunogenicity, and potential cytotoxicity due to broad carbohydrate specificity, which complicate systemic clinical use [39]. Current mitigation strategies focus on protein engineering (domain truncation, point mutations to reduce nonspecific binding), targeted delivery, dose control, and conjugation to carriers to improve selectivity and safety [40]. Looking forward, their clinical future is most promising in highly controlled contexts. Such as topical applications, localized anticancer targeting, antiviral blocking at mucosal surfaces, and use as diagnostic or targeting modules rather than standalone therapeutics, where lectin–glycan specificity can be exploited while minimizing systemic exposure and toxicity [41]. On clinical context, plant lectins have reached clinical evaluation with markedly different levels of translational maturity. Extracts of *Viscum album* containing mistletoe lectins have been investigated as adjunctive therapies in oncology, with several clinical studies reporting improvements in quality-of-life parameters. However, the evidence remains heterogeneous, and methodological limitations and ongoing debate preclude definitive conclusions regarding clinical efficacy [42-44]. While lectins from other sources, such as Griffithsin (from red algae *Griffithsias* sp.), a mannose-binding lectin produced recombinantly in plants, have completed Phase I trials as a topical microbicide, demonstrating excellent safety, minimal systemic absorption, and strong promise for prevention of HIV-1 and other sexually transmitted viral infections [45]. Other plant lectins like Abrin (*Abrus precatorius*) and Ricin-B–containing systems remain at the preclinical stage; despite potent anticancer activity in experimental models, their intrinsic toxicity currently confines their clinical potential to engineered immunotoxins or targeted delivery platforms rather than standalone therapeutics [46].

Ribosome-Inactivating Protein (RIP) Families

Ribosome-inactivating proteins (RIPs) are a class of plant-derived enzymes that irreversibly inhibit protein synthesis by depurinating a highly conserved adenine residue in the sarcin–ricin loop of large ribosomal RNA. This N-glycosidase activity renders ribosomes incapable of interacting with elongation factors, leading to translational arrest and, ultimately, cell death [47]. RIPs are widely distributed in higher plants and are particularly abundant in medicinal species known for their antiviral and anticancer properties [48]. The tissue localization and expression levels of RIPs strongly influence their biological and medicinal effects. RIPs concentrated in seeds and storage tissues are typically associated with defense against predators and pathogens, resulting in high intrinsic toxicity. Conversely, RIPs expressed in vegetative tissues often display more regulated activity and lower toxicity. Intracellular compartmentalization, such as vacuolar sequestration, further limits autotoxicity in plant cells while preserving defensive function [49].

RIPs have been intensively investigated for a broad range of biomedical applications, notably as antiviral agents against HIV-1, HBV, HSV, and emerging viruses such as SARS-CoV-2, as well as anticancer molecules capable of suppressing tumor growth and inducing apoptosis [50]. They have also been developed as immunotoxins for targeted cancer therapy and, in historical and limited clinical contexts, explored as abortifacient and antifertility agents [51], while continuing to serve as powerful experimental tools for studying ribosome function and cell death pathways. The medicinal activity of RIPs is fundamentally based on their ability to depurinate a specific adenine residue in ribosomal RNA, resulting in irreversible inhibition of protein synthesis. In cancer cells, this translational arrest activates apoptotic signaling through both intrinsic mitochondrial and extrinsic death receptor pathways, accompanied by oxidative stress, DNA damage responses, and caspase activation [52]. In antiviral contexts, RIPs suppress viral replication by inhibiting viral protein synthesis and, in some cases, directly targeting viral RNA or replication complexes. Type-II RIPs achieve enhanced cellular entry via lectin-mediated endocytosis, whereas type-I RIPs rely on alternative uptake routes such as pinocytosis and are therefore under active investigation due to their more favorable balance between efficacy and safety [53]. In immunotoxin-based strategies, the catalytic RIP domain is conjugated to targeting molecules to enable the selective elimination of malignant cells, making mechanistic understanding a central factor in the clinical advancement of RIP-derived therapeutics (Figure 2) [54].

RIPs are typically basic proteins with molecular weights ranging from ~25 kDa to over 60 kDa, depending on domain composition. They are exceptionally stable, resistant to proteolysis, and retain activity under a wide range of pH and temperature conditions [55]. These biochemical properties, combined with their potent biological activity, underline both their therapeutic potential and their inherent toxicity. Plant RIPs are traditionally classified into three major types based on their structural organization and cellular targeting capacity, which are summarized in Table 2.

Table 1: Structural aspects of major plant lectin families endowed with medicinal values

Lectin Family	Subunit Size	Oligomeric State	Fold/ Topology	Metal Ion Requirement	Carbohydrate-Binding Site Features	Disulfide Bonds	Structural Stability	Structure–Function Implications	Ref
Legume (L-type) lectins	250–300 aa (~25–30 kDa)	Dimers or tetramers (50–120 kDa)	β-sandwich (jelly-roll–like)	Ca^{2+} and Mn^{2+} essential	Shallow binding pocket formed by loop regions; metal ions stabilize sugar coordination	Few or none	High thermal and pH stability	Multivalency via oligomerization enables receptor cross-linking, immune cell activation, and apoptosis signaling.	22,23
Jacalin-related lectins (JRLs)	15–35 kDa	Monomers, dimers, or tetramers	β-prism I	None required	Compact, deep carbohydrate-binding pocket with aromatic residues for galactose stacking	Rare	Moderate; sensitive to proteolysis	Strong glycan specificity underlies antiviral envelope recognition; mutations reduce mitogenicity without loss of binding.	24,25
Ricin B-like (R-type) lectins	~130 aa per B-chain domain	Part of heterodimeric type II RIPs	β-trefoil fold	None	Two galactose-binding sites per domain; high avidity	None	High	Efficient cell internalization makes them ideal targeting moieties for immunotoxins	26
GNA-type lectins	12–15 kDa	Dimers or tetramers	β-prism II	None	Multiple mannose-binding pockets are arranged symmetrically	None	Very high (protease-resistant)	High affinity for high mannose glycans enables potent viral entry inhibition	27
Hevein lectins	30–45 aa (4–8 kDa)	Monomeric or multimeric	Small cysteine-rich fold	None	Linear groove recognizing GlcNAc/chitin oligomers	Multiple (3–4)	Extremely high	Disulfide-stabilized scaffolds confer resistance to degradation and strong antifungal activity	28,29

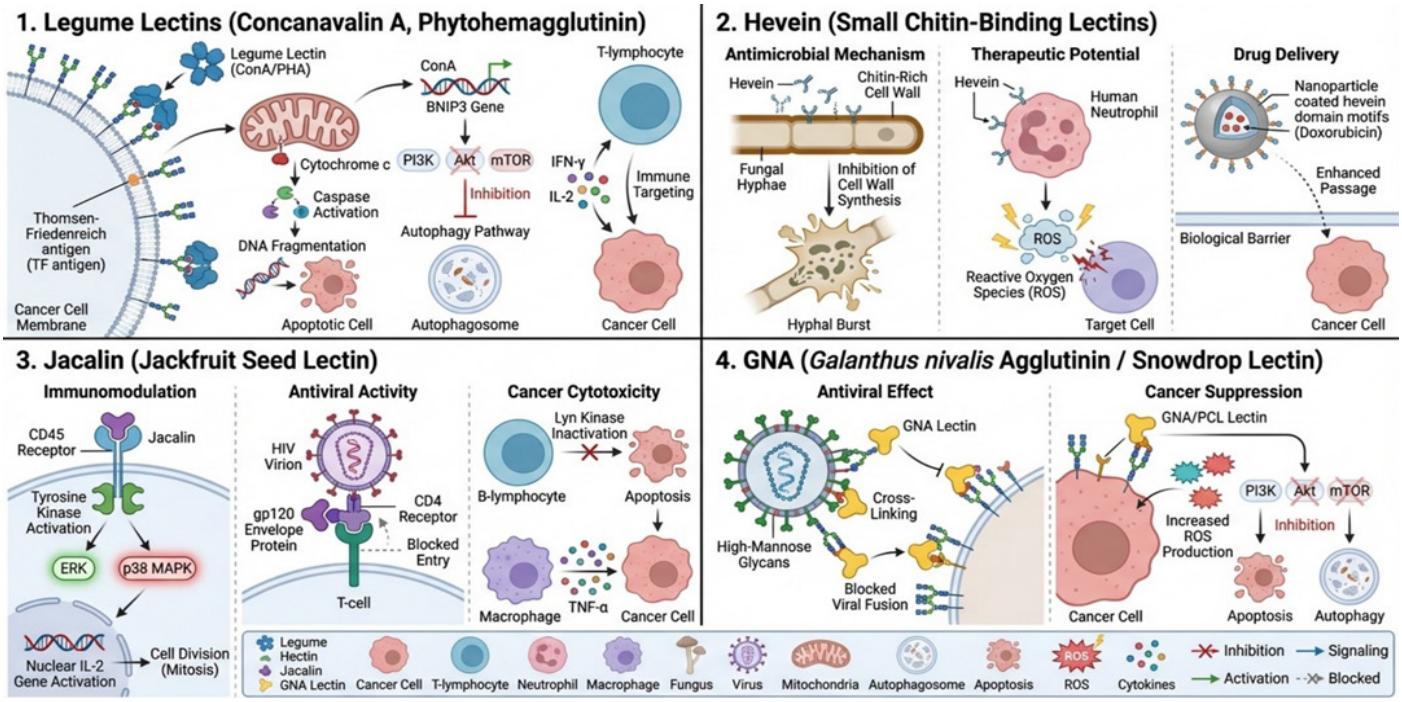

Figure 1: Possible mechanism of action of major plant lectin families: Schematic representation of the principal, experimentally supported mechanisms by which plant lectins exert biological effects, including carbohydrate-mediated cell surface binding, receptor cross-linking, immune cell activation, inhibition of viral entry, and induction of apoptosis. The figure highlights representative pathways common to major bioactive lectin families.

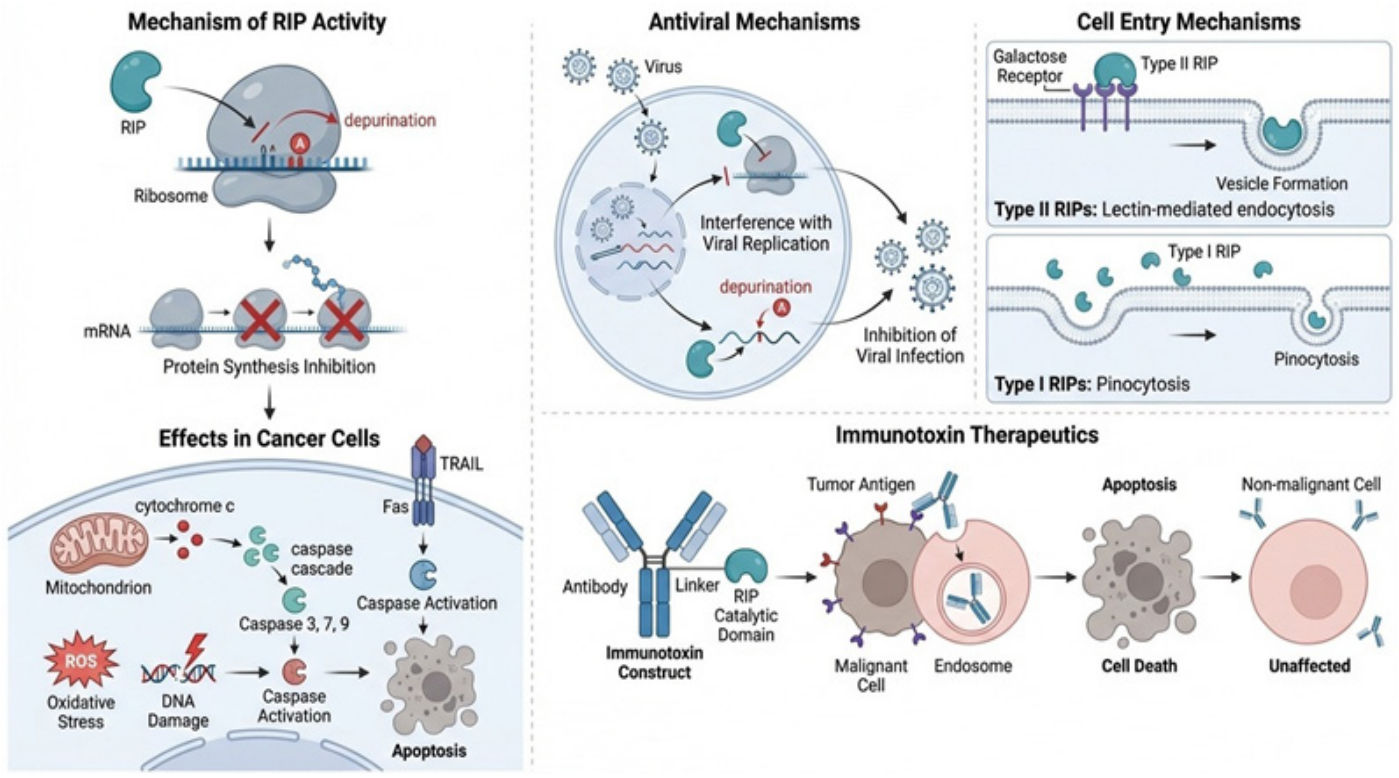

Figure 2: Ribosomal inactivating proteins (RIPs) mechanism of action: Overview of the canonical catalytic mechanism of plant RIPs, illustrating ribosomal depurination, translational arrest, and initiation of apoptotic cell death. The figure emphasizes core molecular events common to RIP classes.

Limitations, mitigation, and clinical trials of RIPs

Plant ribosome-inactivating proteins (RIPs) from classes I, II, and III exhibit substantial therapeutic promise. But their clinical translation is constrained by several well-defined limitations. The principal challenge is toxicity, which is most pronounced for type-II RIPs due to lectin-mediated cellular uptake and efficient retrograde transport, leading

to off-target ribosomal inactivation in healthy tissues. Even type-I RIPs, while lacking a binding subunit, can cause nonspecific cytotoxicity at higher doses, and all RIP classes raise concerns regarding immunogenicity, systemic inflammatory responses, and poor therapeutic index. Additional limitations include inefficient intracellular delivery for type-I and type-III RIPs, rapid clearance, and difficulties in achieving tumor- or virus-specific selectivity. For type-III RIPs, incomplete or uncontrolled proteolytic activation may further reduce predictability and efficacy [63]. To mitigate these limitations, multiple engineering and delivery strategies have been developed. The most advanced approach involves immunotoxin design, where the catalytic RIP domain (commonly the A chain of type-II RIPs or full type-I RIPs) is conjugated to monoclonal antibodies, growth factor ligands, or nanocarriers to enable cell-specific targeting and minimize systemic exposure. Deglycosylation, site-directed mutagenesis, and epitope masking have been used to reduce immunogenicity, while PEGylation and nanoparticle encapsulation improve pharmacokinetics and stability. For type-III RIPs, controlled activation through engineered cleavage sites offers a promising route to enhance safety. Collectively, these mitigation strategies shift RIPs from broadly cytotoxic plant toxins toward precision biological payloads suitable for targeted therapy [63]. In terms of clinical trial status, native plant RIPs have not progressed as standalone therapeutics due to safety concerns. However, RIP-derived immunotoxins have advanced into early-phase clinical trials, particularly in oncology. Ricin A-chain–based and abrin-derived immunotoxins have been evaluated in Phase I/II studies for hematological malignancies and solid tumors, demonstrating proof-of-mechanism but also dose-limiting toxicities that halted broader development. Type-I RIPs, owing to their more favorable safety profile, remain under active preclinical and translational investigation, especially for antiviral and cancer applications. Type-III RIPs are currently confined to experimental and preclinical stages but are increasingly viewed as attractive next-generation scaffolds for safer RIP-based therapeutics. Overall, while no RIP-based drug has yet achieved regulatory approval, continued advances in targeting, delivery, and protein engineering sustain strong clinical interest in RIPs as modular cytotoxic agents rather than conventional drugs [63,64].

Pathogenesis-Related (PR) Protein Families

Pathogenesis-related (PR) proteins constitute a diverse group of plant proteins that are induced in response to biotic stresses such as pathogen infection, herbivory, and wounding, as well as abiotic stresses including salinity, drought, and oxidative stress. Initially characterized in tobacco during hypersensitive responses, PR proteins are now recognized as ubiquitous components of plant innate immunity [65]. Beyond their defensive role in planta, many PR protein families exhibit bioactivities of direct relevance to human health, including antifungal, antibacterial, antiviral, anticancer, and immunomodulatory effects. This arises from multiple mechanisms, including direct membrane disruption, enzymatic degradation

Table 2: Main characteristics of RIP classes

RIP Type	Structure	M.Wt. (kDa)	Domain structure	Catalytic Site	Cellular Uptake	Examples	Biochemical Stability	Intrinsic Toxicity (Structural Basis)	Translational Suitability	References
Type-I RIPs	Single-chain monomeric protein	~25–30	N-glycosidase catalytic domain only	Conserved active-site residues (e.g., Tyr, Glu, Arg) forming the depurination pocket	Inefficient uptake via pinocytosis or non-specific endocytosis	Bryodin I (Bryonia dioica), Luffin-A/B (Luffa cylindrica), Dianthin (Dianthus caryophyllus), Momordin (Momordica charantia)	High thermal and pH stability; protease-resistant	Moderate; absence of lectin domain limits cellular entry	Favorable scaffold for conjugation-based targeting strategies	[47,56-59]
Type-II RIPs	Heterodimer A–B toxin linked by a disulfide bond	~60–65 (A: ~30; B: ~30–35)	A chain: catalytic N-glycosidase; B chain: ricin B-like lectin	Conserved rRNA depurination active site in A chain	Efficient receptor-mediated endocytosis via glycan binding	Ricin (Ricinus communis), Abrin (Abrus precatorius), Volkensin (Adenia volkensii)	Extremely stable; resistant to denaturation	Very high; lectin-mediated uptake enhances cytosolic delivery	A-chain is widely used after detachment from the B-chain	[26,60,61]
Type-III RIPs	Inactive precursor requiring proteolytic activation	~35–45 kDa precursor	Catalytic RIP domain + removable peptide segments	Latent active site exposed after cleavage	Limited uptake; activity dependent on maturation	Maize RIPs (Zea mays)	High stability in precursor form	Low to moderate; restricted activation	Promising platform for engineering safer RIP derivatives	[62]

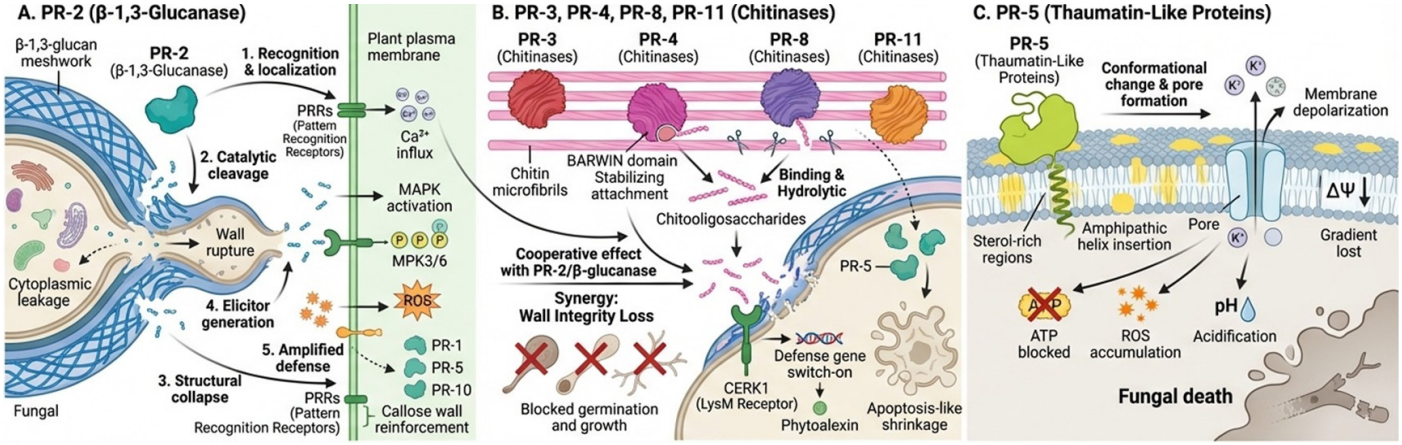

Figure 3: Antifungal activity (cell wall and membrane disruption) of some pathogen-related proteins.

of pathogen structural components, generation of immunogenic fragments, and modulation of host immune responses. Anticancer effects are often mediated through induction of apoptosis, oxidative stress, and interference with cell signaling pathways (Figure 3) [66]. PR proteins are typically low- to medium-molecular-weight proteins (10–40 kDa), often secreted or localized to the apoplast, vacuole, or extracellular matrix. Their stability, inducibility, and frequent enrichment in medicinal plants underpin their prominence among bioactive plant protein families. They are classified into multiple families (PR-1 to PR-17) based on sequence homology, biochemical activity, and immunological properties [67]. Among these, several families have been particularly well-studied for medicinal relevance.

Examples of bioactive PR families

PR-1 proteins (CAP superfamily)

PR-1 proteins are among the most strongly induced defense proteins in plants and belong to the CAP (Cysteine-rich secretory proteins, Antigen 5, and PR-1) superfamily. They are relatively small proteins, typically 14–17 kDa, containing conserved cysteine residues that form disulfide bonds, which stabilize their structure. These proteins are predominantly localized in the extracellular space or apoplast, where they can directly interact with pathogens. Functionally, PR-1 proteins exhibit robust antifungal and antibacterial activities, often acting through sterol-binding and membrane-disruptive mechanisms. While immunomodulatory functions of PR-1 proteins have been primarily characterized in plants, comparative studies of CAP-domain proteins in animals indicate conserved structural features associated with immune modulation, supporting a plausible but indirect mechanistic parallel [68-70].

PR-2 proteins (β-1,3-glucanases)

PR-2 proteins are β-1,3-glucanases that hydrolyze glucans in fungal cell walls, contributing to plant defense. These enzymes typically range from 30 to 40 kDa and possess well-defined catalytic domains that mediate glucan degradation. They are mainly localized in the apoplast and vacuole. Beyond their direct antifungal activity, PR-2 proteins generate bioactive oligosaccharides that function as immune elicitors, promoting immunostimulatory and antimicrobial responses 71.

PR-3, PR-4, and PR-8 proteins (chitinases)

PR-3, PR-4, and PR-8 proteins are chitinase-containing PR proteins that hydrolyze chitin, a key structural component of fungal cell walls and insect exoskeletons. These enzymes typically range from 25 to 35 kDa and often include chitin-binding domains that enhance substrate recognition and catalytic efficiency. They are localized in the apoplast, vacuole, and occasionally within intracellular compartments, allowing them to target pathogens at multiple cellular sites. Beyond their strong antibacterial properties, such as Brassica juncea (BjCHI1) [72], antifungal and insecticidal activities [73]. Chitinases have been explored for antitumor and immunomodulatory applications [74], as their ability to degrade glycan-containing structures can trigger immune responses and disrupt tumor cell integrity.

PR-5 proteins (thaumatin-like proteins)

PR 5 proteins, also called thaumatin-like proteins (TLPs), are small (~20–26 kDa) defense proteins stabilized by multiple disulfide bonds that adopt a conserved β fold related to the intensely sweet thaumatin protein. They accumulate in the apoplast and vacuole, where they interact with invading pathogens, displaying robust antifungal activity. This is achieved through mechanisms that include β-glucan binding and membrane permeabilization, contributing to the disruption of pathogen cell integrity. In addition to their well established role in plant innate immunity, several studies indicate broader bioactivities; specific plant TLP (i.e., Osmotin; Nicotiana tabacum) has been acting as a plant sentinel and has possible functional agonist of mammalian adiponectin, potentially offering therapeutic value for obesity, diabetes, and related metabolic and inflammatory disorders [75,76].

PR-6 proteins (protease inhibitors)

PR 6 proteins comprise plant serine protease inhibitors such as Kunitz-type inhibitors (KTIs) and Bowman-Birk inhibitors (BBIs), typically small proteins of 8–25 kDa often stabilized by disulfide bonds and highly abundant in seeds

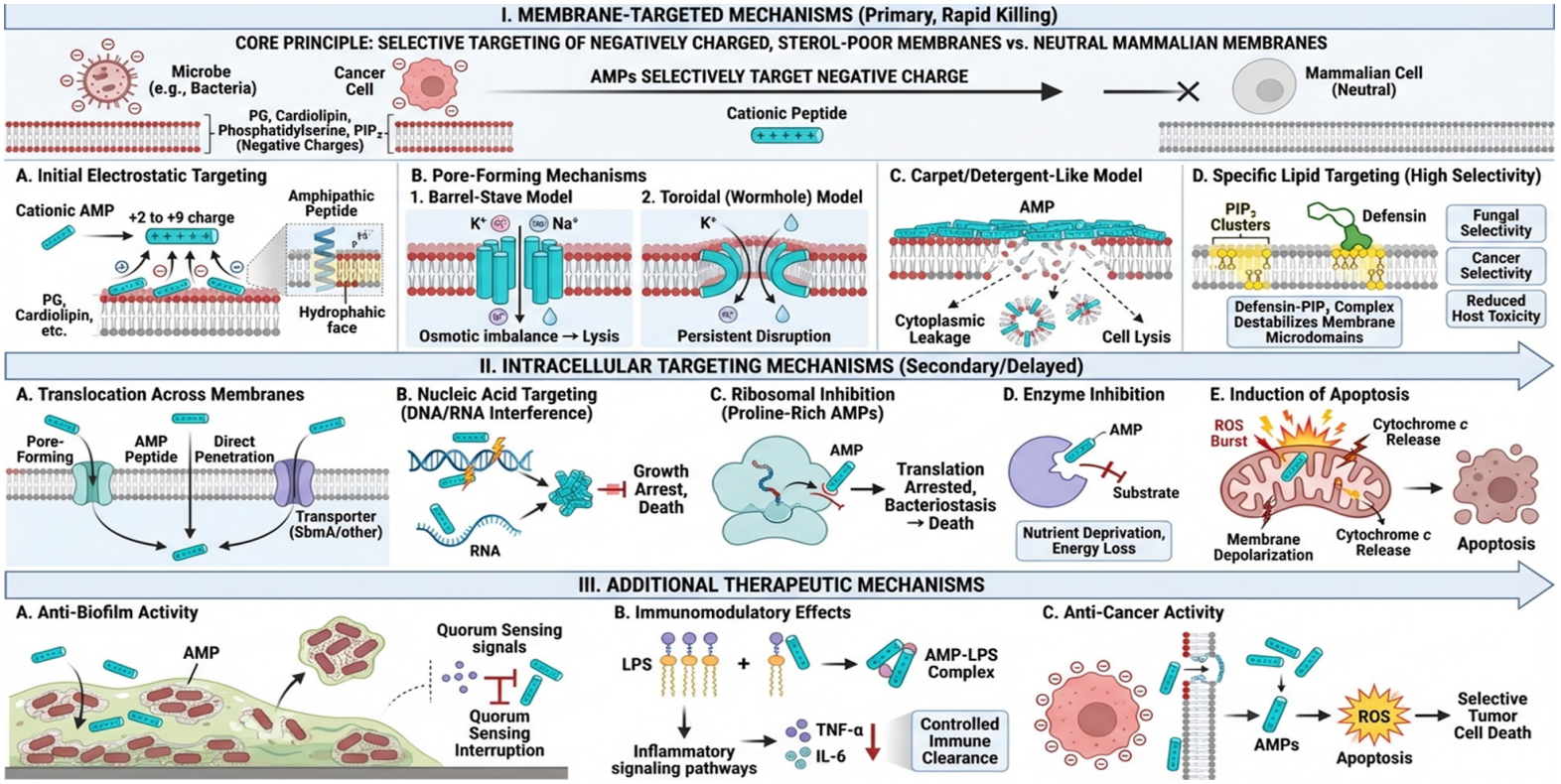

Figure 4: Possible mechanism of action of AMPs/ACPs as therapeutic agents: Conceptual overview of dominant peptide–membrane interactions, including electrostatic binding, pore formation, and membrane destabilization, which underlie antimicrobial and cytotoxic activity. Additional mechanisms such as intracellular targeting, reactive oxygen species induction, and immunomodulatory effects are described in the text and may vary among peptide families.

and storage tissues. These inhibitors block the activity of pathogenic and digestive serine proteases, thereby disrupting protease-mediated processes in microbes and pests and contributing to innate defense. Beyond their plant defensive roles, substantial evidence supports medicinal bioactivity of these inhibitors: soybean-derived BBIs and KTIs have been shown to modulate cell signaling and inhibit proliferation in various cancer models, and they also exhibit anti-inflammatory effects by targeting proteases involved in immune pathways, suggesting utility in inflammatory and tumor contexts [77-79].

Limitations, mitigation strategies, and therapeutic relevance

Plant pathogenesis-related (PR) proteins (PR1–PR6) possess intrinsic antimicrobial, antifungal, and immunomodulatory activities, making them attractive as therapeutic candidates; however, their clinical translation is limited by several factors. Many PR proteins are highly immunogenic or allergenic, having been shown to bind human IgE and elicit hypersensitivity reactions, thereby restricting systemic administration [80,81]. In addition, PR proteins exhibit poor pharmacokinetic properties, including rapid proteolytic degradation, short plasma half-life, and limited tissue penetration, resulting in reduced in vivo efficacy compared with in vitro antimicrobial activity [81,82]. Mechanistic ambiguity further complicates development, particularly for PR1 proteins, whose antifungal or cytotoxic actions have only recently been linked to sterol-binding and membrane disruption, limiting rational therapeutic optimization [70]. To mitigate these challenges, current approaches focus on peptide truncation to remove allergenic epitopes, recombinant expression of minimal active domains, and nanoparticle-based delivery systems to improve stability and targeted activity while reducing immunogenicity [83]. Although no native PR proteins have yet advanced to late-phase clinical trials, PR-derived peptides and engineered variants have been reported to have selective antifungal and anticancer activity in mammalian cell and animal models, supporting their potential as lead scaffolds for next-generation biologics rather than direct therapeutic agents [78].

Antimicrobial and Cytotoxic Peptide Families

Plant antimicrobial and cytotoxic peptides (AMPs and ACPs) are small, typically cysteine-rich proteins ranging from 2 to 10 kDa that serve as first-line defense molecules against a broad spectrum of pathogens, including bacteria, fungi, viruses, and insects. These peptides are highly stable due to extensive disulfide bonding and exhibit amphipathic structures that allow direct interaction with microbial membranes. In addition to antimicrobial activity, many of these peptides display cytotoxicity toward mammalian cancer cells and immunomodulatory properties, highlighting their biomedical potential. AMPs are often constitutively expressed in seeds, roots, leaves, and epidermal tissues, with inducible upregulation under biotic or abiotic stress. Plant AMPs exert their medicinal effects primarily through membrane disruption, pore formation, and induction of oxidative stress. Cationic and amphipathic properties allow selective targeting of negatively charged microbial and tumor cell membranes. Some AMPs additionally interact with intracellular targets, modulate immune responses, and inhibit viral replication, demonstrating multifunctional therapeutic potential (Figure 4) [84,85]. Plant AMPs are classified based on sequence motifs, structural folds, cysteine patterns, and biological activity.

Examples of plant AMPs/ACPs

Defensins

Plant defensins are small (~5 kDa), cysteine-rich peptides of 45–54 amino acids characterized by a conserved cysteine-stabilized α-helix/β-sheet (CSαβ) fold, maintained by four to five disulfide bridges that confer exceptional stability and amphipathicity. These peptides accumulate in multiple tissues, including seeds, leaves, roots, and flowers, and exemplify a versatile class of innate defense molecules. Functionally, plant defensins exhibit broad-spectrum antimicrobial activity against bacteria and fungi, which is attributed to their highly cationic surfaces that preferentially bind to the negatively charged membranes of these microorganisms. This leads to pore formation, membrane depolarization, and subsequent cell death. Beyond antimicrobial effects, defensins have been reported to exert anticancer cytotoxicity by targeting tumor cell membranes and inducing reactive oxygen species (ROS), as well as immunomodulatory effects, including upregulation of chemokine expression in mammalian cells, highlighting their translational potential in biomedicine [86-88].

Thionins

Thionins are small (~5 kDa), highly basic, cysteine-rich peptides stabilized by three to four disulfide bridges and are predominantly found in seeds and vegetative tissues of plants. These peptides exhibit potent antifungal and antibacterial activities through direct interaction with negatively charged microbial membranes, where their amphipathic helices insert into lipid bilayers, leading to membrane permeabilization and cell lysis. In addition to antimicrobial effects, thionins have been reported to display cytotoxicity, in part through similar membrane-disruptive mechanisms and induction of cell death in tumor cells [89,90].

Cyclotides

Cyclotides are a unique class of 28–37 amino acid cyclic peptides characterized by a cystine knot motif in which six cysteine residues form three disulfide bonds, creating exceptional structural stability. They are predominantly found in plant families such as Rubiaceae and Violaceae and accumulate in seeds, leaves, and flowers, where they contribute to innate defense. These peptides exhibit a range of bioactivities, including antimicrobial, antiviral, and anticancer effects, with their cyclic backbone conferring remarkable resistance to proteolytic degradation. Mechanistically, cyclotides interact with lipid bilayers, disrupting membrane integrity and causing leakage and cell death; certain members also interfere with viral entry and replication processes, highlighting their therapeutic potential [91,92].

Hevein-Like Peptides

Hevein-like peptides are a family of small (~4–8 kDa) cysteine-rich plant proteins. They contain conserved chitin-binding domains and are stabilized by multiple disulfide bonds, which gives them a compact and protease-resistant structure. These peptides were first identified in the latex of the rubber tree (Hevea brasiliensis) and are also found in other tissues such as bark and epidermal

layers, where they contribute to innate defense by binding chitin in fungal cell walls and insect exoskeletons, thereby inhibiting pathogen growth and compromising cell wall integrity. In addition to antifungal and broader antimicrobial effects, some hevein-like peptides have been implicated in modulating plant immune signaling and may influence host defense responses through interactions with pathogen enzymes and elicitation of defense pathways [84,93].

Limitation, mitigation strategies, and clinical progress

Despite their strong in vitro antimicrobial and cytotoxic activities, the practical applications of plant-derived AMPs and ACPs remain constrained by several intrinsic limitations. These peptides are highly susceptible to proteolytic degradation in biological and environmental matrices, resulting in short half-lives and poor bioavailability, particularly under in vivo or field conditions [94]. Their cationic and amphipathic nature, while essential for microbial killing, often compromises selectivity, leading to hemolytic activity and cytotoxic effects on mammalian cells at therapeutically relevant concentrations, thereby narrowing the therapeutic window [95]. In addition, peptide activity is frequently reduced in complex biological fluids due to binding to serum proteins or ionic shielding, limiting efficacy beyond controlled in vitro assays. From a production standpoint, chemical synthesis and purification of cysteine-rich plant peptides remain costly, while recombinant expression can suffer from low yields, misfolding, or host toxicity [96]. To address the stability, toxicity, and bioavailability constraints outlined above, current efforts focus on rational peptide engineering and advanced delivery technologies. Chemical modifications such as D-amino acid substitution, peptide cyclization, PEGylation, and sequence optimization substantially improve protease resistance, circulation time, and target selectivity while reducing hemolytic and off-target cytotoxicity. In parallel, encapsulation in nanoparticles, liposomes, or hydrogels protects AMPs/ACPs from degradation and enables controlled or site-specific release, particularly in infection sites or tumor microenvironments [97]. Synergistic combinations with conventional antibiotics further lower effective doses and mitigate toxicity while enhancing efficacy against multidrug-resistant pathogens [98]. Although AMPs have great therapeutic potential, their transition into human clinical trials remains significantly limited compared to animal-derived counterparts. The majority of plant AMP research is currently localized in the preclinical stage or focused on agricultural biotechnology.

Proteases and Protease Inhibitor Families

Plant proteases and protease inhibitor families constitute a diverse and medically significant group of proteins that encompass proteolytic enzymes, antioxidant enzymes, and metabolic enzyme inhibitors, each contributing to distinct therapeutic outcomes. Cysteine proteases such as papain from Carica papaya [99], bromelain from Ananas comosus [100], and ficin from Ficus carica [101] are well-characterized proteolytic enzymes with molecular weights ranging from 20–35 kDa, stabilized by disulfide bridges, and localized primarily in latex, fruit pulp, or vacuoles. These enzymes exhibit anti-inflammatory activity through modulation of cytokine pathways, fibrinolytic activity by cleaving fibrin clots, and anticancer effects by inducing apoptosis and disrupting tumor extracellular matrices [99-101]. Complementing these are plant antioxidant enzymes, including superoxide dismutases, catalases, and peroxidases, which mitigate oxidative stress by converting reactive oxygen species into harmless products. Notable examples, such as horseradish peroxidase (Armoracia rusticana) and Moringa-derived catalases and peroxidases, are reported to have potent radical-scavenging activity, protect cellular components from oxidative damage, and have been investigated for cardiovascular, neuroprotective, and anticancer applications [102-104]. Finally, metabolic enzymes and their natural inhibitors, particularly α-amylase and α-glucosidase inhibitors from Phaseolus vulgaris and Morus alba, contribute to glycemic regulation and obesity management by slowing carbohydrate digestion and reducing postprandial glucose spikes. Across all these protein families, tissue localization, post-translational stabilization, and concentration directly influence bioactivity, with higher accumulation in seeds, fruits, or latex correlating with enhanced therapeutic efficacy [105,106]. Mechanistically, these proteins act by enzymatically cleaving specific substrates, neutralizing reactive oxygen species, and inhibiting key metabolic enzymes.

Limitations, mitigation strategies, and clinical progress

The therapeutic application of plant proteases and protease inhibitor families is constrained by limited specificity, unfavorable pharmacokinetics, and safety concerns. Many plant protease inhibitors exhibit promiscuous inhibition across related protease families, increasing the risk of off-target effects and disruption of essential host proteolytic processes, particularly in coagulation and digestion. Their proteinaceous nature further results in short in vivo half-lives, poor oral bioavailability, and high susceptibility to proteolytic degradation, while immunogenicity and anti-nutritional effects, such as inhibition of human trypsin and chymotrypsin, raise additional safety concerns. Variability in plant-derived preparations and the high cost of producing homogeneous recombinant inhibitors further complicate standardization and scale-up [107]. To overcome these challenges, current strategies emphasize precision engineering and targeted delivery. Structure-based drug design and peptidomimetic approaches are being used to improve specificity and proteolytic stability, while nano-formulations (liposomes, nanoparticles, hydrogels) enhance protection from degradation and extend systemic circulation. Combination therapies that pair protease inhibitors with chemotherapy or immunotherapy reduce required dosages and limit resistance, while targeting compensatory proteolysis pathways (e.g., aggresome–autophagy systems) mitigates adaptive escape mechanisms. Reflecting these advances, several plant-derived or plant-inspired protease inhibitors have progressed into clinical

evaluation, including the Bowman–Birk inhibitor (BBI) for cancer prevention. It has been evaluated in early-phase clinical trials, including Phase II studies for oral leukoplakia, as a potential chemopreventive agent. While these trials have been reported to be safe and have shown some biomarker modulation, definitive evidence for cancer prevention efficacy remains limited [108,109]. Collectively, these developments indicate that while native plant proteases and inhibitors face translational barriers, engineered and selectively delivered derivatives could achieve clinical relevance.

Other Enzymes with Biomedical Applications

Plant-derived enzymes extend beyond proteases to include a variety of catalytic proteins with direct biomedical relevance, such as lipases, phosphatases, and nucleases. These enzymes typically range from 20 to 60 kDa, are stabilized by disulfide bonds or cofactor binding, and are localized in seeds, leaves, or specialized secretory tissues. Lipases from *Coriandrum sativum* and *Elaeis guineensis* exhibit antithrombotic and lipid-lowering activities by catalyzing the hydrolysis of triglycerides and phospholipids, thereby modulating lipid metabolism in vivo. Phosphatases, including acid and alkaline phosphatases from Allium cepa and *Pisum sativum,* have been investigated for their roles in bone health, signal transduction modulation, and detoxification of phosphate-containing xenobiotics. Nucleases, such as ribonucleases and deoxyribonucleases from *Cucumis sativus* and *Ricinus communis*, exhibit antiviral and anticancer properties by degrading nucleic acids in targeted pathogens or tumor cells, inducing apoptosis or inhibiting viral replication. The therapeutic potential of these enzymes is further enhanced by tissue-specific expression, post-translational modifications, and inherent stability under physiological conditions. The biomedical activity is mediated by substrate-specific hydrolysis, modulation of cellular signaling pathways, and induction of programmed cell death, making plant enzymes versatile tools for therapeutic development and industrial biotechnology [110-112].

Storage Proteins and Nutraceutical Bioactive Proteins

Plant storage proteins, traditionally considered nutrient reservoirs, have emerged as important bioactive molecules with medicinal and nutraceutical applications. These proteins, including globulins, albumins, and legumins, generally range from 20 to 70 kDa per subunit and are enriched in seeds, tubers, and nuts, often forming multimeric complexes that confer stability and slow-release properties. Beyond their nutritional value, storage proteins from *Phaseolus vulgaris*, *Glycine max*, and *Arachis hypogaea* exhibit immunomodulatory, antioxidant, and anti-inflammatory effects. Specific subunits can bind carbohydrates or interact with immune receptors, modulating cytokine secretion and enhancing host defense mechanisms. Additionally, certain storage proteins act as natural enzyme inhibitors or carry latent antimicrobial peptides, contributing to protection against microbial contamination and metabolic dysregulation. Their medicinal effects are mediated through interactions with cellular targets, modulation of oxidative stress pathways, and inhibition of pathogenic enzymes, while high stability and abundance in seeds make them amenable to extraction and formulation as nutraceuticals. These features position storage proteins as dual-function molecules that provide both dietary benefits and therapeutic potential [113-115].

Future Perspectives of Bioactive Plant Proteins

Bioactive plant proteins are emerging as a promising frontier in both the pharmaceutical and nutraceutical industries (Table 3). Advances in high-throughput genomics, transcriptomics, and proteomics have enabled rapid identification and functional characterization of novel protein candidates, while recombinant expression systems allow scalable production. Currently, several plant proteins are in preclinical or early clinical phases, including lectin-derived immunotoxins for targeted cancer therapy, enzyme-based antiviral agents, and peptide-based antimicrobial formulations. Nutraceutical applications are also expanding, with seed storage proteins and enzyme inhibitors incorporated into functional foods and dietary supplements for glycemic control, cardiovascular health, and immune support [5].

Despite this progress, several challenges limit broader industrial adoption. Proteins often exhibit limited stability, potential immunogenicity, and batch-to-batch variability, which necessitate careful engineering, formulation, and delivery strategies. Innovations such as protein engineering, glycoengineering, encapsulation, and conjugation to targeting moieties are being applied to improve stability, bioavailability, and specificity. Furthermore, regulatory frameworks for protein-based therapeutics and nutraceuticals are evolving, requiring rigorous safety and efficacy evaluation [116].

Cross-Cutting Strategies for Toxicity Reduction and Functional Preservation

While individual protein families exhibit distinct translational limitations, several cross-cutting biotechnological strategies have emerged to reduce toxicity while maintaining therapeutic bioactivity. These approaches operate at structural, molecular, and formulation levels. At the structural level, protein engineering strategies such as site-directed mutagenesis, domain truncation, and epitope deletion are used to minimize off-target binding and immunogenicity while preserving catalytic and binding domains. Deimmunization algorithms and in-silico epitope mapping further allow rational redesign of plant proteins to reduce T-cell activation potential while preserving functional conformation. Chemical modification approaches, including PEGylation, glycoengineering, and backbone cyclization, improve serum half-life, reduce proteolytic degradation, and mask immunogenic epitopes. PEGylation has been shown to decrease systemic toxicity

and renal clearance while maintaining catalytic efficiency in several protein therapeutics [117].

Advanced formulation systems have become central to translational development. Encapsulation within liposomes, polymeric nanoparticles, micelles, dendrimers, or hydrogel matrices enhances stability, protects against enzymatic degradation, and enables sustained or site-specific release. These delivery systems can reduce systemic exposure while maintaining high local concentrations at disease sites. Targeted delivery represents another critical strategy. Conjugation to monoclonal antibodies, receptor ligands, or cell-penetrating peptides improves cellular selectivity and minimizes collateral toxicity. In RIP-derived immunotoxins, removal of lectin domains combined with antibody-guided targeting significantly enhances therapeutic index [118-120]. Controlled activation systems, including protease-cleavable linkers and tumor microenvironment-responsive designs, further refine safety by ensuring activation occurs predominantly at pathological sites [121].

Collectively, advances in protein engineering, chemical modification, and nanotechnology-based delivery systems are transforming inherently potent yet potentially toxic plant proteins into controllable biologic platforms with improved pharmacokinetics, reduced immunogenicity, and preserved target specificity, thereby enhancing their therapeutic index and translational feasibility. Future progress will depend on genomics-driven discovery of novel protein scaffolds, high-throughput functional screening, precision-targeted and stimulus-responsive delivery strategies, and rational combinatorial approaches with small-molecule drugs. Achieving clinical and industrial impact will require coordinated interdisciplinary collaboration across plant biology, structural biology, pharmacology, and bioengineering, positioning bioactive plant proteins as a sustainable and multifunctional reservoir for next-generation therapeutics and nutraceuticals [122].

Conclusion

Plant-derived proteins represent a versatile and rapidly growing class of bioactive molecules with broad potential for therapeutic and nutraceutical applications. Their inherent specificity, stability, and multifunctional bioactivity provide opportunities for developing novel interventions that complement existing small-molecule drugs. Technological advances in genomics, proteomics, protein engineering, and targeted delivery systems are enabling scalable production and improved safety profiles, bringing many candidates closer to industrial and clinical realization. While challenges such as immunogenicity, stability, and regulatory hurdles remain, ongoing innovations in protein design, formulation, and delivery are mitigating these limitations. The evolutionary diversity of plant protein families offers a rich resource for discovering novel bioactivities and mechanisms, particularly against cancer, infectious diseases, inflammation, and metabolic disorders. Integration of plant protein research with pharmaceutical development, functional foods, and nutraceutical industries promises to expand the pipeline

Table 3: Summary of main plants' bioactive proteins and their reported biological activities, challenges, and possible medicinal translation

Protein Family	Main Indications (Reported/Investigated)	Key Limitations	Furthest Clinical Stage Reported
Lectins	Anticancer (apoptosis induction, immune activation), antiviral (HIV-1, SARS-CoV-2 entry inhibition), immunomodulation, antimicrobial, anti-inflammatory	Off-target glycan binding; mitogenicity; immunogenicity; systemic cytotoxicity; narrow therapeutic index	Early-phase clinical trials (e.g., mistletoe extracts; recombinant griffithsin Phase I as a topical microbicide)
Ribosome-Inactivating Proteins (RIPs)	Anticancer (immunotoxins), antiviral (HIV-1, HBV, HSV), experimental cytotoxic payloads	High intrinsic toxicity (especially Type II RIPs); immunogenicity; delivery challenges; narrow safety margin	Phase I/II trials for RIP-derived immunotoxins; no approved standalone RIP therapeutics
Pathogenesis-Related (PR) Proteins	Antifungal and antibacterial; anticancer (apoptosis induction); anti-inflammatory; metabolic regulation (e.g., BBI chemoprevention)	Allergenicity (IgE binding); limited pharmacokinetics; rapid degradation; incomplete mechanistic clarity (for some PR classes)	Early-phase clinical evaluation for specific derivatives (e.g., Bowman–Birk inhibitor Phase II for oral leukoplakia); most remain preclinical
Antimicrobial / Cytotoxic Peptides (AMPs/ACPs)	Antibacterial; antifungal; antiviral; anticancer (membrane disruption, ROS induction); immunomodulation	Proteolytic instability; hemolysis and off-target cytotoxicity; reduced activity in biological fluids; production cost	Primarily preclinical (in vitro and animal models); no advanced human trials reported
Proteases & Protease Inhibitors	Anti-inflammatory; fibrinolytic; anticancer adjunct; metabolic regulation (glycemic control, weight management)	Limited specificity; off-target proteolysis; short half-life; anti-nutritional effects (for some inhibitors); formulation challenges	Clinical use in nutraceutical contexts; BBI early-phase trials; several enzyme preparations used as supplements rather than regulated drugs
Other Enzymes (Lipases, Phosphatases, Nucleases)	Lipid modulation; bone health; antiviral; anticancer (nucleic acid degradation)	Limited clinical validation; delivery constraints; specificity concerns; immunogenic potential	Preclinical/experimental stage
Storage Proteins / Nutraceutical Proteins	Immunomodulatory; antioxidant; anti-inflammatory; metabolic support	Limited direct therapeutic validation; variability in preparations; regulatory classification challenges	Nutraceutical/functional food applications; not as advanced as regulated biologics

of biologically active agents. Continued interdisciplinary collaboration and strategic investment in research, high-throughput screening, and translational studies will be key to unlocking the full therapeutic and commercial potential of plant-derived bioactive proteins.

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Conflict of interest

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Ethical approval

No specific permits were needed.

Consent to participate

Not applicable.

Availability of data and materials

Not applicable.

Code availability

Not applicable.

CRediT author statement

MEMO: Conceptualization; Data curation; Formal analysis; Validation; Visualization; Writing-original draft; Writing-review and editing.

AID: Formal analysis; Validation; Visualization; Writing-original draft; Writing-review and editing.

RSHO: Data curation; Formal analysis.

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REVIEW ARTICLE

Medicinal Significance, Phytochemical Composition and Pharmacological Properties of *Prunus persica (L.)*: A Review

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Abstract

Peaches, scientifically named *Prunus persica Linn*., are a member of the Rosaceae family and are widely consumed for their nutritional and therapeutic properties.

This review summarizes the phytochemical composition and pharmacological activities of *Prunus persica* based on a structured literature search conducted across PubMed, Scopus, Web of Science, and Google Scholar databases. Evidence indicates that peach contains bioactive compounds, including flavonoids, phenolic compounds, carotenoids, and cyanogenic glycosides, which exhibit antioxidant, anti-inflammatory, anti-microbial, and anti-cancer activities through mechanisms involving oxidative stress modulation, inflammatory pathway inhibition, and regulation of cellular signaling pathways. Most findings are derived in vitro and animal studies, while clinical evidence remains limited. Overall, *Prunus persica* demonstrates promising pharmacological potential; however, further clinical investigations are required to validate therapeutic applications.

Therefore, *Prunus persica* is a versatile medicinal plant with multiple pharmacological importance and may be used therapeutically in the near future. More research is required to examine its comprehensive potential for wellness and health-related to the present sources of bioactive compounds.

Keywords: Peach, *Prunus persica*, Anti-oxidant, Anti-inflammatory, Anti-cancer, Anti-microbial, Anti-allergy

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Introduction

Peach (*Prunus persica L.)* is a member of the Amygdyloideae sub-family of the *Rosaceae* family. It is frequently credited as "*Aaru*" and is extensively consumed globally; in English, it is generally known as "Peach" [1]. According to World Health Organization (WHO), 80% of the population use herbal products and the use of folk medicines is universally followed in cultures. Herbal medicine is the core of Korean Medicine. The literature search reveals that peach has traditionally been widely used for pediatric respiratory illnesses, including the common cold. Author Soo-Dam Kim et al. conducted a literature review of Herbal Medicine to evaluate the efficacy and safety of use of Peach for the common cold. Their findings concluded that significant improvement was observed by the use of the Herbal Medicine of Peach alone, and overall symptoms were improved compared to Conventional Medicine [2]. Since the 17th century, peaches have been grown throughout the United States. They are native to China. Fruits and vegetables are the most widely used products of all the horticulture crops [3]. They are consumed raw or little processed because their nutrients and chemicals promote wellness [4]. A popular fruit enjoyed all over the world, peaches have been a part of the human being's diet for ages.

Peaches are a staple food in the human diet and can be consumed fresh, dried, or frozen. It has a significant role in human nutrition. It is the most popularly consumed fruit in the world due to its flavor and taste and its importance to economics and nutrition [5].

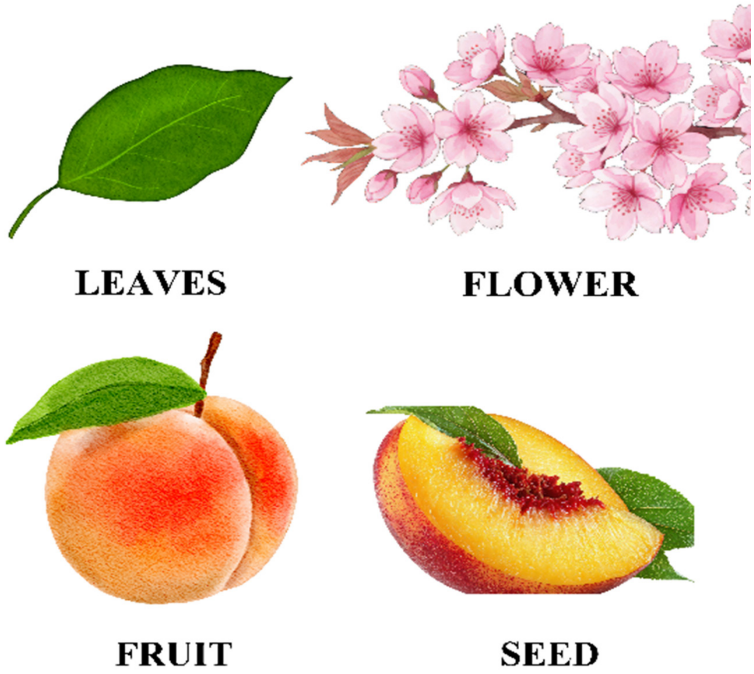


Figure 1: Image of Different Parts of the Peach (Leaves, Flower, Fruit, and Seed)

It is native to temperate zones but originally came from China. [6] A Literature search revealed that trees' Leaves, fruits, and bark have promising pharmacological activities, such as anti-microbial, anti-cancer, anti-inflammatory, anti-oxidant, anti-allergic, and spasmogenic activity.

While the majority of the fruits and seeds of *Prunus* are generally utilized for their anti-oxidant and anti-cancer properties, the green leaves of the plant are also very beneficial as an astringent, demulcent, diuretic, expectorant, febrifuge, laxative, and parasiticide action [7].

This short review focuses on investigating the potential benefits of the pharmacological activities of peach fruit *(Prunus persica L)*. This review was derived from the research published in scientific articles and books over the past 25 years. The central focus was the Phytochemical and Pharmacological properties of the peach due to its significant role in medicinal use. The document emphasizes studies conducted by various national and international researchers; their scientific contributions have enhanced the understanding of the medical significance of Peach in Pharmacological and Phytochemical Properties.

Botanical Description

Plant Morphology

Leaves

The peach plants' leaves are flat, alternate to one another, with pinnately vented glands and small stipules [8].

Flowers

In early spring, peach blossoms bloom long before the leaves. They are single or united, bisexual, pink in color, and pubescent on the outside of the sepals; the petals and stamens are inserted together with the petals into the sepal tube [9].

Fruit

The fruits, also called nectarine is a smooth-skinned cultivator of *Prunus persica* distinguished by the absence of fruit pubescence. The yellow or white pulp covers the seeds, which have a hard shell and a delicate scent. The fruits ripen in August-September [10].

Seeds

Peaches are stone fruits (seeds). Their single, large grain is reddish brown, oval-shaped, and surrounded by wood-like scales.

Method

This review was conducted following a structured literature search strategy to ensure transparency and reproducibility. Scientific databases, including PubMed, Scopus, Web of Science, Google Scholar, and Science Direct, were systematically searched for relevant studies between 2000 and 2026. The search was performed using a combination of keywords such as *Prunus persica*, peach phytochemistry, peach pharmacological activity, antioxidant activity of peach, anti-inflammatory properties of peach, and peach bioactive compounds.

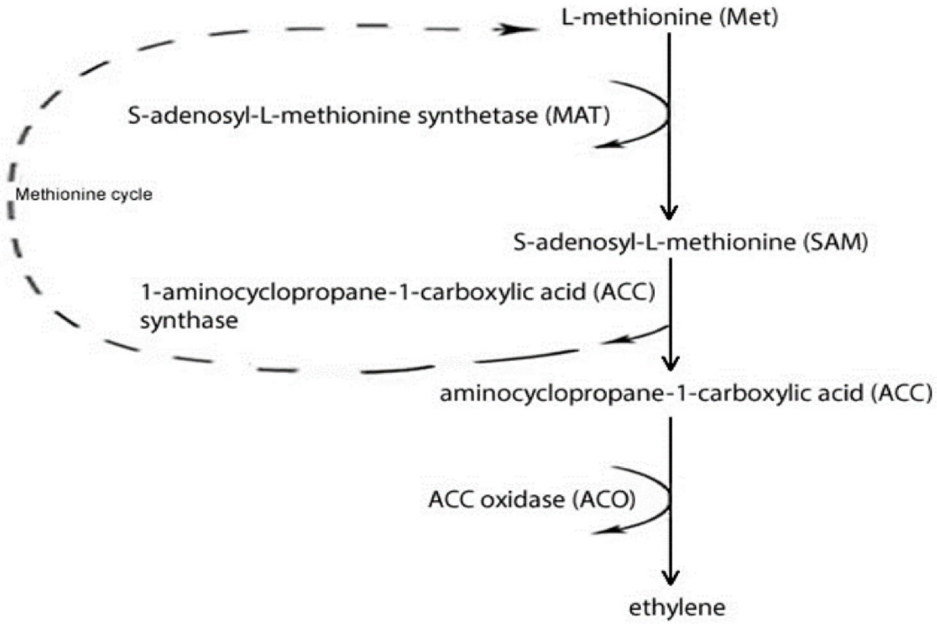


Figure 2: Ethylene Biosynthesis in Peach [11].

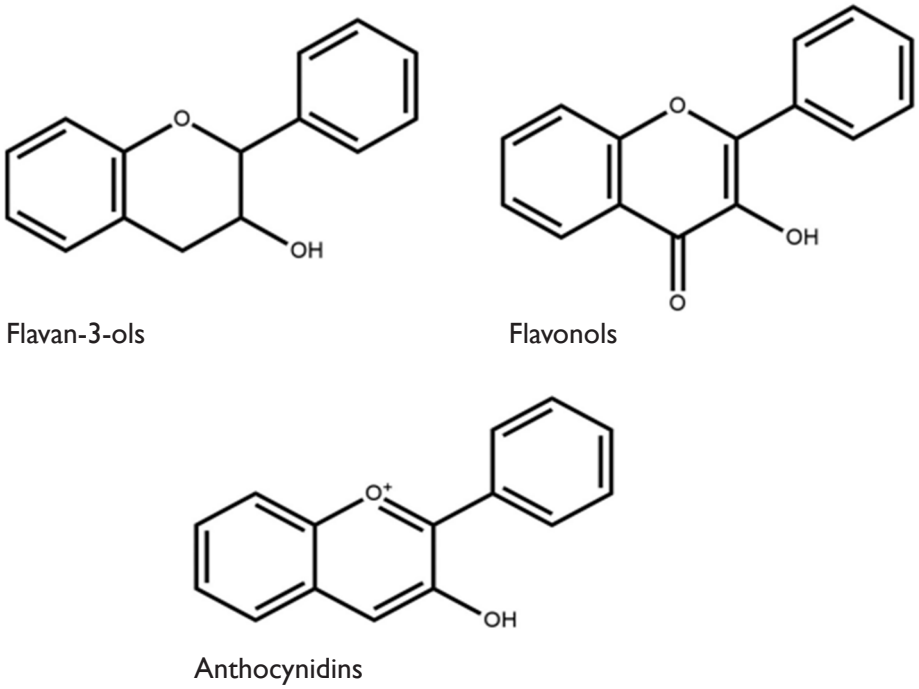


Figure 3: Chemical Structure of Flavanoid classes detected in Peach [26].

The inclusion criteria include peer-reviewed research articles, experimental in vitro, in vivo, or clinical studies, and studies reporting phytochemical or pharmacological properties.

The exclusion criteria follow non-scientific reports, conference abstracts without full data, duplicate studies, and studies unrelated to medicinal properties.

Data extraction focused on phytochemical constituents, pharmacological mechanisms, experimental models, and reported biological effects.

Molecular Mechanism of Peach

Peach *(Prunus persica)* molecular mechanisms are inherently complex and of biological interest as it serves as a model organism of the *Rosaceae* family due to its relatively small, 227.4 Mb haploid genome, which allows the study of the genetic and epigenetic factors determining its developmental process [11]. Peach is characterized as climacteric, and a radically enhanced respiration rate and ethylene synthesis via a dynamic crosstalk of auxin and ethylene occur. In this network, the types of biosynthetic

Table 1: Anti-oxidant activity of *Prunus persica L.*

S.NO.	Author	Chemical Constituents	Mode of Action	Ref
1	Corcoran et al., 2012.	Flavanoids	The existence of conjugated double bonds and carbonyl groups in flavonoids remains stable and delocalized. With numerous hydroxyl groups, these molecules show strong antioxidant and chelating tendencies in vitro.	22
2	Yao, X.-C, et al. 2013.	Hydroxycinnamates (ferulic acid)	It minimizes the effects of free radicals in neuronal cell systems that lead to neuronal impairment. Ferulic acid decreases the oxidative stress on the synaptosomal membrane systems, underlining a rise in hydroxyl and peroxyl radicals.	21
3	Yao, X.-C, et al. 2013.	Peach gum-derived oligosaccharides (PGDO)	The degrees obtained by PGDO and PGDAC demonstrated a high scavenging activity. Hydroxyl radicals (HO•) are highly reactive to all ROS and cause extensive degradation of biomolecules, whereas DPPH• is a stable free radical. HO• and DPPH• have been extensively used to measure anti-oxidants' free radical scavenging activities.	21
4	Lv Q et al., 2021.	Polyphenols	Tartaric and citric acid, which are acidic in nature, cause oxidation, and increased chelation of phenolic compounds with metal ions causes this process.	23
5	Roabaczewskai et al.,	Glutathione	Glutathione contains a highly redox-sensitive sulfhydryl group that affords protection against oxidant damage by anti-oxidant enzymes and other non-enzymatic anti-oxidants. Glutathione can easily be oxidized to Glutathione Disulfide, which can be converted back to GSH in the existence of Nicotinamide Adenine Dinucleotide Phosphate and Flavin Adenine Dinucleotide-dependent Glutathione Reductase.	24
6	Jovanovic et al., 1994	Flavanols (quercitin)	The formation of intramolecular hydrogen bonds directly through the reaction with free radicals, reactions containing enzymes and free radicals. In vivo and in vitro studies have found that quercetin could greatly enhance oxidative damage and suppress NF-κB activation to prevent tissue harm.	25
7	Lv Long et al., 2021	Kaempferol 3-glucoside	It reduces inflammation due to intensive exercise. Thus, Kaempferol exerted a significant anti-oxidation stress effect in the cellular model stimulated by fatty acids.	23

involved are auxin response factors (ARFs) and Aux/IAA genes which are up-regulated by auxin and ethylene respectively [12, 13].

The ethylene biosynthetic pathway involves a three-step enzyme cascade, which is L-methionine to S-adenosyl-methionine (SAM) via methionine adenosyl-transferase (MAT), SAM to 1-aminocyclopropane-1-carboxylate (ACC) via ACC synthase (ACS), followed by ACC oxidase (ACO). Figure 2 explains the Enzyme Cascade Pathway of Peach.

Additional phytohormones are of vital importance: abscisic acid (ABA) peaks at full ripeness, which facilitates fruit softening and sugar accumulation, and gibberellins (GAs) and jasmonates (JAS) tend to retard the ripening process and potentially interact with stress-responsive genes [11, 14].

Phytochemical & Pharmacological Composition of Peach fruit *(Prunus persica L)*

Anti-oxidant Activity

Anti-oxidants can compete with other oxidative substrates by either delaying or inhibiting the reaction to these substrates. These can be enzymes, or compounds such as β-carotene, ascorbic acid, or phenolic compounds [15]. Anti-oxidants have the following properties: reducing potential as electron donors, the ability to delocalize and stabilize the unpaired electron, the ability to chelate metal, and to react with other anti-oxidants [15]. Peaches are rich in natural anti-oxidants such as phenolic compounds flavonoids, and anthocyanins (Table 1).

Phenolic compounds produced from plant metabolism have an anti-oxidant nature due to the presence of hydroxyl groups and a carbonyl group that enhances their anti-oxidant activity [16]. A shown in Figure 3, Flavonoids are molecules with low molecular weight, and their anti-oxidant nature depends on the number and position of hydroxyl groups, the double bond between C-2 and C-3 and 4-oxo group in the C ring, and the catechol group (ortho-dihydroxyl) in the B ring [16-18]. Some flavonoids, such as kaempferol, have an additional hydroxyl group in C-3 that enhances its radical-scavenging activity [16].

Carotenoids are a class of pigments found in all photosynthetic organisms. β-cryptoxanthin and β-carotene are the carotenes present in peaches that can reduce

Table 2: Anti-inflammatory activity of *Prunus persica L.*

S.NO.	Author	Chemical Constituents	Mode of Action	Ref
8	Shin et al., 2010.	Quercetin & Catechin	It controls calcium influx and NF-kB signaling pathways.	30
9	Shin et al., 2010.	Persicaside	The substance blocks the activity of both inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes to reduce nitric oxide (NO) and prostaglandin E2 (PGE2) production.	30
10	Seo et al., 2020.	Cyanogenic glycosides: amygdalin and prunasin	According to Western Blot and reverse transcription polymerase chain reaction analysis, PPB down-regulated the expression of diverse evidence of pro-inflammatory enzymes, including nitric oxide synthase and cyclooxygenase.	31
11	Elshamy et al., 2019.	Flavonoids	Both naringenin and apigenin glycosides have an anti-inflammatory effect on both phases of carrageenan-induced edema. The early phase is linked to changes in vascular permeability, and the late phase with increased synthesis of prostaglandins.	32

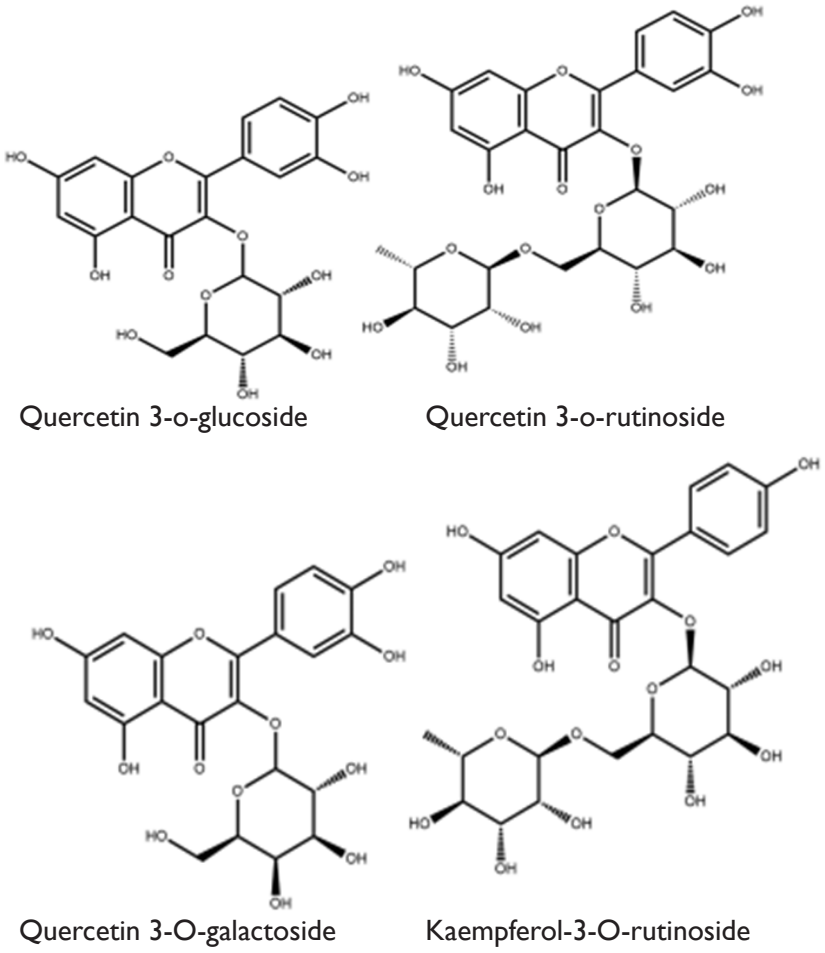


Figure 4: Chemical Structure of Phenolic Compounds reported in Peach [26].

chronic and degenerative diseases, including diseases triggered by reactive oxygen species (ROS) [19, 20].

The anti-oxidant assays analyzed peach gum-derived oligosaccharides' (PGDO) free radical-scavenging ability. The PGDO has shown positive results for both hydroxyl radical-scavenging activity and DPPH radical-scavenging activity [21].

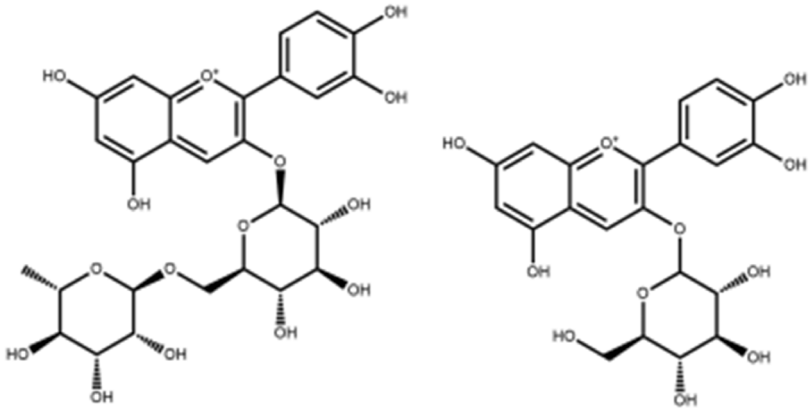
Although multiple studies demonstrate the antioxidant potential of *Prunus persica*, most evidence originates from in vitro assays, which may not accurately represent physiological conditions. Variations in extraction methods and cultivar differences also contribute to inconsistent findings across studies. Therefore, standardized experimental designs and clinical validation are necessary.

Anti-inflammatory Activity

Inflammation is a pathophysiological phenomenon of the human body featuring redness, edema, fever, aches, and functional loss that can be due to pathogenic infection or tissue damage [27, 28]. This natural response occurs when

Table 3: Anti-inflammatory activity of *Prunus persica L.*

S.NO.	Author	Chemical Constituents	Mode of Action	Ref
12	Noratto et al., 2014	Polyphenols (*cyaniding 3β-glucoside, quercetin 3β-rutinoside, chlorogenic acid, neochlorogenic acid, quercetin 3β- glucoside and catechin derivatives*)	A literature search shows that polyphenolics isolated from peaches suppress the number of tumors formed and the MDA-MB-435 breast cancer cells' metastasis along with the angiogenesis process.	[33]
13	Heo et al., 2001	Flavonoids Anthocyanins	They demonstrated a decrease in tumor rate, tumor number, and tumor latency period. UVB causes keratinocyte DNA damage both in p53+ and p53—cells, forming cyclo-butane dimers and photoproducts. A part of this DNA damage is now known to be rectified through enzymatic pathways catalyzed through endonucleases. The aforementioned open lesions leave undemolished mutations in the target genes and trigger skin cancer.	[38]
14	Hernández et al., 2018	Peptides	Peptides can bind to target receptors on the tumor cell surface and exert effects that suppress growth. They may also stimulate signal-transduction routes that result in the death of cancer cells through apoptosis. These peptides can also inhibit protein Synthesis and, therefore, slow down or halt the growth of cancer cells.	[39]
15	Fukuda et al., 2003	Glycosides	A literature search revealed that Glycosides in *Prunus persica* showed EBV-EA activation without exhibiting cytotoxicity and had a relatively strong anti-tumor-promoting effect	[40]
16	Koyu et al., 2020	Phenols	They suppress tumor cells and promote apoptosis by controlling cell cycle arrest, signal transduction pathways, carcinogen metabolism and enzymes, oncogene gene expression, and angiogenesis.	[41]
17	Shen et al., 2017	Flavanols and Fatty Acids (ethanol extract)	These constituents can reduce HepG2 cell viability and suppress cell and tumor growth by inhibiting migration through cell cycle arrest. It can also exert anti-tumor effects on freshly implanted HepG2 cells derived in vivo tumors. This data shows that the possible antecedent of HepG2 growth inhibition by *Prunus persica* extract was cell cycle arrest and migration inhibition.	[42]



Cyanidin-3-O-rutinoside Cyanidin-3-O-glucoside

Figure 5: Chemical Structure of Anthocyanins reported in Peach [26].

the body's immune system notices invaders like microbes and triggers and produces an inflammatory response. Any disruption in the innate immune system can lead to an inflated response, and inflammation occurs, which can lead to more damage and chronic disorders.

Phenolic compounds (Figure 4) have been suggested as a replacement for preventing or treating chronic inflammatory diseases with their anti-inflammatory and immunomodulatory activity [27, 28] as described in Table 2. Among them, flavonoids are the most associated with this property [27, 28]. Examples include quercetin, kaempferol, and rutin, which have been studied in animal models and cell cultures. Rutin was effective in treating arthritis, and quercetin was shown to reduce edema in animal models [28, 29]. Flavonoids can lower inflammation by scavenging free radicals, preventing cell damage, and regulating pro-

Table 4: Anti-microbial activity of *Prunus persica L.*

S.NO.	Author	Chemical Constituents	Mode of Action	Ref
18	Atef, Nagwa M., et al., 2019	Flavonoids, Phenolics, Sterols	The leaves exhibit anti-bacterial activity, displaying potent effects against both Gram-negative (*E. coli, P. aeruginosa*) bacteria and Gram-positive (*B. subtilis, S. aureus*).	45
19	Abderrahmane Mokrani., 2011	Phenol	The compounds counteract reactive oxygen species (ROS) generated during fruit ripening, providing protection against oxidative damage. The anti-oxidant power is linked to the total phenolic content, which varies among different peach varieties and ripening stages.	46

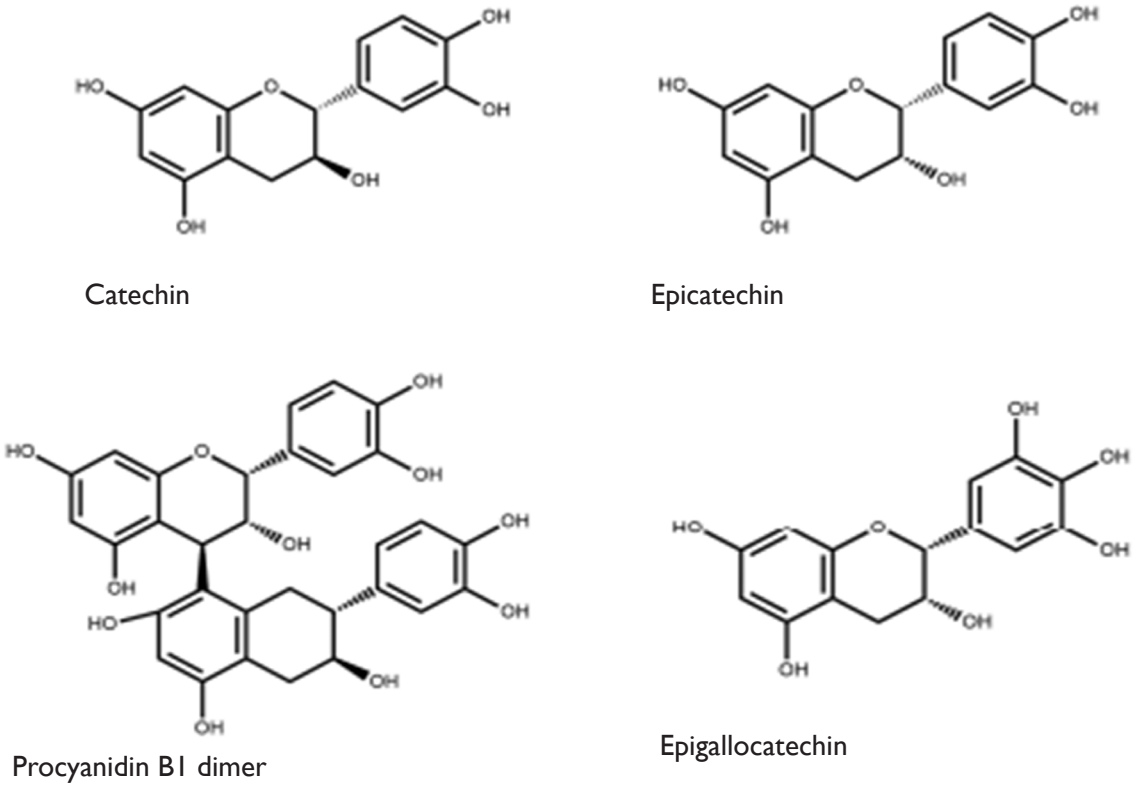


Figure 5: Chemical Structure of Anthocyanins reported in Peach [26].

inflammatory enzymes. They can also downregulate pro-inflammatory gene expression, thereby inhibiting the production of arachidonic acid, prostaglandins, leucotrienes, and NO, which are key mediators of the inflammatory response [27, 28]. Quercetin inhibits the liberation of arachidonic acid, which is the precursor of inflammation, by blocking the phospholipase A2 enzyme in human neutrophils [27,28].

Peach peel and fresh pulp-derived products inhibit inflammation mediators such as tumor necrosis factor-α (TNF-α), interleukin IL-1β and nuclear factor κB (NF-κB) [19, 28] . Mast cells have been targeted in in-vivo models and showed anti-allergic inflammatory effects of the fruit extract [30].

Although multiple studies demonstrate the anti-inflammatory activity of *Prunus persica*, most evidence originates from in vitro assays, which may not accurately represent physiological conditions. Variations in extraction methods and cultivar differences also contribute to inconsistent findings across studies. Therefore, standardized experimental designs and clinical validation are necessary.

Anti-Cancer Activity

Tumors are abnormal cells that grow and multiply abnormally. Normally, the growth and proliferation of cells in mammals are regulated by a cycle, and any alteration can cause cell abnormality [33, 34]. Transformed cells grow in a stroma, controlled by tumor cells. Endothelial cells move to new areas and form blood vessels called angiogenesis, as this process supplies nutrients and oxygen to the carcinoma [33]. Angiogenesis transitions to metastasis and spreads to other parts of the body [33, 35].

As described in Table 3, Phenolic compounds show their ability to stop cancer development while slowing down the progression of cancer tumors. Flavonoids block carcinogenic factor metabolism by inhibiting cytochrome P450 enzymes as well as phase I enzymes [36]. Studies have established critical therapeutic effects for flavonoids parallel to non-flavonoids in breast cancer care. The breast tumor cells receive antitumor benefits from flavan-3-ols, flavonols, anthocyanins, and hydroxycinnamate acid derivatives through chlorogenic acid, neoclorogenic acid, epicatechin, catechin, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, and cyanidin-3-O-glucoside (Figures 5 and 6) [33, 37].

A study using yellow-fleshed peach "Rich Lady" extracts in female mice showed promising results in inhibiting tumor growth, reducing neo-angiogenesis, and inhibiting the expression of certain MMPs genes [33]. The Literature search investigated in another study the cytotoxic effects of peach extracts against estrogen-dependent MCF-7, together with estrogen-independent MDA-MB-435 cancer cell lines [37].

Although multiple studies demonstrate the anticancer activity of *Prunus persica*, most evidence originates from in vitro assays, which may not accurately represent physiological conditions. Variations in extraction methods and cultivar differences also contribute to inconsistent findings across studies. Therefore, standardized experimental designs and clinical validation are necessary.

Anti-Microbial Activity

Plants use an anti-microbial nature as a part of their defense mechanism and have many anti-microbial components. Phytochemicals in the peach confer strong anti-microbial activity (Table 4). Peachtree fruit and branches produce gum with complex structures in response to any microbial attack or mechanical injury [21]. Different studies have reported the anti-microbial efficacy of peach extracts against some pathogens.

A methanolic extract was prepared by implementing the disc diffusion method on both gram-positive bacteria and gram-negative bacteria specimens. The extract demonstrated both blocking behavior along with inhibition zones for *S. aureus, B. subtili, S. epidermidis,* and *Klebsiella pneumoniae* against the bacteria strains tested. The bioactive compounds consisting of β-sitosterol and phenolic compounds together with organic acids and others are responsible for this effect. [43].

Similarly, ethyl acetate extract from peach leaves showed significant anti-bacterial activity against E. coli, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Results demonstrated that anti-microbial activity is due to the presence of triterpenoids in ethyl acetate extract [44].

Although multiple studies demonstrate the anti-microbial activity of *Prunus persica*, most evidence originates from in vitro assays, which may not accurately represent physiological conditions. Variations in extraction methods and cultivar differences also contribute to inconsistent findings across studies. Therefore, standardized experimental designs and clinical validation are necessary.

Conclusion

Peach (*Prunus persica L.)* has been observed to possess several pharmacological activities, all of which have potential health implications. Based on nutrient composition, flavonoids are known to have anti-oxidant effects which reduce peach's ability to form free radicals and perform oxidative damage to cells. The anti-inflammatory properties of the fruit are due to the presence of quercetin, catechin, persic acid, and cyanogenic glycosides that reduce inflammation and inflammation-related enzymes.

Peach also shows significant anti-cancer effects, where components such as polyphenols, flavonoids, peptides, glycosides, and phenols have been reported to show effects, including inhibition of tumors, causing cell destruction, and hindering metastases. Also, the antibiotic effect of the fruit against different pathogens is established because of flavonoids, phenolics, and sterols. The diverse pharmacological effects of Peach suggest potential applications in health promotions.

Future research should focus on identifying and describing other bioactive compounds present in peaches and on their mode of action. Other researchers indicate that an enhanced understanding of how peach phytochemicals interact with other therapeutic compounds may lead to the discovery of new therapies.

Further studies on the phytochemical content of peaches are important for discovering additional uses in treatment and gaining further insight into peaches' beneficial properties. This Literature search reveals that other bioactive components of peaches can be used as drugs and supplements to unlock the maximum therapeutic potential of peaches and support disease risk reduction. Despite promising pharmacological findings, most available evidence is limited to pre-clinical studies. Well-designed clinical trials are required before therapeutic recommendations can be established.

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Conflict of interest

The authors declare that they have no competing interests that can influence the work reported in this work.

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REVIEW ARTICLE

Wound Healing and Pharmacological Properties of the Siddha Formulation *Mathan Thailam*: A Narrative Review

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Abstract

Wound healing remains a significant concern in clinical practice, especially in chronic and infected wounds, where delayed healing can lead to serious complications and increased healthcare costs. The rising problem of antimicrobial resistance has encouraged a renewed interest in traditional systems of medicine as alternative options for wound care, even though conventional treatments are often effective. The Siddha system of medicine offers several medicated oils for external application, one of which is *Mathan Thailam*, a formulation that has been used for a long time in the treatment of burns, infected wounds, and ulcers. In this review, a comprehensive literature search was conducted using PubMed and Google Scholar with keywords including "*Mathan Thailam*," "wound healing," and "Siddha medicine." Studies published in English up to 2025 were considered, including preclinical experiments, traditional Siddha texts, and selected case reports; studies lacking relevant data were excluded. Evidence was synthesized narratively to evaluate pharmacological properties, mechanisms of action, and clinical outcomes. *Mathan Thailam* formulation is prepared using Datura leaf extract, purified copper sulfate, and coconut oil. Earlier reports indicate that this preparation shows antimicrobial, anti-inflammatory, antioxidant, and wound-healing effects. Preclinical data suggest that topical application of *Mathan Thailam* accelerates wound healing through increased collagen deposition, greater tensile strength, and improved epithelialization. It is thought that these effects are linked to changes in inflammatory mediators(TNF-α, IL-10) as well as better antioxidant defense systems and angiogenesis. Clinical evidence however remains limited and is largely confined to observational case reports documenting symptomatic improvement and reduction in wound size in chronic ulcers. In the absence of controlled clinical trials, such observations should be interpreted with caution. Overall, the existing literature indicates that *Mathan Thailam* possesses wound-healing potential that is supported mainly by preclinical data. Further well-designed clinical studies are required to establish its safety, efficacy, and therapeutic relevance within evidence-based wound-care practice.

Keywords: *Mathan Thailam*; Siddha medicine; wound healing; pharmacological attributes.

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Introduction

Hemostasis, inflammation, proliferation and remodeling are the four overlapping stages of wound healing a complicated and tightly controlled biological process that restores the structural and functional integrity of damaged tissue [1]. After an injury clot formation stops blood loss and makes it easier for growth factors to be released which attracts immune cells to the wound site. Neutrophils and macrophages infiltrate during the inflammatory phase eliminating necrotic tissue and microbial pollutants to prepare the wound environment for healing. Fibroblast migration, collagen synthesis, angiogenesis and granulation tissue formation take place during the proliferative phase. During the remodeling phase collagen reorganization and tissue strengthening take place[1]. Acute wounds often heal in a predictable amount of time, while chronic and infected wounds frequently show delayed healing and necessitate further therapeutic procedures. A significant clinical and financial burden is associated with chronic wounds, especially in individuals with diabetes mellitus, vascular insufficiency, advanced age, and weakened immune systems [2]. Conventional wound management strategies including antibiotics, antiseptics, and synthetic dressings, are effective in many cases, however rising antimicrobial resistance, side effects from treatment, longer healing times and high healthcare costs have sparked a resurgence of interest in complementary and alternative therapies [2,3].

In these regards, traditional medical systems have their topical preparations that have been long employed to treat wounds and are currently receiving a new scientific focus based on their ability to exhibit a multi-target phenotype in wound-healing mechanisms. For wound care, traditional medical systems like Ayurveda, Siddha, and Traditional Chinese Medicine have traditionally used natural products, medicinal oils, and preparations based on plants and minerals [4]. Numerous of these formulations show several biological activities related to wound healing such as pro-regenerative, antibacterial, anti-inflammatory and antioxidant properties. In preclinical models, experiments using herbal remedies like *Centella asiatica, Curcuma longa, Calendula officinalis* and honey-based dressings have shown improved wound contraction, collagen deposition, angiogenesis, and epithelialization[5–7]. Similarly, preclinical trials using polyherbal formulations derived from traditional systems have Suggested potential for better granulation tissue development and quicker wound healing suggesting their potential use as adjuncts in wound management [7,8]. The Siddha system of medicine rooted in classical Tamil traditions emphasizes holistic wound care using herbal, mineral, and animal-derived formulations based on tridosha principles. A number of medicated oils (thailams) that are recommended for burns, ulcers, infected wounds and persistent non-healing skin lesions are described in classical Siddha scriptures [9]. Among them, Siddha practice frequently uses formulations like *Mathan Thailam*, Mahamegarajanga Thailam, and associated medicinal oils for external wound care [9–11]. Many Siddha wound-healing compositions are still understudied from a contemporary pharmacological and mechanistic standpoint despite their continued traditional and clinical use. *Datura metel* leaf juice, refined copper sulfate, and coconut oil are the basic ingredients of *Mathan Thailam*, a traditional Siddha herbomineral preparation[10]. Traditionally, it has been utilized topically to treat burns, diabetic ulcers, infected wounds, and cuts [10]. Preliminary experimental studies in animal models have suggested that *Mathan Thailam* may improve wound contraction, epithelialization, collagen deposition and tensile strength in animal wound models possibly by modifying inflammatory cytokines, antioxidant defense mechanisms, angiogenic signaling and antimicrobial activity, according to preliminary experimental studies[11]. However the available evidence remains predominantly preclinical, and clinical data are largely limited to observational case reports rather than controlled clinical trials. Although several Siddha medicated oils are indicated for wound management, *Mathan Thailam* meritsfocused scientific evaluation due to its distinctive herbomineral composition, incorporation of copper ions with established roles in angiogenesis and tissue remodeling, and preliminaryexperimental observations suggesting potential multi-targeted effects relevant to wound-healing activity. At the same time, significant gaps remain regarding its exact mechanism of action, safety in the long-term, especially its standardization in terms of Datura-derived alkaloids, consistency between batches, and the strength of the overall clinical evidence on its therapeutic use. Thus, the purpose of this narrative review is to critically summarize and review the existing classical Siddha literature, experimental studies and documented clinical results regarding *Mathan Thailam*. This review combines available information on the phytochemical and mineral makeup, pharmacological properties, and proposed mechanisms of action of *Mathan Thailam* to relate traditional Siddha knowledge to contemporary science, while also highlighting gaps in evidence, safety concerns, and directions for future research. To have a better perspective on the therapeutic merit of *Mathan Thailam*, it can be first useful to explore the history and traditional context of its usage in the Siddha medicine or system. The traditional background gives this conceptual framework with which its composition, method of preparation and clinical application are informed. Thus, the next section is the summary of classical descriptions and conventional use of *Mathan Thailam* and then pharmacological properties and chemical composition are presented.

Traditional Background of *Mathan Thailam*

Wound care was a well-known part of the Siddha system of medicine, in which internal and external methods of treatment are used to recover the integrity of the tissue and the humoral balance. Classical Siddha texts describes several medicated oils preparartions (*thailams*) that are traditionally indicated for infected wounds, ulcers, burns, and inflammatory skin disorders. These are mainly topical preparations and are aimed at cleaning wounds, decreasing inflammation, preventing infection and promoting tissue regeneration [9]. *Mathan Thailam* (also known as Mattan Thailam or Pachai Ennai) is a well-recognized Siddha medicated oil used traditionally for infected wounds, chronic ulcers, diabetic wounds, and inflammatory skin conditions, as described in classical texts and the *Siddha Formulary of India* [9,10]. Traditional Siddha wound care involves a step-by-step approach that engages wound cleansing, hemostasis, application of medicated oils traditionally signs and signs that symbolize treating the inflammatory and infectious issues, and subsequent application of supportive interventions that are supposed to assist the granulation and epithelialization. These are practices of *Aruvai Maruthuvam*, the surgical and para-surgical branch of Siddha medicine which focuses on repairing the health of tissues whilst balancing *Vatham*, *Pitham*, and *Kabam* [9].

Mathan Thailam is a classical herbomineral formulation prepared using fresh leaf juice of *Datura metel* L., purified copper sulphate (*thurusu*), and coconut oil. The formulation procedure and the compositional information are explained in Siddha pharmacopeial texts and have been standardized in contemporary research in line with the procedures as stipulated in the *Siddha Formulary of India* [10]. Contemporary standardization research has shown that the formulation can be reproduced and has given physicochemical, phytochemical and analytical validation to the quality and consistency of the formulation [10]. It is still found in classical Siddha texts and in contemporary Siddha medical practice, which points to the fact that it

still belongs to the historical wound care methods. Even though its conventional use has been informed mainly by the practical observations and written traditions, some new studies have initiated preliminary systematic evaluation of its composition, short-term safety, and its probable pharmacological properties. Such initiatives offer a basis of establishing the relationship between the traditional knowledge of Siddha and modern scientific testing and go to the next level of researching *Mathan Thailam* as a possible topical agent in wound care [9,10]. Although the usual literature explains how *Mathan Thailam* is prepared and when it is used, a scientific review of the matter needs to have a clear look at the chemical and mineral content used. This is necessary to conduct such correlations whenever no purely biological mechanism is involved and to maintain the standard and safety of formulations by use of such compositional analysis. In this line, the following part will cover the phytochemical and mineral profile of *Mathan Thailam* with reference to the modern analytical researches.

Phytochemical and Mineral Composition of *Mathan Thailam*

Mathan Thailam is a classical Siddha herbomineral preparation made by processing fresh leaf juice of *Datura metel* L. with purified copper sulphate ($CuSO_4 \cdot 5H_2O$) in coconut oil, based on procedures in the *Siddha Formulary of India* and tested in modern standardization studies [10]. It is important to know the chemical composition of this formulation as it helps in determining the therapeutic actions of use reported and defining a scientific basis of its use in the traditional wounds treatment. Traditional indicators of formulation completion, such as disappearance of froth and characteristic changes in colour and odour, are used to ensure effective extraction of lipophilic phytoconstituents into the oil medium. The chemical characterization of the standardized formulation indicates that several classes of bioactive phytochemicals, primarily derived from *Datura metel* leaves, are retained in the final oil.The major constituents have been identified to be alkaloids, saponins, coumarins, steroids/phytosterols, triterpenoids, phenolics and flavonoids, through qualitative phytochemical screening and chromatographic analyses [10].GC–MS profiling further indicates that coconut oil forms a fatty-acid-rich lipid base, dominated by lauric, capric, palmitic, oleic, and stearic acids, which may facilitate skin penetration and offer potential antimicrobial and barrier-protective benefits[10]. Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP–OES) elemental analysis shows that the finished formulation contains low, measurable amounts of copper (around 33 ppm), reflecting careful control of mineral incorporation during processing [10] . Notably, the heavy metals like lead, arsenic and mercury were not found, which means that purification and standardization measures are effective in reducing heavy metal contamination. This observation reflects Siddha pharmacopeial principles, which focus on the safe and controlled use of mineral components through proper detoxification [10].

The phytochemical profile identified in *Mathan Thailamis* consistent with literature on *Datura metel*, which is known to contain tropane alkaloids (e.g., atropine, scopolamine, hyoscyamine), flavonoids, saponins, and other secondary metabolites that have been reported to have biological activities [12,13]. It should also be noted though that most mechanistic understanding is based on the extrapolation of studies on individual plant constituents as opposed to the entire formulation. Thus, the combination of plant compounds, a lipid carrier, and copper ionspresents a profile that may be relevant to wound healing, , although we still lack clear evidence of how it works at the overall formulation level.Comprehensively, physicochemical testing, chromatographic fingerprinting (HPTLC, GC–MS), and elemental analysis (ICP–OES) provide a reliable control system of quality control and safety assessment of *Mathan Thailam* [10].These analytical results confirm the formulation's consistency, but they do not demonstrate its clinical effectiveness, highlighting the need for further controlled pharmacological and clinical studies. These phytochemical classes and mineral elements have been identified, which allows postulating the potential biochemical explanations of the processes whereby *Mathan Thailam* can have an effect on various stages of wound healing. Compositional determination of these findings is therefore used as a basis to study the pharmacological properties of the formulation which are discussed in the next section.

Pharmacological Basis of Wound Healing Activity

Due to its complicated phytochemical and mineral structure, *Mathan Thailam* has been recommended to have various biological activities in wound healing. The subsequent subsections present in short, the experimental and non-experimental evidence that exists on its anti-inflammatory, antimicrobial and tissue-regenerative effects as it pertains to the various phases of wound healing.

Anti-inflammatory and Antioxidant Mechanisms

The wound healing process commences with an inflammatory phase, where immune cells such as neutrophils and macrophages work to remove dead tissue and fight off invading pathogens. In this phase, reactive oxygen species (ROS) are produced to counteract infections and initiate repair mechanisms. Nevertheless, chronic inflammation and high reactive oxygen species production can harm healthy tissue and hinder epithelial regeneration and collagen synthesis. Components of *Mathan Thailam* have been reported in related studies to potentially mitigate these effects and help restore balance [14]. The activity of *Datura metel* leaves is attributed to its phytochemical constituents. Supercritical CO_2 extracts of D. metel have been reported the ability to diminish nitric oxide production, inhibit MMP-2 activity, and support fibroblast migration crucial activities that may modulate inflammation and facilitate prompt wound healing [14]. Copper, a mineral component of *Mathan Thailam* is included in the formulation as described in classical

Siddha texts and confirmed by modern standardization studies [10]. The particular effects of *Mathan Thailam* on oxidative stress, inflammatory cytokines, epithelialization, and collagen maturation have not been experimentally verified in these investigations despite the fact that copper is known in general biology to boost antioxidant enzymes like superoxide dismutase [15,16]. Along with the regulation of inflammation and oxidative stress, the control of the microbial colonization is also crucial in the process of the organized wound healing. This factor is specifically significant in chronic and diabetic wounds whereby infection can drastically retard healing. The possible antimicrobial effects of *Mathan Thailam* are thus addressed under the following subsection.

Antimicrobial and Antiseptic Properties

Microbial colonization and wound infection are established factors that impede healing, mostly due to prolonged inflammation, heightened oxidative stress, and advancing tissue damage. The antibacterial and antiseptic efficacy of *Mathan Thailam* is proposed to stem from the synergistic effects of its phytoconstituents and mineral ingredients, rather than from a singular active principle[10,17] . In vitro investigations have reported that methanolic extracts of *Datura metel* exhibit extensive antibacterial activity against both Gram-positive and Gram-negative bacteria, thus aligning with its traditional application in infected wounds [18,19]. Limited preclinical investigations have further suggested antibacterial activity of *Mathan Thailam* against *Escherichia coli,* including resistance-modifying effects [20]. Nevertheless, the antibacterial activity of the entire formulation has not been thoroughly verified using standardized assays or comparison controls and these conclusions are based on laboratory-level research. The majority of the clinical data pertaining to *Mathan Thailam*'s antibacterial activity is found in observational studies and individual case reports. In addition to reported granulation tissue production and epithelialization, observational investigations detailing its topical treatment in diabetic foot ulcers have described decreased clinical indicators of infection [21]. However, the interpretability of these results is limited due to the lack of comparator therapies, standardized outcome measures, and controlled clinical trials. Therefore, strong clinical confirmation is still needed even though preliminary experimental and anecdotal clinical observations point to possible antibacterial advantages. In addition to the potential part in infection control, successful wound healing involves cellular interactions between fibroblast-proliferation, extracellular-matrix-deposition, and angiogenesis. The evidences that are available concerning these tissue-repair processes in the case of *Mathan Thailam* have been summarised in the subsection that follows.

Cellular and Molecular Mechanisms of Tissue Repair: Collagen Synthesis and Angiogenesis

Preliminary experimental observations suggest that *Mathan Thailam* may contribute to tissue repair by creating a wound microenvironment conducive to collagen synthesis and angiogenesis, primarily through modulation of inflammation and oxidative stress. Extracellular matrix (ECM) production and fibroblast activation may be facilitated by reducing prolonged inflammatory responses. This may support the synthesis and deposition of collagen, especially types I and III, which are critical for early wound matrix formation and the development of tensile strength [10,22]. Additionally, preclinical research suggests that bioactive phytochemical-containing wound healing formulations may affect angiogenic processes, including the control of pro-angiogenic growth factors like vascular endothelial growth factor (VEGF). Increased VEGF signaling supports the neovascularization of the wound bed by encouraging endothelial cell migration, proliferation, and capillary sprout creation within the temporary extracellular matrix [23,24]. Nevertheless there is still little direct molecular data supporting these routes for the full *Mathan Thailam* formulation. Granulation tissue production depends on the coordinated interaction of endothelial cells, macrophages, and fibroblasts. Together, fibroblast-derived collagen deposition, macrophage-mediated debris removal, and endothelial reactions to integrin- and growth factor-mediated signaling enhance the regenerating tissue's vascularization and structural structure [25,26]. These procedures give the healing wound mechanical stability and improve the supply of nutrients and oxygen. In order to restore tissue integrity and functional strength during the later stages of wound healing, collagen remodeling and maturation as well as the stability of newly generated microvasculature are crucial. The proliferative and remodeling phases may be supported by *Mathan Thailam*, according to available experimental observations; however these conclusions are mainly based on indirect evidence and extrapolation from related wound-healing studies, highlighting the need for focused mechanistic investigations.

Safety Considerations and Potential Risks of Datura Alkaloids

Mathan Thailam is enriched with fresh leaf juice of *Datura metel L.* which is a medicinal herb known to have biologically active tropane alkaloids including atropine, hyoscyamine, and scopolamine. These substances are commonly known anticholinergic drugs which act through their pharmacological actions by competitive antagonism of muscarinic acetylcholine receptors, both in the peripheral and central nervous systems. The behaviors of this kind lead to a number of the therapeutic properties, but are also thought to explain the toxicological profile of Datura species whenoral intake or systemic intake happens at more significant doses [26,27]. Accidental or even defaulted ingestion of Tropane alkaloid may cause anticholinergic syndrome, the clinical scenario associated with having peripheral symptoms such as tachycardia, mydriasis, dry mucous membranes, urinary retention and hyperthermia as well as central nervous system symptoms such as agitation, delirium, hallucinations, confusion and in the worst case situation, seizure or coma [28–30]. The formulation to be adopted in the case of *Mathan Thailam* is directed solely to topical use and is conventionally made by means of processing of *Datura metel* leaf juice in coconut oil and

Table 1. Major Phytochemical Classes Identified in *Mathan Thailam* and Reported Biological Relevance

S. No.	Phytochemical class	Source component	Reported biological relevance*	Supporting literature
1	Alkaloids	*Datura metel* leaves	Antimicrobial, analgesic; modulation of inflammatory mediators	[10,12]
2	Saponins	*Datura metel* leaves	Surface-active properties; reported role in tissue repair	[13]
3	Coumarins	*Datura metel* leaves	Antioxidant, anti-inflammatory activity	[12]
4	Steroids / phytosterols	*Datura metel*, coconut oil	Membrane stabilization; anti-inflammatory effects	[10]
5	Triterpenoids	*Datura metel* leaves	Reported association with collagen modulation	[13]
6	Phenolics / flavonoids	*Datura metel* leaves	Free radical scavenging; vascular protection	[12,13]

Table 2. Fatty Acid Components Detected in *Mathan Thailam* by GC–MS

S. No.	Compound identified	Chemical class	Probable source	Reference
1	Lauric acid	Saturated fatty acid	Coconut oil	[10]
2	Capric acid	Saturated fatty acid	Coconut oil	[10]
3	Palmitic acid	Saturated fatty acid	Coconut oil	[10]
4	Oleic acid	Monounsaturated fatty acid	Coconut oil	[10]
5	Stearic acid	Saturated fatty acid	Coconut oil	[10]

Table 3. Mineral Profile of *Mathan Thailam* (ICP–OES Analysis)

Element	Detection status	Approx. concentration (ppm)	Reference
Copper (Cu)	Detected	~33.2 ppm	[10]
Lead (Pb), Arsenic (As), Mercury (Hg)	Not detected	Below detection limit	[10]

purified copper sulphate of the Siddha pharmacopeial. It is assumed that the processes help to extract the lipophilic constituents and may reduct the toxicity of the crude plant material but it is not possible to rule out the chances of systemic exposure [10]. Percutaneous absorption of lipophilic tropane alkaloids may occur with application over open wounds or damaged skin barriers and over extended use or when applied over make widely accessible surfaces, alkaloids may be highly varied based on environmental conditions, maturity of the plant, geographic origin and changes in processing, which can influence the consistency and safety profile of the formulations derived using this plant [27,31]. Physical and chemical standards of *Mathan Thailam* have been presented that have given acceptable values and non-toxic heavy metals like lead, arsenic and mercury and contain controllable amounts of copper ions at approximately 33 ppm [10]. Also, existing preclinical experiments and few isolated clinical observations did not report obvious symptoms of anticholinergic toxicity during topical application. Yet, these results are initial and detailed toxicological analyses such as dermal absorption research, pharmacokinetic research studies, and long-term safety persons checkups are not yet accessible. Considering all these, *Mathan Thailam* should be used cautiously in clinical practice especially in patients with extensive wounds, impaired skin breaks or those using drugs with an anticholinergic effect. Watching against possible indicators of systemic absorption e.g. tachycardia, dry mouth, or neurological symptoms may be indicated in long time use. Quantitative profiling of tropane alkaloids in standardized batches, dermal permeability studies, systematic pharmacovigilance studies should be a priority in future research to determine evidence-based ranges of safety of clinical use of this traditional Siddha formulation.

Limitations of Preclinical Evidence

Although preclinical studies and experimental observations give preliminary evidence on the possible wound-healing functions of *Mathan Thailam* (e.g. anti-inflammatory, antioxidant, antimicrobial and tissue healing) available data are overwhelmed by various methodological limitations that diminish the reliability and generalizability of the available data (i.e. small sample sizes often n=6-10 animals per group) lack of appropriate controls (e.g. vehicle-only group, control with standard treatment e.g. povidone-iodine or silver sulfadiazine. Moreover, there has been no systematic study of variations in the oil preparation used in the different studies (e.g. variations in heating time, ratio of ingredients, batch-to-batch reproducibility of *Datura metel* leaf or method of extract preparation) which may cause inconsistent findings [10,33]. There is no systematized study of the variability in the oil preparation between different studies (e.g. variations in heating time, ratios of ingredients

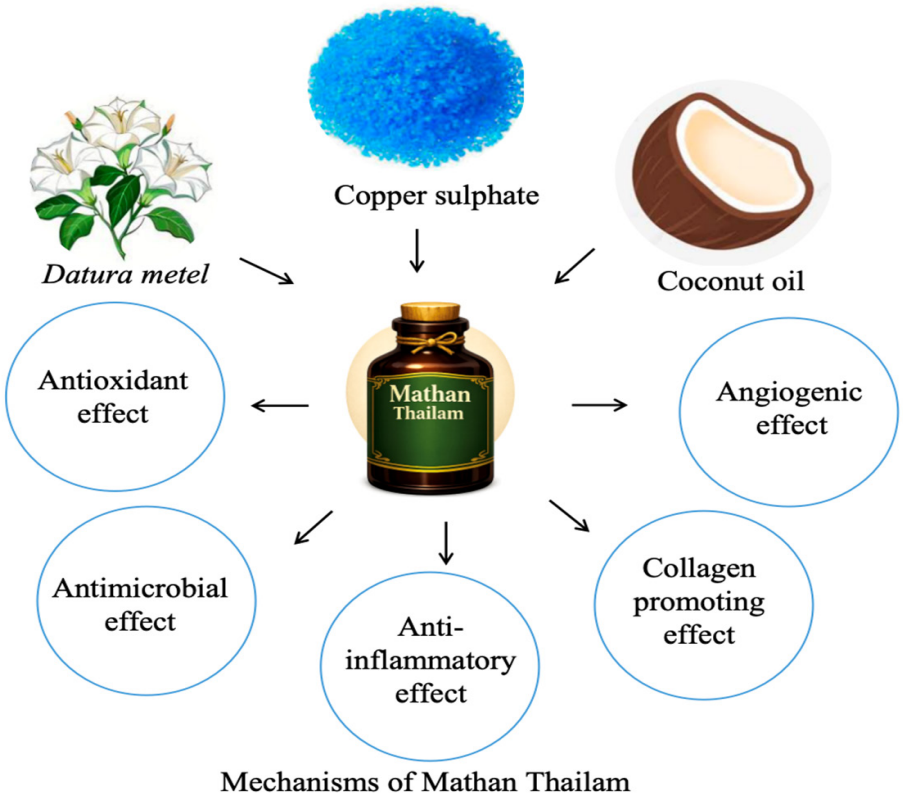

Figure1: Synergistic mechanisms of *Mathan Thailam*. Integration of *Datura metel*, Copper sulphate and Coconut oil results in a multi-targeted pharmacological effect including antioxidant, antimicrobial and anti-inflammatory activities that accelerate wound healing.

used, consistency between batches of *Datura metel* leaf, or different methods of extract preparation), which can contribute to incons. Such limitations are typical of early-stage herbal and traditional formulation research, and suggest that the evidence is encouraging and consistent with traditional use, but cannot be called definitive evidence of efficacy and mechanisms until advanced rigorous and standardized protocols with larger cohorts, adequate controls, and replication are done.

Case Studies

There is only limited clinical evidence on *Mathan Thailam* mostly as individual case reports and small case series of observations. These give its topical applications, such as in conjunction with other Siddha preparations (e.g., Triphala Chooranam wash, Palagarai Parpam, or Muthuchippi Parpam) to a range of chronic non-healing ulcers, such as varicose, venous, diabetic foot, and arterial ischemic ulcers [21 33].

The reports have given examples such as: In one case, a 60-year-old diabetic man reported having a diabetic foot ulcer, which was managed using *Mathan Thailam* dressing and Triphala Chooranam wash, and the ulcer was completely treated in four months [35]. In another case, a 52-year-old diabetic, hypertensive male patient who exhibited a seven-month-old deep leg ulcer; *Mathan Thailam* and Palagarai Parpam therapy had a seven-month reduction in Leg Ulcer Measurement Tool (LUMT) of 18/68 to 12/68 in six weeks [21]. Similarly, a 49-year-old man with painful arterial ischemic ulcer on the right fourth toe; *Mathan Thailam* and Palagarai Parpam produced improvement in the LUMT score of 21/68 to 06/68 in six weeks [21]. In another case, a 55-year-old male, having a venous ulcer on the left leg; every alternate-day *Mathan Thailam* usage six-week intervention led to the reduction of the LUMT score of 28/68 to 14/68 [21]. Similarly, a 58-year-old female who experienced a varicose ulcer (Naala Vibatha Pun) around the right ankle; *Mathan Thailam* application on alternate days, six weeks was accompanied by an improvement of LUMT score between 17/68 to 12/68, coupled with actual pain reduction, oozing and swelling [21]. In another case, a 48-year-old male patient with a chronic venous ulcer on the left ankle; *Mathan Thailam* 10mg a day, Muthuchippi Parpam 10mg a day on November 1-3 every week the LUMT score decreased to 21/68 by 05/68 [21].

Such observations also create some initial ideas about what may happen in chronic wound management when working with the traditional Siddha protocols. Nonetheless, the evidence is anecdotal, limited by significant limitations, such as extremely small sample sizes (in most cases, isolated instances or small series), failure to control or match, lack of randomization, blinding, or standardized protocols other than of LUMT scoring, and can also be confounded (e.g. by co-administration of systemic treatment, natural variability of wounds, or adjunctive wound care measures). There is further limited interpretability by retrospective reporting and potential publication bias of the desirable

Table 5: Reported Case studies on *Mathan Thailam*

S. No.	Case Description	Observation / Outcome	Reference
1	Diabetic foot ulcer in a 60-year-old male treated with *Mathan Thailam* dressing and cleansing with Triphala Chooranam	Complete recovery of the ulcerative foot after 4 months of treatment	[27]
2	A 52-year-old male with diabetes and hypertension presenting with a 7-month-old deep ulcer on the left leg, treated with *Mathan Thailam* and Palagarai Parpam	Reduction in Leg Ulcer Measurement Tool (LUMT) score from 18/68 to 12/68 after 6 weeks of treatment	[21]
3	A 49-year-old male farmer diagnosed with a severely painful blackish arterial ischemic ulcer on the right fourth toe, treated with *Mathan Thailam* and Palagarai Parpam for 6 weeks	LUMT score reduced from 21/68 to 06/68, indicating marked healing and improved quality of life	[21]
4	A 55-year-old male with a venous ulcer on the left leg for 3½ months, treated with topical application of *Mathan Thailam* on alternate days for 6 weeks	LUMT score reduced from 28/68 to 14/68, suggesting significant ulcer healing and improved quality of life	[21]
5	A 58-year-old female diagnosed with Naala Vibatha Pun (varicose ulcer) around the medial malleolus of the right ankle for 5 months, treated with *Mathan Thailam* on alternate days for 6 weeks	LUMT score reduced from 17/68 to 12/68, with reduced pain, oozing, and swelling, and improved mobility	[21]
6	A 48-year-old male with a 12-month-old venous ulcer on the left ankle joint, treated with *Mathan Thailam* and Muthuchippi Parpam on alternate days for 6 weeks	LUMT score reduced from 21/68 to 05/68, showing marked wound contraction and significant improvement in quality of life	[21]

Footnote*: The information is obtained by uncontrolled case reports of observation and small series. Results are tentative, prone to numerous biases and are not universal. Validation must be done via randomized controlled trials.

Table 6: Marketed Products of *Mathan Thailam*

S. No.	Brand / Manufacturer	Reported Uses*
1	Moolihai Ayurveda	External wounds, skin infections, minor burns, itching, and ulcerated lesions
2	Medisiddh Thaila (Oil)	Skin disorders, wounds, ulcers, and inflammatory skin conditions
3	IMPCOPS (Govt. of Tamil Nadu)	Diabetic ulcers, carbuncles, fissures, eczema, and chronic non-healing wounds
4	Vadalur Arutjothi Vaidyasalai	Diabetic ulcers, chronic wounds, non-healing ulcers, and ear infections
5	AEON Wellness – *Mathan Thailam*	Weeping eczema, itching, chronic ulcers, bedsores, and wound healing
6	SKM Siddha & Ayurveda – *Mathan Thailam*	Eczema, inflammatory skin conditions, redness, and minor wounds

outcomes. By definition, it means that these reports must be taken as hypothesis generating and not evidence of efficacy. They are neither strong clinical evidence nor can be used to make general therapeutic arguments relating to *Mathan Thailam*. The safety, efficacy and generality in clinical practice can only be assessed appropriately by use of rigorous randomized controlled trials (RCTs)- with sufficient sample sizes, objective primary endpoints (e.g., complete healing rates, time to healing, infection rates), comparators and long term follow-up.

Marketed product

With increasing public interest in traditional systems of medicine, several Siddha and Ayurveda-based pharmaceutical manufacturers have developed and commercialized formulations derived from classical texts. Many manufacturers have embraced *Mathan Thailam*, a traditional Siddha medicinal oil that is today sold under many brand names. These formulations are mainly meant to be used topically to wounds, ulcers, and certain dermatological diseases. Table 6 shows a typical list of *Mathan Thailam* items that are sold commercially. It should be mentioned that the stated indications are not supported by data from controlled clinical trials, but rather by conventional usage claims and product information from manufacturers.

Conclusion

Traditionally, burns, infected wounds, and chronic non-healing ulcers have been treated topically using *Mathan Thailam*, a traditional Siddha herbomineral preparation. In order to assess its potential for wound healing within a modern scientific framework, the current narrative review critically integrates information from experimental pharmacology, recent standardization research, traditional Siddha literature, and accessible clinical case reports. Purified copper sulphate, coconut oil, and *Datura metel* leaf juice make up the formulation, which is a multi-component therapeutic system that can have complimentary biological effects related to wound healing. According to preclinical research, *Mathan Thailam* has antibacterial, anti-inflammatory, antioxidant, and collagen-promoting qualities that may help with wound contraction,

angiogenesis, epithelialization, and tensile strength restoration. Although there is still no direct molecular evidence at the formulation level, mechanistic studies suggest that *Mathan Thailam* may influence pro-angiogenic signaling, oxidative stress pathways, and inflammatory cytokines. Importantly, when prepared according to pharmacopeial guidelines, modern analytical tests have demonstrated consistent quality, physicochemical stability, and controlled incorporation of mineral components, without detectable harmful heavy metals, supporting both safety and formulation reliability. On the clinical side, the current evidence comes mainly from observational case reports and small case series, which report symptomatic improvement and reduced ulcer severity—particularly in chronic, diabetic, venous, and arterial ulcers. While these findings are encouraging, they should be interpreted cautiously due to the absence of randomized controlled trials, standardized comparators, and long-term safety data. Therefore, in the context of evidence-based wound care, the therapeutic claims related to *Mathan Thailam* cannot yet be deemed definitive. The idea that *Mathan Thailam* has true wound-healing capacity is generally supported by the literature, which is mostly based on preclinical research and conventional use. In order to address safety concerns about alkaloid variability and batch-to-batch consistency, future research should focus on well-designed clinical trials, mechanistic investigations at the molecular and cellular levels, and strong pharmacovigilance. Such initiatives are necessary to confirm its therapeutic effectiveness and to make it easier to logically include *Mathan Thailam* into contemporary wound-management techniques.

Credit authorship contribution statement

The authors confirm sole responsibility for the following: review conception and design (BK, MP,N), data collection (BK, N, MP), first draft of manuscript (BK, N, MP, TK), analysis and interpretation of collected data (BK,MP), editing and review (BG, MP, N, TK).

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this.

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REVIEW ARTICLE

A Comprehensive Review of the Nutritional Composition, Bioactive Compounds, and Health-Promoting Potential of *Eleusine coracana* (L.) Gaertn (Finger Millet)

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Abstract

Eleusine coracana (L.) Gaertn (finger millet), is a traditional cereal extensively cultivated in India and parts of Africa, is gaining attention as a nutrient-dense food with multiple health-promoting properties. It is rich in polyphenols, flavonoids, tannins, dietary fiber, essential amino acids, vitamins, and minerals, which contribute to its antioxidant, antidiabetic, antimicrobial, and anticancer activities. Processing methods such as germination, fermentation, malting, and roasting have been shown to enhance the bioavailability and efficacy of these bioactive compounds, further improving the nutritional and functional quality of finger millet-based foods. Scientific studies indicate that finger millet regulates oxidative stress, improves enzymatic antioxidant defenses, lowers postprandial glucose, modulates gut microbiota, inhibits pathogenic bacteria and fungi, and exhibits cytotoxic and chemopreventive effects against various cancer cell lines. Its prebiotic properties also support beneficial gut microbes, contributing to overall metabolic and immune health. This review consolidates current research on the phytochemical composition, biological activities, and therapeutic potential of *E. coracana*, emphasizing its role as a functional food and a natural source of health-promoting compounds. Future research should focus on detailed characterization of bioactive constituents, optimization of processing techniques, and the development of nutraceuticals to fully harness the functional, therapeutic, and disease-preventive potential of finger millet.

Keywords: Anticancer, Antimicrobial, Antioxidant, Polyphenols, Finger millet

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Introduction

E. coracana, commonly known as ragi, is a highly nutritious cereal renowned for its diverse health-promoting properties [1]. India is the leading global producer of finger millet, with an annual production of approximately 1.8 million tons, followed by Ethiopia, Nepal, Uganda, and Tanzania, with production levels ranging from approximately 1.2 million tons to 0.10 million tons [2]. *E. coracana* is an important traditional Indian millet mainly cultivated under rainfed conditions in peninsular regions, and is valued for its stress tolerance, high nutritional content, and health benefits [3]. The grain of finger millet consists mainly of starch (about 70%), supplemented by proteins, lipids, vitamins, and minerals, while bioactive compounds contribute substantially to the nutritional value and quality of finger millet-based foods [4]. Finger millet is traditionally malted, yielding a malt that is a good source of α- and β-amylases, proteases, and phytase, and that offers readily digestible carbohydrates and proteins with enhanced mineral bio-accessibility [1]. It is also a rich source of thiamine, riboflavin, iron, methionine, isoleucine, leucine, phenylalanine, and other essential amino acids. The high content of these phytochemicals enhances its nutraceutical value, establishing finger millet as a nutrient-dense, health-promoting grain S [5]. Renowned for its health-promoting properties, *E. coracana* derives these benefits from its rich polyphenol content, dietary fiber, and calcium, contributing to antidiabetic, antitumor, antiatherogenic, antioxidant, and antimicrobial activities [6].

This review aims to provide a comprehensive overview of

Eleusine coracana, emphasizing its bioactive constituents and associated health-promoting properties. It critically examines the relationship between its phytochemical profile and reported therapeutic potential, highlighting its value as a nutrient-dense functional food and a promising natural resource for disease prevention. Furthermore, the review addresses current limitations, including variability in phytochemical composition, bioavailability challenges, and gaps in clinical validation. It also explores future prospects for the integration of finger millet into the food industry, with particular focus on product development, functional food innovation, and strategies to enhance its nutritional and commercial value.

Methodology: Literature Search Strategy

A systematic literature search was conducted using Google Scholar, ScienceDirect, and PubMed to identify relevant studies published between 2002 to 2025. Keywords used included *Eleusine coracana*, finger millet, phytochemistry, antioxidant, antidiabetic, anticancer, antimicrobial, and pharmacological activities. Peer-reviewed systematic reviews, meta-analyses, and relevant experimental and observational studies published in English were included. Articles were screened based on title, abstract, and full text for relevance to the phytochemistry and therapeutic potential of *E. coracana*. Studies with insufficient data, duplicates, non-peer-reviewed sources, or those not directly relevant were excluded. This approach ensured the inclusion of reliable and scientifically relevant literature.

Phytochemicals of *E. coracana* and Their Role in Antioxidant Activity

Phytochemicals derived from plants serve as potent anti-inflammatory agents, with studies demonstrating that both crude extracts and isolated compounds can effectively modulate inflammatory responses[7].Rising awareness of nutrition underscores the health benefits of phytochemicals like polyphenols and dietary fiber. Finger millet, a minor cereal, is a promising source of such compounds, contributing to its multiple health-promoting properties [8]. Finger millet and kodo millet (*Paspalum scrobiculatum*), both rich in phenolic compounds and tannins [9]. Malting finger millet alters phenolic composition, increasing antioxidant activity of free phenolics (770 to 1686) while reducing that of bound phenolics (570 to 448), showing that malting modulates the grain's antioxidant potential [10]. In a study evaluated tannin and polyphenol content in finger millet germplasm and varieties KOPN-330, MR-6, and RAU-8. Polyphenols averaged 156.34 mg GAE/100 g and tannins 99.26 mg TAE/100 g. The phytic acid to iron molar ratio ranged from 16.18 to 20.01, suggesting low iron bioavailability, as ratios below 10:1 are preferred for higher absorption [11]. Finger millet is rich in phenolic compounds and antioxidants, but thermal processing affects their levels and activity. Roasting flour and foods increased total phenolic content (21.58–28.63 µmol FAE/g) and enhanced radical scavenging, reducing power, ferrous ion chelation, and β-carotene/linoleate emulsion antioxidant activity. Steaming reduced these effects, while adding spices like garlic and cinnamon boosted antioxidant activity in open-boiled porridges. These findings highlight finger millet's potential as a functional food ingredient for promoting health[12]. Fermentation of light and dark brown finger millet flours for 96 hours enhanced bioactive compounds and antioxidant activity. Total polyphenols and flavonoids increased 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and lipid peroxide inhibition improved, while anthocyanins decreased. Protein and acidity rose, whereas fiber, fats, moisture, and carbohydrates declined. Porridges from 24 hours fermented flours showed the best sensory quality, highlighting fermentation as an effective way to boost antioxidant properties[13]. Finger millet seed coat and endosperm are rich in phytonutrients, and drum drying alters their composition. While catechin and DPPH activity decreased in the seed coat (30%), they increased in the endosperm (18%), with total antioxidant activity maintained, highlighting these fractions as potential functional foods with strong antioxidant properties [14]. Finger millet exhibits strong antioxidant properties. During 48 hours of germination, its total phenol content increased significantly from 230.9 ± 1.01 to 262.3 ± 1.10 mg/100 g, with ferric reducing antioxidant power (FRAP) also showing an increasing trend, highlighting its potential for promoting health [15]. Flavonoids from *E. coracana* (ECFs) were extracted and analyzed, revealing 82.6% total flavonoid content with 16 major components identified. ECFs demonstrated strong *in vitro* antioxidant activity (IC_{50}: 22.75 µg/mL in the ABTS assay). This value is lower than that reported for vitamin E in comparable assays, suggesting a potentially higher radical scavenging capacity; however, such comparisons should be interpreted with caution, as they depend on consistent experimental conditions and must be supported by appropriate references. Furthermore, ECFs enhanced in *vivo* antioxidant defenses in *Caenorhabditis elegans*, increasing the activities of catalase, superoxide dismutase, and glutathione, while reducing malondialdehyde (MDA) and lipofuscin levels. These effects were associated with improved mobility and a 17.86% extension in lifespan, demonstrating significant antioxidant and anti-aging potential [16]. Further detailed information on phytochemicals and their reported mechanisms of action is provided in Table 1.

Exploring the Antidiabetic Potentials of *E. coracana*

The worldwide incidence of diabetes mellitus is increasing, posing serious health risks and higher mortality. Poor blood glucose regulation can lead to severe complications. While conventional antidiabetic drugs are effective, they often have side effects, highlighting medicinal plants as promising alternatives with potentially fewer adverse effects [17]. Finger millet, a nutrient-rich cereal, is a low glycemic index food (GI 54–60) that helps reduce blood glucose levels, making it beneficial for diabetic patients. Its consumption also offers

Table 1. Chemical compounds therapeutic properties and mechanism of action of *E. coracana*

Class of Compounds	Specific Compounds	Chemical Type	Therapeutic Properties	Mechanism	Quantitative Data (examples)
Hydroxybenzoic acids	Gallic acid, Protocatechuic acid, Vanillic acid, Syringic acid	Polyphenols	Antioxidant, Antimicrobial, Anticancer	ROS scavenging, enzyme inhibition	Total phenolics: ~0.3–3 mg GAE/g [39]
Hydroxycinnamic acids	Ferulic acid, p-coumaric acid, Caffeic acid, Sinapic acid	Polyphenols	Antioxidant, Anticancer	Inhibits lipid peroxidation, chemoprevention	Ferulic acid (18.60 mg/100 g) [40]
Flavonoids	Quercetin	Flavonoid	Antioxidant, Antimicrobial, Anticancer	Apoptosis induction, anti-inflammatory	Low concentration (3µg/g) [41]
Tannins / Proanthocyanidins	Procyanidins	Condensed polyphenols	Strong antioxidant, Antimicrobial, Anticancer	Radical scavenging, microbial membrane disruption	Tannins: 0.04–3.74% [42]
Total Polyphenols	Seed coat phenolics	Polyphenol mixture	Antioxidant, Antimicrobial	Reducing power, DPPH scavenging	Up to 85% in seed coat fraction [43]
Tannins (general)	Catechin equivalents	Polyphenols	Antioxidant, Anticancer	ROS suppression	340–500 mg/100g [44]
Alkaloids, terpenoids, steroids	Various	Secondary metabolites	Antimicrobial, Antioxidant	Broad bioactivity	Qualitative presence [45]
Phytates and lignans	Phytic acid, lignans	Phytonutrients	Antioxidant, Anticancer	Metal chelation, DNA protection	Phytic acid ~571 mg/100 g [46]

antioxidant, hypocholesterolemic, and chronic disease–preventive effects, highlighting its potential as a functional food for diabetes management [18]. Seed coat matter (SCM) of black *E. coracana* demonstrated significant antidiabetic and antioxidant effects in streptozotocin-induced diabetic rats. Treatment with 20–40% SCM reduced blood glucose by up to 45%, improved liver enzyme profiles increased catalase and superoxide dismutase activities, lowered lipid peroxidation (TBARS), and protected pancreatic, liver, and kidney tissues from damage, highlighting its therapeutic potential [19] *E. coracana* contains bioactives like polyphenols, phytic acid, and dietary fiber that reduce postprandial glucose and improve insulin sensitivity. Its prebiotics support gut microbes such as *Bifidobacterium*, *Lactobacillus*, and *Akkermansia muciniphila*, producing short-chain fatty acids and proteins that enhance anti-diabetic effects. Millet compounds also suppress harmful gut bacteria, lowering inflammation and diabetes risk [20]. Anti-diabetic potential of finger millet using *in vitro* absorption and intestinal permeability models. Finger millet extract showed superior absorption and permeability compared to Metformin, likely due to its high phenolic and flavonoid content, which inhibit carbohydrate-digesting enzymes. These results highlight finger millet as a promising natural alternative for diabetes management [21]. Reduced-sugar cookies formulated with Finger Millet, Jamun, and stevia showed high phenolic content and antioxidant activity. These cookies have potential as a functional food for managing diabetes and metabolic disorders [22]. Phenolic compounds extracted from Finger millet seed coats showed potent inhibition of α-glucosidase and pancreatic amylase, key enzymes involved in postprandial hyperglycemia. The phenolics exhibited non-competitive inhibition with low IC_{50} values, indicating strong therapeutic potential for natural diabetes management [23].

Antimicrobial Properties of *E. coracana*

Many currently available drugs suffer from significant limitations, including adverse side effects, diminished efficacy against emerging or re-emerging fungal strains, and the rapid development of antimicrobial resistance[24.] Consequently, natural dietary products have gained attention as promising sources for the discovery of novel antibacterial agents [25]. In this context, natural products derived from medicinal plants, particularly *E. coracana*, represent a valuable and promising resource for the development of new antimicrobial agents [26]. Studies indicate that the seed coat of *E. coracana* is rich in polyphenols with strong antimicrobial activity. Methanol–acid extracts of the seed coat showed greater inhibition of *Bacillus cereus* and *Aspergillus flavus* than whole flour extracts, primarily due to phenolic acids such as daidzein, gallic, coumaric, syringic, and vanillic acids. These results highlight the potential of *E. coracana* seed coat as a natural antimicrobial agent for therapeutic and food preservation applications [27]. Finger millet from northern Nigeria exhibited concentration-dependent antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. typhi*, with the highest inhibition (8 mm) observed against *P. aeruginosa* at 100 mg ml^{-1}. Phytochemical analysis revealed the presence of tannins/phenols, flavonoids, alkaloids, saponins, glycosides, terpenoids, and steroids, with measurable phenolic and flavonoid contents. These results indicate that finger millet possesses notable *in vitro* antimicrobial potential along with nutritional benefits [28]. In a study, the incorporation of 20% finger millet into a fermented milk–millet composite product significantly enhanced its antimicrobial activity compared to the control

without finger millet ($P < 0.05$). The composite product exhibited higher inhibitory effects against all tested pathogens, demonstrating that finger millet enrichment substantially improves the antimicrobial potential of fermented dairy-based functional foods [29]. β-Glucan (Ec-β G) isolated from finger millet (*E. coracana*) demonstrated potent antimicrobial activity against both Gram-positive (*Lysinibacillus fusiformis*, *Enterococcus faecalis*) and Gram-negative (*Proteus vulgaris*, *Shigella sonnei*) bacteria, with minimum inhibitory concentrations below 70 µg/mL. At 100 µg/mL, Ec-βG significantly reduced bacterial viability and inhibited biofilm formation, as confirmed by light and confocal microscopy, highlighting its potential as a natural antimicrobial agent [30]. *E. coracana* a nutrient-dense staple of arid and semi-arid regions, exhibits notable health-promoting properties. Extracts from 12-hours germinated seeds showed bactericidal activity against *Escherichia coli*, indicating potential applications in managing infectious diarrhea and as a natural food preservative. The study also reported antioxidant activity and high sensory acceptability, supporting the use of germinated finger millet as a functional food with antibacterial benefits [31]. Ethanolic and methanolic extracts of whole-grain flours from Ravi, Rawana, and Oshadha finger millet varieties were evaluated for antibacterial, antifungal, and β-lactamase inhibitory activities. The extracts exhibited dose-dependent antibacterial effects against both antibiotic-sensitive and antibiotic-resistant bacteria, with greater activity against Gram-positive strains, particularly *Staphylococcus aureus* and *Bacillus subtilis* (MICs: 2.1 and 1.8 mg/mL, respectively). However, the extracts showed limited antifungal and β-lactamase inhibitory activities and were less effective than standard drugs, indicating moderate antibacterial but minimal antifungal potential[32]. Five fungal endophytes were isolated from finger millet roots, three of which exhibited activity against *Fusarium* species. The most potent strain, WF4, was identified as *Phoma* sp. and produced four bioactive compounds viridicatol, tenuazonic acid, alternariol, and alternariol monomethyl ether that disrupted the hyphae of *Fusarium graminearum*. As reported by Mousa et al. [33], except for tenuazonic acid, these compounds were newly reported from *Phoma* sp. WF4 (identified at the genus level) and were also newly associated with anti-*Fusarium* activity, highlighting finger millet as a promising source of natural antifungal agents [33].Polyphenols extracted from the seed coat of finger millet demonstrate strong antimicrobial activity against several pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Quercetin was identified as a major bioactive compound responsible for these antibacterial effects, underscoring the potential of finger millet polyphenols as natural antimicrobial agents [34].

Chemopreventive and Anticancer Properties of *E. coracana*

Cancer represents a major global public health concern, affecting individuals across all socioeconomic groups. Medicinal plants offer potential in cancer prevention and treatment, largely due to their antioxidant properties and ability to inhibit tumor growth [35]. Phenolic acids from finger millet were isolated and tested for anticancer activity. Cytotoxicity assays showed that millet polyphenols exhibited activity against HepG2 liver cancer cell lines, suggesting their potential as natural compounds for cancer prevention and therapy [36]. A finger millet (*E. coracana*) formulation was developed using *Levilactobacillus brevis* MTCC 4460 and optimized via response surface methodology and artificial neural networking. The optimized formulation (2% bacterial inoculum, 2% glucose, 3.3 days fermentation) yielded 5.98 mg/mL lactic acid and 3.38 $\log_{10}$ CFU/mL viable bacteria. The fermented millet showed antiproliferative and antimigratory effects on MDA-MB-231 and HCT116 cancer cell lines, inducing apoptosis via Bcl-2 family proteins, while exhibiting no toxicity to normal HEK293 cells [37]. Finger millet (*E. coracana*) contains high levels of proteins, dietary fiber, and polyphenols, which are known for their various health benefits. This study comparatively evaluated the anticancer potential of its polyphenols through molecular docking against important cancer-related targets such as CDKN1A, FOXO1, FGFR2, CTNNB1, and GST-PI. The docking analysis revealed relatively strong binding affinities, ranging from -116.56 to -105.07 kcal/mol, indicating favorable interactions between millet polyphenols and these targets. In comparison, these results suggest that finger millet polyphenols demonstrate notable anticancer potential and may serve as promising compounds for further investigation in cancer therapy [38].

Conclusion and Future Scope

E. coracana is a nutrient-rich cereal with considerable health-promoting properties, attributed to its high content of polyphenols, flavonoids, tannins, dietary fiber, essential amino acids, and minerals. Its bioactive compounds exhibit strong antioxidant, antidiabetic, antimicrobial, and anticancer activities, which are further enhanced by processing methods such as germination, fermentation, malting, and roasting. Evidence from *in vitro, in vivo,* and computational studies highlights finger millet's potential as a functional food for managing oxidative stress, regulating blood glucose, combating microbial infections, and providing chemopreventive effects. These findings establish finger millet as a valuable natural resource for promoting health and preventing chronic diseases.

Future research should focus on validating the pharmacological effects of finger millet through human clinical trials to establish safe and effective dosages for therapeutic applications. Exploration of its bioactive compounds, particularly polyphenols and peptides, can lead to the development of novel nutraceuticals and functional foods. Studies on its prebiotic effects and modulation of gut microbiota may uncover additional mechanisms for disease prevention. Furthermore, breeding programs for nutritionally superior and stress-tolerant varieties, along with optimized processing techniques, can maximize both the health benefits and sensory quality of finger millet products. Integration of finger millet into functional diets and therapeutic interventions holds significant potential for sustainable nutrition-driven health promotion and

chronic disease management.

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Conflict of Interest

Authors declare no competing or conflict of interest.

Data Availability

None

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