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Hibiscus rosa sinensis
Gurhal

Oxalis dehradunensis
Dehradun wood sorrel

Coccinia grandis
Kundru

Cedrus deodara
Deodar

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Editorial

Herbal Antioxidants: Nature's Defense Mechanism Modern biomedical research increasingly validates what Ayurveda has advocated for centuries: herbs rich in antioxidants play a pivotal role in protecting the body from oxidative stress. Oxidative stress, caused by free radicals, weakens immune defenses and accelerates aging. Herbal antioxidants neutralize these free radicals, thereby enhancing immune function protect from various types of cancers and inflammatory diseases.

Synergy of Tradition and Science The convergence of Ayurveda and contemporary science offers a powerful narrative. While Ayurveda prescribes holistic regimens including diet, lifestyle, and herbal formulations, modern research provides molecular insights into their efficacy. For instance: **turmeric (Curcuma longa):** Curcumin exhibits potent anti-inflammatory and immunomodulatory properties. **Tulsi (Ocimum sanctum):** Known as the "Queen of Herbs," tulsi enhances stress resilience and immune regulation.

Herbs not only strengthen immunity but also contribute to overall well-being, bridging preventive and therapeutic healthcare. The integration of Ayurveda with modern immunology can pave way for evidence-based, globally accepted protocols that honour tradition while meeting contemporary standards.

Ayurveda's wisdom, enriched by the science of herbal antioxidants, offers a holistic pathway to immunity enhancement. This editorial greets researchers, clinicians, and policymakers to embrace integrative approaches that harmonize ancient knowledge with modern science—ensuring health, resilience, and vitality for generations to come.

This issue of UJPAH is devoted to antioxidants and antimicrobial herbs. My best wishes to all those scientists, Research scholars, students and teachers who contributed for bringing out this issue and I also express my sincere gratitude to all board members who make this issue a memorable for science fraternity of the Uttarakhand and the people of science at large.

Dr. S. Farooq
Chief Editor
UJPAH

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In -Vitro Antioxidant and Nitric Oxide Scavenging Activity of *Oxalis dehradunensis* Raizada Plant Leaves

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Abstract- The current study was conducted to assess the in-vitro antioxidant activity of the methanolic extracts of leaves *Oxalis dehradunensis* Raizada, a plant with medicinal properties traditionally utilized in Indian folk drug. Reactive oxygen species (ROS) are known to induce oxidative stress, resulting in cellular injury and the development of multiple chronic disease. Therefore, the identification of natural antioxidants derived from plant sources are of considerable importance. Preliminary phytochemical screening of the extract indicated the presence of important biologically active compounds including alkaloids, flavonoid, tannin, phenolic compounds, terpenoids, and carbohydrate, while saponins were absent. The antioxidant potential of the extracts was evaluated using the DPPH assay radical scavenging and the nitric oxide scavenging activity was evaluated at different concentration levels (20–100 µg/mL), with ascorbic acid served as the standard comparison of reference. The results demonstrated that the extract showed notable free radical scavenging activity in a concentration de-pendent manner in both the assays. In the DPPH assay, the extract showed a maximum inhibition of 79.14% at 100 µg/mL, whereas ascorbic acid shows 93.18% inhibition. Similarly, in the nitric oxide scavenging assay, the extract exhibited 73.33% inhibition at the highest concentration, which was comparatively lower than the standard. Although the extract showed lower activity than ascorbic acid, it demonstrated considerable antioxidant potential, possibly attributed to the presence of phenolic and flavonoid compound. This result supports the traditional uses of the plant and suggest that it may serve as a promising organic source of antioxidants. Addi-tional studies are needed to isolate and characterize the active compounds responsible for this effect.

Key words: *Oxalis dehradunensis*, Antioxidant activity, DPPH assay, Nitric oxide scavenging.

Introduction

Reactive oxygen species (ROS) are generated within the body, and when their generation exceeds the capacity of intracellular defence systems to failure to neutralize them in a condition termed oxidative stress. This type of imbalance damage essential biomolecules like lipids, proteins, DNA, and RNA[1]. Such types of damage is linked to the development of various disorder such as cancer, oxygen toxicity, aging, lipofuscin accumulation, and liver damage[2-3]. Anti-

oxidants play a crucial role in safe-guarding against disease related to free radicals[4]. These compounds help in reducing oxidative stress by inhibiting oxidation and neutralizing reactive oxygen species. The antioxidant potential of plants are mainly attributed to the pre-sence of phytochemicals, which interact synergistically to produce beneficial effects. In addition to their antioxidant properties, plant derived phytochemicals have shown the ability to interact with microorganisms in the environment, thereby inhibiting the growth of bacteria and fungi. Due to their antimicrobial activity and relatively low toxicity to-wards hosts cells, these com-pounds are considered promising potential sources for the development of novel anti-microbial agents[5]. Plants have served long as significant sources of medicinal agents for centuries. They are commonly employed in traditional systems of medicines and have contributed for the discovery of many effective drugs. Research in this field is largely based on traditional knowledge, especially the use of medicinal plants, which hold significant importance in folk medicine systems[6-7].

In India, *Oxalis dehradunensis* Raizada is widely acknowledged as a valuable medicinal plant due to its adaptability and broad range of physiological activities. Commonly referred to as creeping wood sorrel, this naturally occurring plant is rich in essential constituents that contribute to maintainning normal health and well-being in humans[8]. The plant is a stemless herb without a rootstock, forming bulb-like ovoid structures (about 5 × 2 cm). It produces several basal stolons with small scales that develop into bulbils. Petioles grow up to 20 cm long, and the leaflets are nearly equal, obdeltoid, and deeply incised. The inflorescence is umbellate with 5–12 flowers, supported by peduncles up to 25 cm long. Bracts are small and ovate, while pedicels are slender and reach up to 2 cm. Sepals are oblong-lanceolate with faint veins. Petals are narrow, pink to reddish-purple with a green base, and quickly wither after flowering[9]. In traditional Indian folk medicine, many easily available plants are used for treating fever, pain, inflammation, and wound healing. *Oxalis dehradunensis* Raizada has been selected for the present study due to its medicinal importance. It is a very common weed found in warm regions of India as well as other parts of the world[10].

Commonly known as garden pink sorrel or broadleaf sorrel, this flowering plant classified under the family of Oxalidaceae. The plant is a perennial herb and is native to regions such as Mexico, Central or South America, parts of the southeastern world[11]. Chemical studies have identified the presence of fatty acids, glycolipids, neutral lipids, vitexin, and isovitexin in *Oxalis dehra-dunensis* Raizada. Phytochemical in-vestigations also report compounds such as tannins, palmitic, linoleic, linolenic, and stearic acids. The methanolic extract of the plant contains proteins, amino acids, carbohydrates, glycosides, and volatile oils, along with tannins and calcium rich fiber. The leaves are known to possess tannins, citric acid, calcium oxalate, and various flavonoids inc-luding apigenin, quercetin, orientin, and vitexin. Due to the high oxalate content,

the plant exhibits a distinctly acidic taste[12].

Leaves part of *Oxalis Dehradunensis* **Raizada**

Material and Methods

For the in-vitro antioxidant and nitric oxide scavenging studies of *Oxalis dehradunensis* Raizada leaves, various chemicals and reagents were employed. In the DPPH radical scavenging assay, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), methanol, or ascorbic acid served as procured from the Shaila Enterprise, while all others reagents used were of analytical grade quality.

In the nitric oxide scavenging assay, sodium nitroprusside was utilized as a source for generating nitric oxide in solution, and the formation of nitrite was measured using Griess reagent at 546 nm. The plant extract and reagents were prepared using methanol or phosphate buffer (pH 7.4), depending on the requirement. All experimental procedures were carried out using standard laboratory apparatus such as glassware, pipettes, and cuvettes. Each experiment was carried out three times to ensure reliability and accuracy of the result. Distilled water was used for preparing all dilutions.

Collection- *Oxalis dehradunensis* Rai-zada plant leaves have been taken from the local area of Dehradun, Uttarakhand and were shade dried at room temperature.

Authentication- Authentication of *Oxalic dehradunensis* Raizada was done by BSI, Dehradun, UK.

Plant extraction- Dried and powdered plant (50g) of *Oxalis dehradunesis* was extracted by using a Soxhlet apparatus, the sub-stance was neutralized with 100 ml of petroleum-based ether and extraction with 100 ml of methanol over the course of 24 hours was carried out. A rotating vacuum evaporator was used to dry the extract, which was be kept refrigerated until needed again.

In-vitro Antioxidant activities

Antioxidant Assay- The antioxidant activity of the plant extract was assessed using the DPPH radical scavenging method. Each experiment was performed three times and average values were taken for analysis. Additionally, the capacity of the extract to scavenge nitric oxide radicals was assessed. In phosphate buffer, sodium nitro prusside served as the source for nitric oxide generation and its activity was determined using Griess reagent.

DPPH Radical Scavenging Assay- The methanolic extract of the plant exhibited free radical scavenging activity. *Oxalis dehradunesis* leaves were evaluated. A 0.004% (w/v) solution was made using 95% ethanol. Stock solutions (10 mg/100 mL) of the plant extraction were prepared by dissolving the methanolic extract in 95% ethanol or distilled water as required. Different aliquots (2, 4, 6, 8, and 10 mL) were pipetted from the stock solution into individual test tubes. The volume in each test tube was then adjusted to 10 mL using the respective solvent to obtain concentrations of 20,

40, 60, 80, and 100 µg/mL. Each test tube was then treated with a freshly prepared solution of DPPH (0.004% w/v). After an incubation period of ten minutes or absorbance was recorded at 517 nm using a spectrophotometer. Ascorbic acid was used as the reference standard, prepared in a similar manner using distilled water. A control sample containing the same reagents without extract was also maintained. The %age of free radical scavenging activity was calculated using the appropriate formula[13-15].

% radicals-scavenging= Absorbance of control - Absorbance of test Sample) X 100
(Absorbance of control)

Nitric Oxide Scavenging Assay-The leaf extract demonstrated nitric oxide scavenging activity assessed at concentrations of 20, 40, 60, 80 and 100 µg/mL, using ascorbic acid, the standard reference. Sodium nitroprusside (5mM) was incubated with various concentrations of the extract or standard in phosphate buffer (pH 7.4) at room temperature for 150 minutes. Following incubation, an equal vol. of Griess reagents was added to every reaction mixture to react with the nitrite generated from nitric oxide. The resulting pink-colored solution was analyzed spectrophotometrically at 546 nm. The % age inhibition of nitric oxide radicals was calculated by comparing the absorbance of the test's samples with that of a control solution lacking the extract.

% Inhibition = (Absorbance of control - Absorbance of test Sample) X 100
(Absorbance of control)

Formula for sample absorbance for % inhibition- Absorbance sample – Absorbance of Control X (1- % inhibition/100)

Results

Preliminary phytochemical investigation of *Oxalis dehradunensis* Raizada - The phytochemical testing of methanolic extraction of leave of *Oxalis dehradunensis* **Raizada** shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

Table-1 Result of phytochemical test

Tests	HAE
Tests for Alkaloid	
1. Mayer's Tests	+
2. Wagner's Tests	+
3. Hager's Tests	+
4. Dragendroff's tests	+
Tests for Saponins	
1. Foam test	-
Test for Flavonoid	
1. Alkaline reagent tests	+
2. Lead Acetate tests	+
Tests for Tannins	
1. Gelatin + extract	++
Tests for Phenolic compound	
1. Ferric chloride solution	+
Tests for Terpenoids	
1. Salkowski test	+
Tests for Carbohydrates	

Molisch test	++

+ represents presence; ++ represents present in more concentrations; - represents absence.

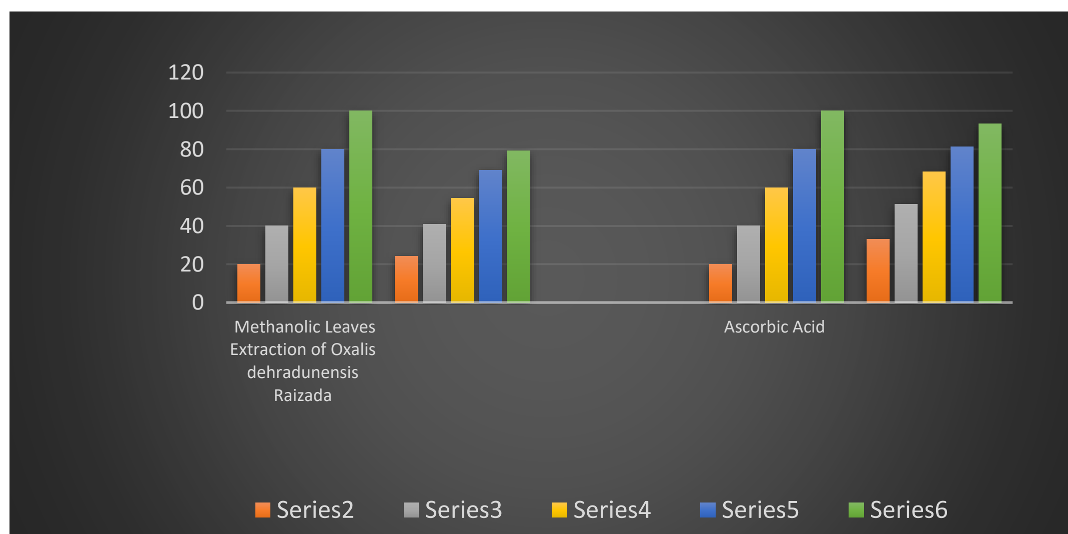
Phytochemical analysis- Initial phytochemical analysis of *Oxalis dehradunensis* Raizada leaves extracts revealed that the plant contains a wide range of bioactive compounds (secondary metabolite) Screening results indicated a strong to moderate presence of important constituents such as alkaloids, flavonoids, saponins, and carbohydrates (Table-I).

In-Vitro Antioxidant Activity

DPPH radical scavenging activity- DPPH radicals scavenging strength of *Oxalis dehradunensis* Raizada leaves extract at different two concentrations investigated in this current study was examined along with the standard antioxidants (Ascorbic Acid) in a similar concentration. *Oxalis dehradunensis* Raizada leaves extract (methanolic extracts) presented significant scavenging effects on DPPH free radicals in concentration dependent manner. When it was compared to the standard antioxidant used in the experiments, extracts showed fairly lesser DPPH free radicals scavenging potentials.

Table-2 Antioxidant activities of methanolic extracts of leaves parts of *Oxalis dehradunensis* Raizada by DPPH method

S.NO.	Methanolic Leaves Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	*DPPH % Inhibition*	*Concentrations (µg/mL)*	*Percentage Inhibition*
01	20	24.16	20	33.14
02	40	40.98	40	51.23
03	60	54.45	60	68.19
04	80	69.13	80	81.18
05	100	79.14	100	93.18

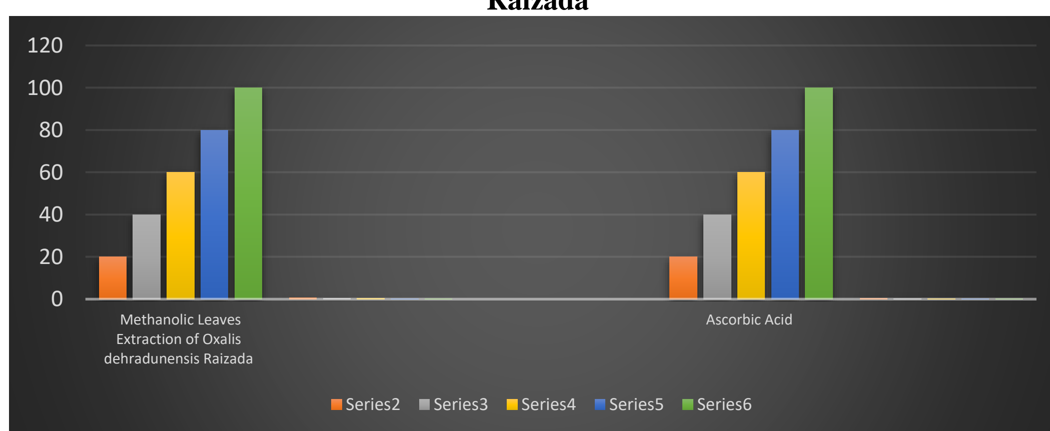


Graph -1 shows the % inhibition of DPPH radicals by extraction of *Oxalis dehradunensis* Raizada

Table-3 Antioxidant activities of methanolic extraction of leaves part of *Oxalis dehradunensis* Raizada by DPPH method

S.NO.	Methanolic Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	Absorbance at 517nm	Concentration (µg/mL)	Absorbance at 517nm
01	20	0.619	20	0.499
02	40	0.489	40	0.302
03	60	0.398	60	0.199
04	80	0.219	80	0.118
05	100	0.119	100	0.021

Graph -2 showing the absorbance of DPPH radicals by extracts of *Oxalis dehradunensis* Raizada



Nitric Oxide (NO) Scavenging Activities- In the current study, the nitric oxide radical scavenging activity of ***Oxalis dehradunensis*** **Raizada** leaf extract was evaluated at various concentrations or compared it with a standard antioxidant, ascorbic acid, at corresponding concentrations. The methanolic extract of *Oxalis dehradunensis* **Raizada** leaves demonstrated notable nitric oxide scavenging activity, which increased in a concentration-dependent way. However, when relative to the standard antioxidant applied in the study, the extracts exhibited comparatively low scavenging efficacy against nitric oxide radicals.

Table-4 Antioxidant activities of methanolic extraction of leaves part of *Oxalis dehradunensis* Raizada by Nitric Oxide (NO) scavenging method

S. no.	Methanolic Leaves Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	***Nitric Oxide % Inhibition***	***Concentrations (µg/mL)***	***Percentage Inhibition***
01	20	16.24	20	28.12
02	40	30.18	40	48.12
03	60	45.97	60	69.56
04	80	59.12	80	69.25
05	100	73.33	100	80.13

Graph- 3 shows the % inhibition of Nitric oxide radicals by extraction of *Oxalis dehradunensis* **Raizada**

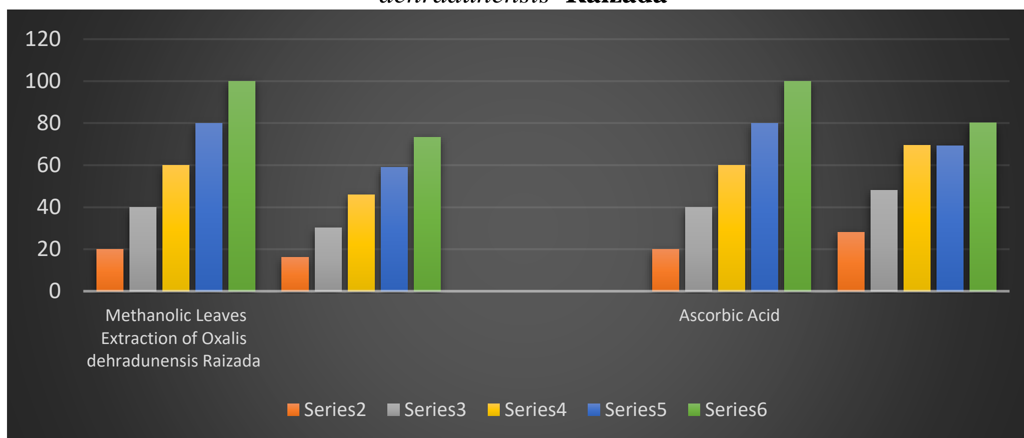
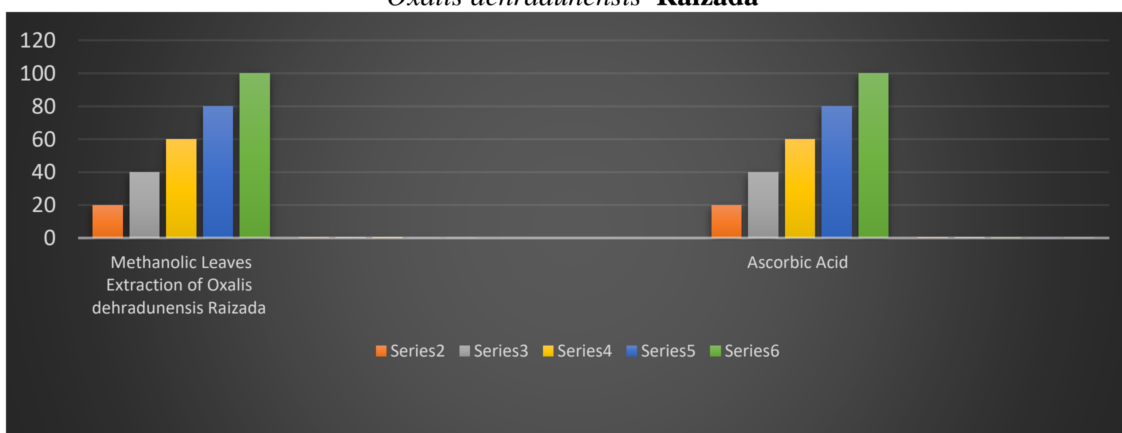


Table-5 Antioxidant activities of methanolic extraction of leaves part of ***Oxalis dehradunensis*** **Raizada by Nitric Oxide (NO) Scavenging method.**

S.NO.	Methanolic Leaves Extraction of *Oxalis Dehradunensis* Raizada		Ascorbic Acid	
	Concentrations (µg/mL)	Nitric Oxide Absorbance at (546nm)	*Concentrations (µg/mL)*	*Absorbance at (546nm)*
01	20	0.599	20	0.589
02	40	0.512	40	0.399
03	60	0.476	60	0.243
04	80	0.288	80	0.115
05	100	0.132	100	0.087

Graph -4 shows the % inhibition of Nitric oxide radicals by extraction of *Oxalis dehradunensis* **Raizada**



Discussion

In this current study, in-vitro anti-oxidant potential of the methanol used leaf extract of *Oxalis dehradunensis* Raizada was evaluated using both DPPH or nitric oxides scavenging assay. The finding revealed that the extracts exhibited considerable free radical sca-venging activities in both the methods, with effect increasing as the concentration rise. This suggested that

higher doses of the extract are more efficient in neutralizing reactive species. On comparison with the standard anti-oxidant, ascorbic acid, *Oxalis dehradunensis* Raizada extract showed comparatively lower percentage inhibition across all tested concentrations. This could be attributed to the facts that plant leaves extracts contain a complex mixture of phytoconstituents, which may act synergistically but are generally less potent than isolated standard compounds under in-vitro conditions. Despite this, the extract demonstrated appreciable anti-oxidant activity, suggesting its potential as a natural antioxidant source.

In the DPPH assay, which evaluate the extract's hydrogen donation potential to stabilize free radicals, the extract achieved a maximum inhibition of about 79.14% at a conc. of 100 µg/mL, whereas ascorbic acid exhibited 93.18% inhibition at the same concentration. Similarly, within the nitric oxide scavenging test, the extract showed effective inhibition of nitric oxide radical, which are reported to play a role in oxidative stress or inflammatory processes.

However, the activity observed in the nitric oxide assay was slightly lower than that in the DPPH method, indicating that the extract may possess stronger hydrogen donating capacity than nitric oxide scavenging ability. These observations agree with earlier reports on plant derived antioxidants, where methanolic leaf extracts are known to be rich in phenolic and flavonoid compounds responsible for scavenging free radicals. The prog-ressive increase in activity with concentration further highlights the potential of *Oxalis dehradunensis* Raizada leaves as a promising natural anti-oxidant source.

Overall, although the extract showed lower potency compared to ascorbic acid, it still exhibited significant anti-oxidant and nitric oxide scavenging activities. These results support its traditional medicinal uses and suggest the needs for further study to explore its healing potential.

Conclusion

The present investigation revealed that the leaves of *Oxalis dehradunensis* Raizada are rich in diverse secondary metabolites. These phytoconstituents could serve as valuable sources of pharmacologically active compounds, suggesting that this plant species holds considerable potential for management of many long-term diseases. In addition, crude extract demonstrated promising antioxidant activity, thereby providing scientific support for its traditional medicinal use. However, further detailed studies are necessary to facilitate the development of novel antioxidant therapeutics.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Antioxidant-Mediated Hepatoprotective Potential of Methanolic Leaf Extract of *Cedrus deodara* against CCl_4-Induced Oxidative Liver Injury in Wistar Rats

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Abstract- Hepatotoxicity driven by oxidative stress remains a major unresolved clinical challenge. *Cedrus deodara* (Roxb.) G. Don, a Himalayan medicinal conifer, possesses a phytochemical repertoire rich in flavonoids, terpenoids, and phenolics known to modulate free radical pathways. However, hepatoprotective investigation of its leaf fraction remains unreported. To evaluate the antioxidant-mediated hepatoprotective activity of the methanolic leaf extract of *C. deodara* against carbon tetrachloride (CCl_4)-induced oxidative hepatic injury in male Wistar rats, using hepatic glutathione (GSH) and lipid peroxidation (LPO) as primary antioxidant endpoints.

Following OECD 423 acute oral toxicity assessment, extract doses of 250 and 500 mg/kg were administered orally for 14 days. CCl_4 (1 ml/kg, i.p., 1:1 v/v in olive oil) was given on days 7 and 14. Silymarin (100 mg/kg, oral) served as the reference standard. Hepatic GSH and LPO, liver weight, body weight, and histopathology were assessed. Phyto-chemical screening was performed using standard qualitative assays. CCl_4 administration caused a significant depletion of hepatic GSH (15.83 ± 1.47 vs. 22.83 ± 1.94 U/L in normal control; $p<0.001$) and reduction of LPO to below normal levels (16.00 ± 1.41 nmol/mg protein; $p<0.001$), accompanied by hea-ptomegaly and body weight loss. Treatment with the extract at 500 mg/kg significantly restored GSH (19.67 ± 1.03 U/L; $p<0.001$) and normalized LPO (21.33 ± 1.21 nmol/mg protein; $p<0.001$) in a dose-dependent manner, with efficacy statistically comparable to sily-marin. Histopathology confirmed reduc-ed hepatocyte degeneration, decreased steatosis, and regenerative changes at 50 0 mg/kg.

The methanolic leaf extract of *C. deodara* confers significant dose-depen-dent, antioxidant-driven heap-to protection in CCl_4 injured rats, establishing scientific justification for its further development as a plant-based heapto-therapeutic agent.

Keywords: *Cedrus* deodara; Heapto-protection; Oxidative stress; Gluta-thione; Lipid peroxidation; Carbon tetra-chloride; Antioxidant; Phytochemistry.

Introduction

Liver disease constitutes one of the foremost causes of morbidity and mortality worldwide, with toxic and oxidative hepatic injury being implicated across a spectrum of pathologies ranging from drug-induced liver injury (DILI) to non-alcoholic steatohepatitis and chronic hepatitis[1-2]. The hepatocyte, as the primary functional unit of the liver, is uniquely exposed to chemical insults by virtue of its central role in xenobiotic biotransformation. Cytochrome P450 enzymes within the hepatocyte endoplasmic reticulum convert drugs and environmental toxins into reactive electrophilic and radical intermediates, which, when generated in excess, overwhelm endogenous defenses and initiate cellular injury[3]. Oxidative stress, the net imbalance between reactive oxygen species (ROS) generation and antioxidant neutralization capacity, is the convergent mechanistic pathway through which most hepatoxins exert their damage[4]. Among endogenous antioxidants, hepatic reduced glutathione (GSH) occupies a pivotal position: it functions as a direct free-radical scavenger, a cofactor for glutathione peroxidase in the detoxification of lipid hydroperoxides, and a substrate for glutathione-S-transferase-mediated conjugation of reactive metabolites[5]. Depletion of hepatic GSH is therefore both a reliable biomarker of oxidative injury and a pathogenic amplifier that sensitizes hepatocytes to further damage. Simultaneously, lipid peroxidation of polyunsaturated fatty acids in cell membranes, measured as malondialdehyde (MDA) equivalents via the thiobarbituric acid reactive substance (TBARS) assay, reflects the downstream consequences of unchecked ROS activity on membrane integrity[6].

Carbon tetrachloride (CCl_4) is the most extensively validated experimental model of oxidative hepatic injury. Following hepatic uptake, CCl_4 undergoes cytochrome P450 2E1-catalyzed homolytic cleavage to yield the trichloromethyl radical ($CCl_3•$), which rapidly reacts with dissolved oxygen to form the trichloromethylperoxy radical ($CCl_3OO•$). These radical species initiate lipid peroxidation cascades, deplete hepatic GSH, and cause hepatocyte necrosis indistinguishable histologically from human toxic hepatitis, making this model uniquely suitable for pre-clinical hepatoprotection evaluation[7-8].

Current pharmacotherapeutic options for toxic liver injury remain limited. Silymarin, a standardized flavonolignan complex from Silybum marianum, is the most widely used heaptoprotective agent in clinical practice, primarily through free-radical scavenging and GSH preservation[9].

Nevertheless, silymarin's modest oral bioavailability, inconsistent efficacy in advanced liver disease, and the complete absence of any regulatory approved synthetic agent specifically for toxic hepatic injury collectively define a substantive therapeutic gap that has sustained global investigation into plant derived hepatoprotective compounds[10-11].

Medicinal plant research has yielded numerous hepatoprotective leads, with polyphenols, flavonoids, and terpenoids consistently identified as the principal

active compound classes[12]. These phyto-constituents exert multi-mechanistic antioxidant effects: direct hydrogen atom donation to terminate radical chain reactions, chelation of pro-oxidant metal ions, inhibition of lipid peroxidation chain propagation, and transcriptional up regulation of endogenous antioxidant enzymes via the Nrf2/ARE pathway[13-14]. This multi-target profile is recognized as pharmacologically superior to single mechanism antioxidants and aligns with the complex, self-amplifying pathogenesis of oxidative hepatotoxicity. *Cedrus Deodara* (Roxb.) G. Don (family Pinaceae), the Himala-yan deodar cedar, is a large evergreen conifer distributed between 1,200 and 3,200 metres across the western Himala-yan range[15]. It occupies a well docu-mented position in Ayurvedic thera-peutics, referenced in the Charaka Samhita under multiple drug categories relevant to inflammation, metabolic dis-orders, and detoxification[16]. Phyto-chemical investigations of its wood and bark have identified sesquiterpene alcohols (himachalol, allo himachalol, beta-himachalene), di-terpenes, bioact- ive lignans (deodarone, *Cedrus*in), flavonoids and phenolic acids[17-18]. Documented pharmacological activities include anti-inflammatory[19], anti-oxidant[20], anti-hyperglycemic[21] and anti-cancer effects[22].

Despite this pharmacological profile, the leaf fraction of *C. deodara* has received minimal scientific attention and has never been investigated for heapto-protective activity. This represents a clear gap in the literature, since leaves offer a renewable, sustainably harvested plant resource and are known to concentrate bioactive phenolics amenable to methanolic extraction[23]. The present study was designed specifically to address this gap by evaluating the anti-oxidant mediated hepatoprotective potential of the methanolic *C. deodara* leaf extract in the CCl_4 rat model, with hepatic GSH and LPO as primary endpoints, alongside liver weight, body weight, and histo-pathological assessment.

The mechanistic basis of CCl_4 heapto-toxicity has been characterized with precision. Weber et al; established the molecular sequence from CYP2E1-mediated radical generation to self-propagating lipid peroxidation and centrilobular necrosis. Recknagel et al. confirmed that depletion of hepatic GSH and membrane lipid peroxidation are the earliest and most sensitive biochemical events, preceding overt histological damage, making them ideal pre-clinical efficacy endpoints for hepatoprotective agents.

Glutathione biochemistry in the context of hepatic oxidative injury was comprehensively characterized by Meister and Anderson, who demonstrated that intracellular GSH depletion below a critical threshold eliminates the heaptocyte's principal defence against reactive metabolites. Subsequent investigations established that the Nrf2/ARE signalling pathway governs transcriptional induction of GSH synthesis and antioxidant enzyme expression in response to oxidative challenge, and that plant flavonoids and polyphenols are potent Nrf2 activators, suggesting a molecular mechanism for their GSH-restorative hepatoprotective effects.

Silymarin, the reference hepato-protective compound, achieves hepato-protection primarily through free radical scavenging, inhibition of lipid per-oxidation, and GSH preservation, as demonstrated in multiple CCl_4 and other hepatotoxin models[24]. Vargas-Mendoza et al[25]; confirmed that silymarin maintained hepatic GSH and attenuated lipid peroxidation markers in experi-mentally stressed animals. Satyam et al[26]; further showed that antioxidant-anti-inflammatory combinatorial appro-aches produced superior histological recovery in CCl_4 models compared to silymarin alone, supporting the trans-lational advantage of multi-mechanism plant extracts. Investigative work on Himalayan medicinal plants has gene-rated relevant parallels. Nirmalkar et al[27]; demonstrated that the methane-olic leaf extract of *Lathyrus sativus* restored hepatic antioxidant status and serum biochemical markers in paracetamol hepatotoxic Wistar rats over 14 days with confirmed histopathological tissue recovery, providing a method-logical template for leaf extract evaluation. Within the genus *Cedrus*, Adinarayana and Subbaraju[28] isolated bioactive lignans from *C. deodara* wood with antioxidant structural features, and Zhang and Shi[29] provided a compre-hensive review identifying sesqui-terpenes as the pharmacologically dominant fraction. Sharma and Singh[28] confirmed the presence of flavonoids, alkaloids, tannins, and saponins in *C. deodara* leaves, establishing the phytochemical basis for the antioxidant pharmacology expected from this fraction. Sharma and Prasad reported significant anticancer activity mediated through antioxidant mechanisms in vivo. No published study, however, has evaluated the hepatoprotective efficacy of the leaf fraction, identifying the specific scientific gap this work addresses.

Materials and Methods

Ethical Clearance and Animal Care

All experimental procedures were conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of Siddhartha Institute of Pharmacy, Dehradun (IAEC Form B)[29]. Healthy adult male Wistar rats (150-200 g; 10-12 weeks) were procured from Shri Guru Ram Rai University, Dehradun, and housed under standard laboratory conditions (25 ± 2°C, 12-hour light/dark cycle) with free access to standard pellet diet and drinking water. A 7-day acclimatization period preceded all procedures.

Plant Material and Authentication

Fresh leaves of *Cedrus Deodara* were collected from Berinag, Uttarakhand, India, shade-dried, and coarsely powdered. The plant material was authenticated by the Botanical Survey of India, Dehradun (Authentication No. 1775).

Preparation of Methanolic Extract

Forty grams of dried leaf powder was subjected to Soxhlet extraction using methanol (400 mL) as solvent over 48 hours until the siphonate became colourless[30]. The extract was concen-trated under reduced pressure and stored at 4°C. OECD 423 acute oral toxicity evaluation at 2000 mg/kg revealed no mortality or observable toxicity, and working doses of 250 and 500 mg/kg were selected accordingly[31].

Phytochemical Screening

Qualitative phytochemical tests were performed on the extract for alkaloids (Dragendorff's, Mayer's, and Hager's tests), flavonoids (alkaline reagent and lead acetate tests), carbohydrates (Molisch's, Benedict's, and Fehling's tests), glycosides (Keller Killiani and Legal's tests), and tannins (ferric chloride test[32]).

Experimental Methods

Thirty male Wistar rats were randomly assigned to five groups (n = 6):

Group I (Normal Control): No treatment.
Group II (Disease Control): CCl_4 (1ml/ kg i.p., 1:1 v/v in olive oil) on days 7 and 14.
Group III *C. deodara* extract 250 mg/kg/day (oral, 14 days) + CCl_4.
Group IV *C. deodara* extract 500 mg/kg/day (oral, 14 days) + CCl_4.
Group V (Standard): Silymarin 100 mg/kg/day (oral, 14 days) + CCl_4.

Doses were calculated daily on body weight. Confirmatory hepatotoxicity induction was verified by retro-orbital blood sampling from one disease control animal after day 7.

BiochemicalandAntioxidant Estimation

At study termination (day 14), animals were sacrificed under anesthesia. Liver tissue was homogenized in 0.1M phosphate buffer (pH 7.4) and used to estimate:

(a) Hepatic GSH by Ellman's method[33] (U/L).
(b) Hepatic LPO by the TBARS assay[34] (nmol MDA/mg protein).

Liver weight was recorded immediately after excision. Body weights were measured on days 1, 7, and 14.

Histopathological Examination

Liver specimens were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 5 µm, and stained with haematoxylin and eosin. Sections were examined at 45× magnification under light microscopy[35].

Statistical Analysis

Data are expressed as mean ± SEM (n = 6). Statistical significance was assessed by one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ was considered significant[36].

Results

Phytochemical Screening

Phytochemical screening of the methanolic leaf extract confirmed the presence of alkaloids, flavonoids, carbohydrates, glycosides, and tannins **(Table-1)**. The presence of flavonoids, tannins, and phenolic constituents is of particular relevance to the antioxidant pharmacological profile of the extract.

Table-1 Phytochemical screening of methanolic leaf extract of *Cedrus deodara*

Phytochemical Class	Test Applied	Result
Alkaloids	Dragendorff's, Mayer's, Hager's tests	Present (+)
Flavonoids	Alkaline reagent test, Lead acetate test	Present (+)
Carbohydrates	Molisch's, Benedict's, Fehling's tests	Present (+)
Glycosides	Keller-Killiani test, Legal's test	Present (+)
Tannins	Ferric chloride test	Present (+)

(+) denotes presence of phytoconstituent

Body Weight- Normal control animals showed consistent weight gain throughout the study period. Disease control animals receiving CCl_4 exhibited progressive body weight loss from day 7, reflecting systemic catabolic effects of hepatotoxic inflammation. Both extract-treated groups and the silymarin group showed attenuated weight loss, with the 500 mg/kg group maintaining body weight most closely to the normal trajectory (**Table-2**).

Table-2 Body weight (g) of rats across treatment groups on days 1, 7, and 14

Group	n	Day 1 (g)	Day 7 (g)	Day 14 (g)
Normal Control	6	132 ± 25.6	145 ± 26.8	138 ± 31.9
Disease Control (CCl_4)	6	130 ± 18.4	118 ± 19.2	110 ± 21.5
C. deodara 250 mg/kg	6	128 ± 17.3	125 ± 18.4	120 ± 19.7
C. deodara 500 mg/kg	6	131 ± 16.8	138 ± 15.2	125 ± 14.9
Silymarin 100 mg/kg	6	161 ± 12.2	163 ± 12.4	149 ± 10.8

Values expressed as mean ± SEM. Individual rat weights given in thesis raw data.

Liver Weight

CCl_4 caused significant hepatomegaly in the disease control group (17.39 ± 0.34 g) versus normal control (11.02 ± 0.31 g; $p<0.001$). Extract treatment reduced liver weight dose-dependently (250 mg/kg: 8.18 ± 0.16 g; 500 mg/kg: 10.24 ± 0.31 g; both $p<0.001$ vs. disease control). The 500 mg/kg group was comparable to silymarin (11.21 ± 0.14 g; $p<0.001$ vs. disease control) (Table-3, Figure-1).

Table-3 Effect of various treatments on liver weight (g) (mean ± SEM, n=6)

S. No.	Group	Liver Weight (g)	Significance
1	Normal Control	11.02 ± 0.31	---
2	Disease Control (CCl_4)	17.39 ± 0.34	p<0.001 vs. NC
3	*C. deodara* 250 mg/kg + CCl_4	8.18 ± 0.16	p<0.001 vs. DC
4	*C. deodara* 500 mg/kg + CCl_4	10.24 ± 0.31	p<0.001 vs. DC
5	Silymarin 100 mg/kg + CCl_4	11.21 ± 0.14	p<0.001 vs. DC

NC = Normal Control; DC = Disease Control. One-way ANOVA + Tukey's post-hoc.

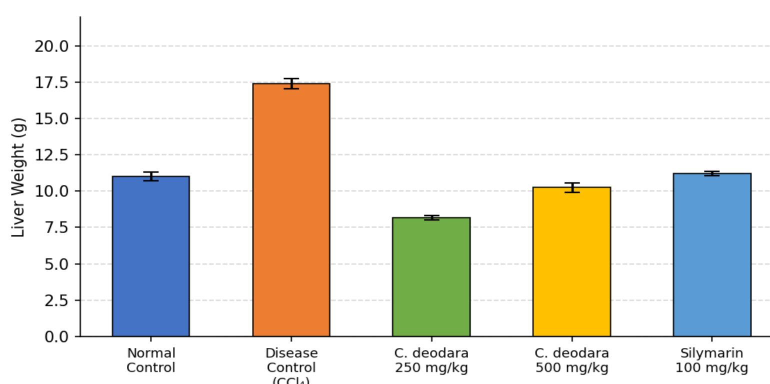

Figure-1 Effect of *C. deodara* extract on liver weight in CCl_4 induced hepatotoxicity

Hepatic Glutathione (GSH)

Hepatic GSH was significantly depleted in the disease control group (15.83 ± 1.47 U/L) relative to normal control animals (22.83 ± 1.94 U/L; $p<0.001$), reflecting consumption of the glutathione pool during free-radical detoxification. Treatment with the extract produced dose dependent, statistically significant GSH restoration: 250 mg/kg yielded 18.50 ± 1.05 U/L ($p<0.001$ vs. disease control), and 500 mg/kg yielded 19.67 ± 1.03 U/L ($p<0.001$ vs. disease control). Importantly, there was no significant difference between the 500 mg/kg group and silymarin (21.50 ± 1.05 U/L; $p=0.165$), confirming comparable antioxidant efficacy at this dose (Table-4, Figure-2).

Table-4 Effect of various treatments on hepatic GSH level (U/L) (mean ± SEM, n=6)

S. No.	Group	GSH (U/L)	Mean Diff.	Adjusted p Value
1	Normal Control	22.83 ± 1.94	---	---
2	Disease Control (CCl_4)	15.83 ± 1.47	+7.00 vs. NC	<0.001
3	*C. deodara* 250 mg/kg + CCl_4	18.50 ± 1.05	-2.67 vs. DC	<0.001
4	*C. deodara* 500 mg/kg + CCl_4	19.67 ± 1.03	-3.83 vs. DC	<0.001
5	Silymarin 100 mg/kg + CCl_4	21.50 ± 1.05	-5.67 vs. DC	<0.001

500 mg/kg vs. Silymarin: mean diff. = -1.83; p = 0.165 (NS). One-way ANOVA + Tukey's post-hoc.

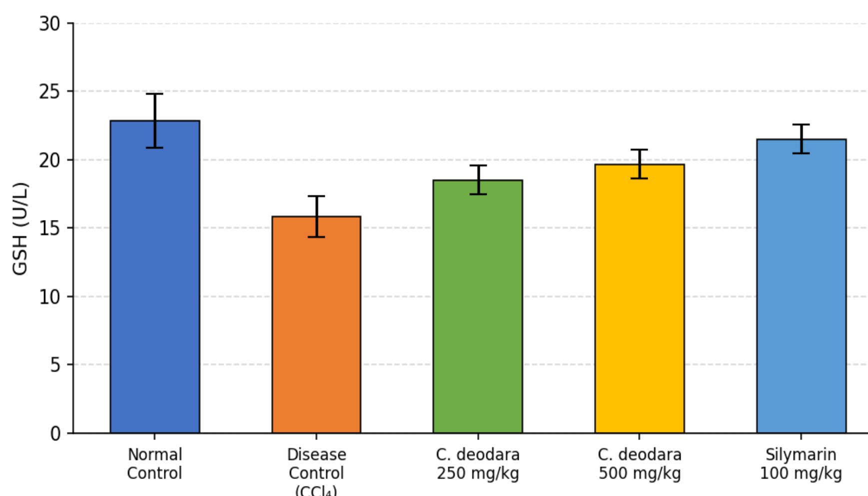

Figure-2 Effect of *C. deodara* extract on hepatic GSH level (U/L)

Hepatic Lipid Peroxidation (LPO)

Hepatic LPO values in the disease control group (16.00 ± 1.41 nmol/mg protein) were significantly lower than normal controls (24.00 ± 1.79 nmol/mg protein; $p<0.001$). This pattern reflects the known consequence of severe CCl_4-induced necrosis: depletion of peroxidable lipid substrate and suppression of microsomal enzyme activity eliminate the substrate for ongoing peroxidation at the 14-day endpoint. Extract treatment restored LPO values toward normal in a dose-dependent manner: 250 mg/kg produced 17.33 ± 1.63 nmol/mg ($p=0.562$ vs. disease control; NS), while 500 mg/kg produced 21.33 ± 1.21 nmol/mg ($p<0.001$ vs. disease control). The silymarin group achieved 25.33 ± 1.51 nmol/mg, near normal. LPO normalization in treated groups reflects

recovery of membrane lipid homeostasis during hepatocellular regeneration, confirmed histopathologically (Table-5, Figure-3).

Table-5 Effect of various treatments on hepatic LPO level (nmol/mg protein) (mean ± SEM, n=6)

S. No.	Group	LPO (nmol/mg)	Mean Diff.	Adjusted p Value
1	Normal Control	24.00 ± 1.79	---	---
2	Disease Control (CCl_4)	16.00 ± 1.41	+8.00 vs. NC	<0.001
3	*C. deodara* 250 mg/kg + CCl_4	17.33 ± 1.63	-1.33 vs. DC	0.562 (NS)
4	*C. deodara* 500 mg/kg + CCl_4	21.33 ± 1.21	-5.33 vs. DC	<0.001
5	Silymarin 100 mg/kg + CCl_4	25.33 ± 1.51	-9.33 vs. DC	<0.001

NS = Not significant. One-way ANOVA + Tukey's post-hoc.

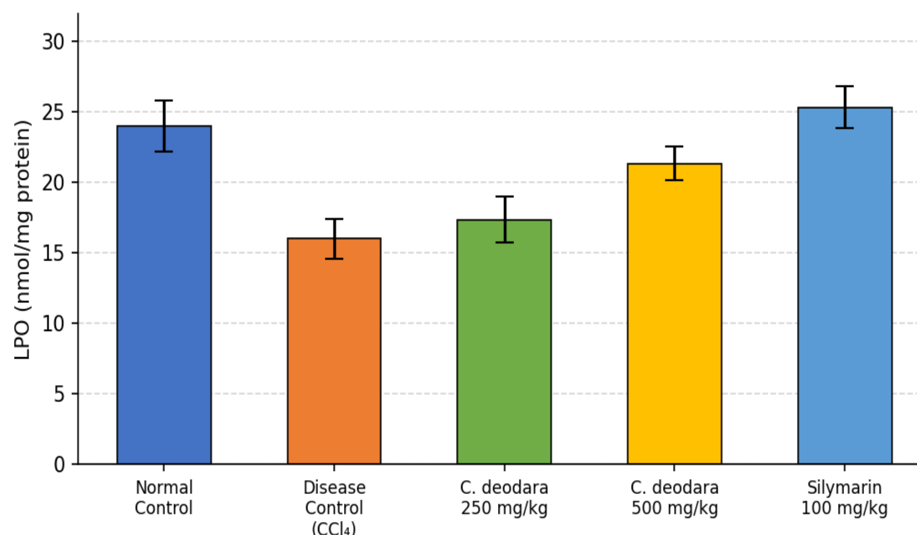

Figure-3 Effect of *C. deodara* extract on hepatic LPO level (nmol/mg protein)

Histopathology

Normal control liver sections (Fig.a) showed intact hepatic cords, well-defined central veins, and normal portal triads with no cellular damage. Disease control sections (Fig.-b) revealed moderate hepatocyte degeneration with dropout necrosis, ballooning predominantly in the periportal zone, prominent fatty change, mononuclear inflammatory infiltration, congestion, and marked cholestasis, consistent with classical CCl_4 oxidative hepatic injury. The 250 mg/kg group (Fig. c) showed mild-to-moderate degeneration with vesicular change, reduced but persistent necrosis and steatosis. The 500 mg/kg group (Fig.d) demonstrated minimal hepatocyte degeneration, moderate Kupffer cell hyperplasia, mild fatty change, and scant hemorrhage, indicating substantially preserved hepatic architecture. The silymarin group (Fig. e) showed only occasional hepatocyte degeneration with regenerative changes and prominent Kupffer cell hyperplasia, a near normal histological profile closely paralleled by the 500 mg/kg group.

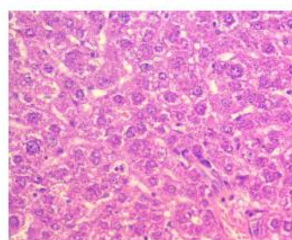

Fig- (a) Normal Control

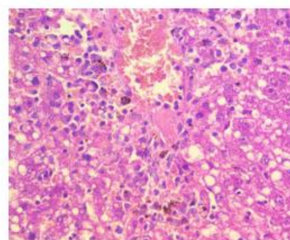

Fig- (b) Disease Control

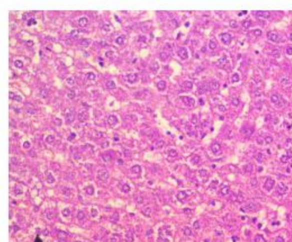

Fig- (c) C. Deodara 250mg/kg

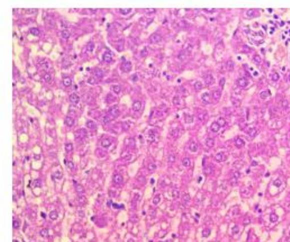

Fig- (d) C. Deodara 500mg/kg

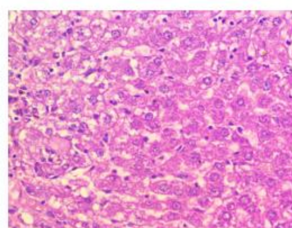

Fig-(e) Silymarin 100mg/kg

Figure-4 Photomicrographs (45×) of H&E-stained rat liver sections. (a) Normal Control: normal hepatic architecture; (b) Disease Control (CCl_4): necrosis, ballooning degeneration, steatosis, cholestasis; (c) *C. deodara* 250 mg/kg: mild-moderate degeneration with reduced injury; (d) *C. deodara* 500 mg/kg: minimal degeneration, Kupffer cell hyperplasia; (e) Silymarin 100 mg/kg: regenerative changes, near-normal architecture.

Discussion

This study provides the first systematic pre-clinical evidence that the methanolic leaf extract of *Cedrus Deodara* confers significant antioxidant mediated heaptoprotection against CCl_4 induced oxidative liver injury in a dose-dependent manner. The coherent pattern of GSH restoration, LPO normalization, liver weight reduction, and histopathological recovery across both treatment doses, with the 500 mg/kg dose achieving equivalence with silymarin on the primary antioxidant endpoint, constitutes robust pre-clinical evidence of hepatoprotective efficacy. The hepatic GSH depletion observed in the group is the predictable biochemical signature of CCl_4 toxicity. As trichloromethyl radicals are generated by CYP2E1 biotransformation, GSH is consumed both enzymatically through glutathione peroxidase catalyzed hydroperoxide reduction and non-enzymatically through direct adduct formation with radical intermediates. Once depleted, the glutathione pool can no longer buffer subsequent oxidative insults, creating a vicious cycle of amplifying damage. The significant and dose dependent restoration of hepatic GSH by *C. deodara* extract, with statistical equivalence to silymarin at 500 mg/kg, indicates that the

extract's polyphenolic and flavonoid constituents either scavenge radicals upstream, sparing GSH from consumption, or activate Nrf2/ARE-driven transcriptional induction of GSH synthesis, as reported for structurally similar flavonoids. The LPO pattern requires contextual mechanistic inter-pretation. Reduced LPO in the disease control relative to normal animals at the 14 day endpoint, rather than the elevated values often assumed, reflects known biophysical consequences of severe heaptocellular necrosis: extensive cell-ular destruction depletes the poly-unsaturated lipid substrate available for peroxidation and suppresses the CYP 2E1 driven radical generation that sus-tains the peroxidative cascade. The acute MDA burst characteristic of CCl_4 injury resolves substantially within 48-72 hours, leaving reduced residual values at the chronic endpoint that reflect substrate depletion rather than anti-oxidant recovery. The dose dependent restoration of LPO toward normal values in extract treated animals, most signify-cantly at 500 mg/kg, is therefore corr-ectly interpreted as a marker of membrane lipid replenishment and resto-ration of normal hepatocellular lipid metabolism during regeneration, a concl-usion fully supported by the parallel histopathological observations of redu-ced steatosis and improved hepatocyte architecture.

The multi-component phytochemical profile of the extract provides a mech-anistically coherent basis for the obser-ved effects. Flavonoids, the principal antioxidant compound class identified, act through at least four complementary mechanisms: direct hydrogen atom donation to terminate lipid radical chain reactions, chelation of redox-active iron and copper ions that catalyze Fenton-type hydroxyl radical formation, allosteric inhibition of prooxidant enzymes including xanthine oxidase and NADPH oxidase, and Nrf2-mediated transcriptional upregulation of glutamate cysteine ligase and glutathione reduc-tase[37]. Tannins contribute additional membrane stabilizing and astringent eff-ects that limit hepatocyte membrane per-meabilization. Sesquiterpene terpe-noids characteristic of C.deodara , particularly himachalol, exert anti-inflammatory activity through inhibition of prosta-glandin synthesis and neutrophil margination, addressing the inflame-matory amplification phase of CCl_4-induced injury. Alkaloids may further contribute through membrane stabilizing effects that reduce lysosomal enzyme release. Kupffer cell hyperplasia in the 500 mg/kg and silymarin groups reflects active phagocytic clearance of necrotic debris and initiation of hepatocyte regeneration, and may additionally reflect immune-modulatory modulation of the resident macrophage pool by the extract's terpenoids and flavonoid constituents[38]. The absence of acute toxicity at 2000 mg/kg (OECD 423) and excellent tolerability at therapeutic doses over 14 days establishes an adequate pre-clinical safety profile for further development.

Conclusion

The methanolic leaf extract of *Cedrus Deodara* demonstrates significant, dose-dependent, antioxidant mediated hepato-protection against CCl_4 induced oxidative liver injury in male Wistar rats. The extract significantly restored hepatic

GSH and normalized LPO in a dose-dependent fashion, with the 500 mg/kg dose-achieving efficacy statistically equivalent to silymarin (100 mg/kg) on the primary antioxidant endpoint. Liver weight normalization, body weight pre-servation, and histopathological evi-dence of reduced necrosis, decreased steatosis, and hepatocyte regeneration comprehensively corroborate these find-ings. The phytochemical profile, inclu-ding flavonoids, tannins, alkaloids, and terpenoids, provides a mechanistically coherent multi-target antioxidant and anti-inflammatory basis for these effects. These results validate, for the first time, the hepatoprotective potential of the *C. deodara* leaf fraction and establish a rigorous pre-clinical foundation for further pharmacological development.

Future Perspectives

Future research should employ HPLC-MS and GC-MS-based phytoconstituent profiling to identify the specific bio-active molecules responsible for hepato-protection and enable standardization of the extract. Mechanistic pathway studies targeting Nrf2/ARE, NF-κB, and TNF-α signaling will clarify the molecular basis of action. Sub-chronic and chronic toxi-city evaluation across multiple species is essential before clinical translation. Nano-formulation or other delivery system development may improve the oral bioavailability of the active constituents. Parallel investigation of nephron-protective and neuro-protective activities would capitalize on the established antioxidant profile of the extract. Ultimately, well-designed pilot clinical trials in patients with hepatic dysfunction, subject to regulatory app-roval, represent the translational goal that these pre-clinical findings now justify.

Conflict of Interest

The authors declare no conflicts of interest.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Quantitative Estimation of Phenolic and Flavonoids Contents and Antioxidant Activity of Aerial Parts of *Morina logifolia*

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Abstract-Since ancient times plants have served as vital components of traditional, folk medicine, and fulfilling essential nutritional and dietary needs. The extracts of various plant species have been reported to possess anti-oxidant activities to scavenge free radicals. Therefore, the present study was aimed to determine the total phenolic, flavonoid content, free radical scavenging potential, and reducing power of chloroform extract (MCF), ethyl acetate extract (MEA), acetone extract (MAT) and ethanol extract (MAL) of aerial parts of *Morina longifolia*. The results of the present study showed that different extracts of aerial parts of *M. longifolia* showed significant antioxidant activity. The acetone extract of aerial parts showed maximum total phenolic contents (MAT 15.47 ± 0.187 mg GAE/100mg) while ethyl acetate showed maximum flavonoid contents (MEA 4.27 ± 0.086 mg quercetin/100mg). Maximum reducing power was observed in ethyl acetate extract (MEA 0.556 ± 0.003, at 500µg/ml) and highest free radical scavenging activity was observed in acetone extract (MAT 57.40 ± 0.592 at 500µg/ml).

Keywords: *Morina longifolia*, Falvonoid, Phenolic, Antioxidant Activity, DPPH, Reducing Power Assay.

Introduction

Plants are important source of medicines because they contain a wide range of secondary metabolites. The crude extracts of plants are used in traditional medical system while in modern and herbal medical systems plants have been exploited for the isolation of specific bioactive compounds[1,2]. Plants rich in phytochemicals like flavonoids, phenols, alkaloids, tannins, terpenoids shows many biological activity including antioxidant activity without any side effects[3,4]. Several studies have shown that the plant extracts obtained using different solvents such as methanol, ethanol and aqueous media exhibit potent antioxidant activities. The antioxidant activity is correlated with total phenolic and flavonoid content of the extracts. The extracts of various medicinal plant shows strong free radical scavenging activities comparable to synthetic antioxidant[5-9].

The genus *Morina* belongs to the family *Caprifoliaceae* formerly *Dipsacaceae* is

a genus of flowering plants. It includes 14 species natives to Eurasia ranging from southern Europe through Western and Central Asia. Morina species, namely *M. chinensis, M. chlorantha, M. kokonorica, M. longifolia, M. lorifolia and M. nepalensis* have been traditionally used in Tibetan, Himalayan and Central Asian systems of medicine. Phytochemical studies reveal the presence of iridoids, phenolic acids, flavonoids, terpenoids, aromatic glycosides, essential oils and other bioactive compounds which contribute to their wide-ranging pharmacological potential including anti-oxidant, anti-inflammatory, analgesic, anti-cancer, anti-microbial, antidiabetic and wound healing activities[10-15]. *M. longifolia* commonly known as 'Whorlflowers' is a small perennial herb, found in temperate and alpine regions of Himalayas from Kashmir to Bhutan at an altitude of 2400-4200 meters[16]. The antioxidant activity of *M. longifolia* of Uttarkashi region have not been carried out previously therefore the present study was aimed to determine the free radical scavenging activity and reducing potential of different extracts of aerial parts of *M. longifolia.*

Material and Methods

Preparation of extracts- The aerial parts of *M. longifolia* were collected from Dayara Bugyal (3300-3500 m asl), District Uttarkashi, Uttarakhand, India in August 2024. Plant species were identified by Dr. Pratibha Baluni, Department of Botany, Govt. Degree College Dehradun Shahar, Dehradun, Uttarakhand. The air dried and powdered aerial parts of *M. longifolia* was packed in a Soxhlet apparatus and extracted sequentially with chloroform, ethyl acetate, acetone and ethanol. The organic extracts were dried in a vacuum evaporator to obtain the chloroform extract (MCF), Ethyl acetate extract (MEA), Acetone Extract (MAT) and ethanol extract (MAL). The extracts were dissolved in dimethyl sulfoxide (DMSO) or ethanol prior to analysis depending upon their solubility. The extracts were subjected for further analysis and all the assays were done in triplicate

Determination of Total Phenolics and Total Flavonoid Contents- The total phenolic contents (TPC) of the different extracts of areal parts of *M. longifolia* was determined by modified Folin-Ciocalteu method [17] using gallic acid as standard. Folin-Ciocalteu reagent and Na_2CO_3 was added to the various extracts and after 20 min of incubation at room temperature, the mixture was centrifuged and the absorbance of the supernatant was measured at 730 nm. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g) (Table-1 and Figure-1).

The total flavonoid content (TFC) was determined by slightly modified colorimetric method [18]. The extracts were dissolved with suitable solvent and then potassium acetate and aluminium nitrate was added to it. After 40 min of incubation at room temperature, the absorbance was measured at 415 nm using quercetin as standard and results are presented in (Table-1 and Figure-1).

Table-1 Total phenolic and flavonoid contents of different extracts of aerial parts of *M. longifolia*

Extract	Total flavonoids mg Quercetin/100mg	Total phenolics mg GAE/100mg
MCF	0.70 ± 0.015	10.22 ± 0.095
MEA	4.27 ± 0.086	14.68 ± 0.124
(MAT)	3.70 ± 0.031	15.47 ± 0.187
(MAL)	3.59 ± 0.014	12.72 ± 0.280

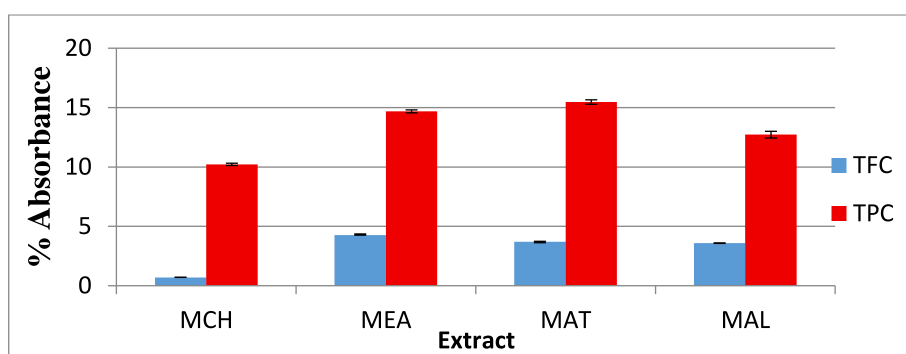

Figure- 1 Total Flavonoid and Phenolic Contents of different extracts of aerial parts of *M. longifolia*

Antioxidant Activity

Determination of reducing potential (RP)- In this method, the reduction potential of the extracts was carried out by reduction of potassium ferricyanide to potassium ferrocyanide using ascorbic acid as standard. Five concentrations (100, 200, 300, 400 and 500 µg/ml) of each extract were prepared by dissolving with suitable solvent and the reducing potential each extract was determined by the method described by Oyaizu, 1986 [19]. In one ml of each concentration of each extract 2.5 ml phosphate buffer and 2.5ml of potassium ferricyanide (10mg/ ml) solution was added. The content was heated at 50^0C for 30 min and then 2.5 ml of 10% trichloroacetic acid was added to it and the mixture was centrifuged for 10 min at 3000-4000 rpm. In 2.5 ml of supernatant was diluted with 2.5 ml double distilled water and 0.5 ml of 0.1% $FeCl_3$ solution was added to it and the absorbance was recorded at 700 nm. The results were compared with ascorbic acid taken as positive control and represented as ascorbate equivalents (Table-2, Figure- 2).

Table-2 Reducing potential of different extracts of aerial parts of *M. longifolia*

CONC	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
STD	0.23	0.43	0.53	0.63	0.66
MCF	0.033 ± 0.000	0.070 ± 0.001	0.093 ± 0.000	0.105 ± 0.001	0.124 ± 0.002
MEA	0.116 ± 0.002	0.240 ± 0.003	0.336 ± 0.002	0.438 ± 0.004	0.556 ± 0.003
MAT	0.076 ± 0.002	0.162 ± 0.003	0.256 ± 0.002	0.339 ± 0.004	0.381 ± 0.006
MAL	0.108 ± 0.002	0.239 ± 0.004	0.309 ± 0.004	0.347 ± 0.003	0.367 ± 0.003

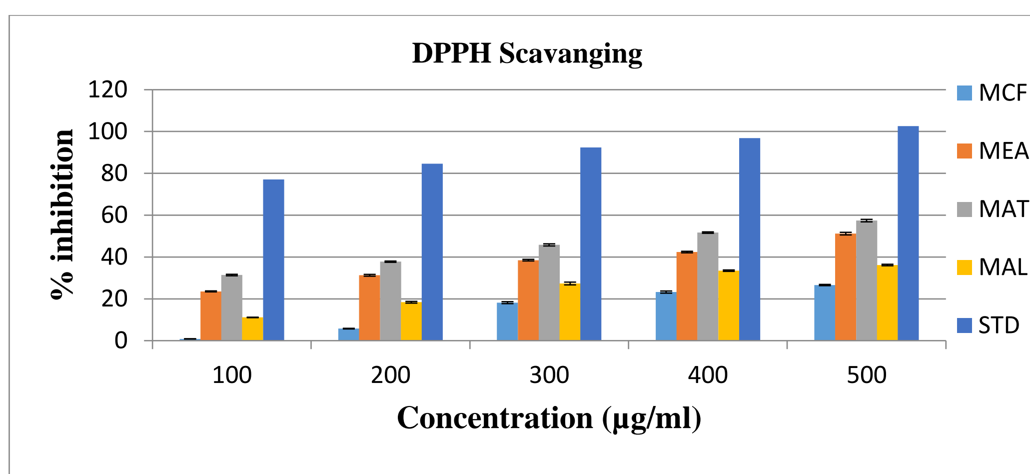

Figure-2 DPPH Assay of different extracts of aerial parts of *M. longifolia*

DPPH Radical Scavenging Activity (DPPH)- 2,2-Diphenyl-1-picrylhydrazyl commonly known as DPPH is composed of stable free radical molecules used as indicator for evaluation antioxidant activity of various compounds and extracts. It is deep blue coloured dyes changes to light yellow to colorless on reduction depending on anti-oxidant activity of the material under investigation. The redical scavenging activity was determined by the method described by **Cuendet** [20] with slight modifications. Five concentrations (100, 200, 300, 400 and 500 µg/ml) of each extract were prepared with suitable solvent and the antioxidant activity of each plant extracts was assessed using the stable DPPH radical scavenging with ascorbic acid as standard. Two ml of freshly prepared ethanolic solution DPPH (0.1 M) solution was added to the different concentrations of the extracts. After 30 min of incubation in dark, the absorbance was recorded at 517 nm. The results were expressed as percentage inhibition of DPPH.

$$\% \text{ Inhibition} = [\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}] \times 100$$

The results were compared with ascorbic acid as the positive control and are presented in (Table-3, Figure-3).

Table-3 DPPH assay of different extracts obtained from aerial parts of *M. longifolia*

CONC.	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
STD	77.03	84.56	92.33	96.86	102.6
MCF	0.817 ± 0.014	5.747 ± 0.142	18.16 ± 0.463	23.22 ± 0.506	26.59 ± 0.292
MEA	23.55 ± 0.231	31.26 ± 0.421	38.49 ± 0.387	42.36 ± 0.364	51.17 ± 0.616
MAT	31.37 ± 0.360	37.76 ± 0.289	45.78 ± 0.506	51.67 ± 0.341	57.40 ± 0.592
MAL	11.10 ± 0.105	18.35 ± 0.409	27.33 ± 0.660	33.46 ± 0.336	36.17 ± 0.355

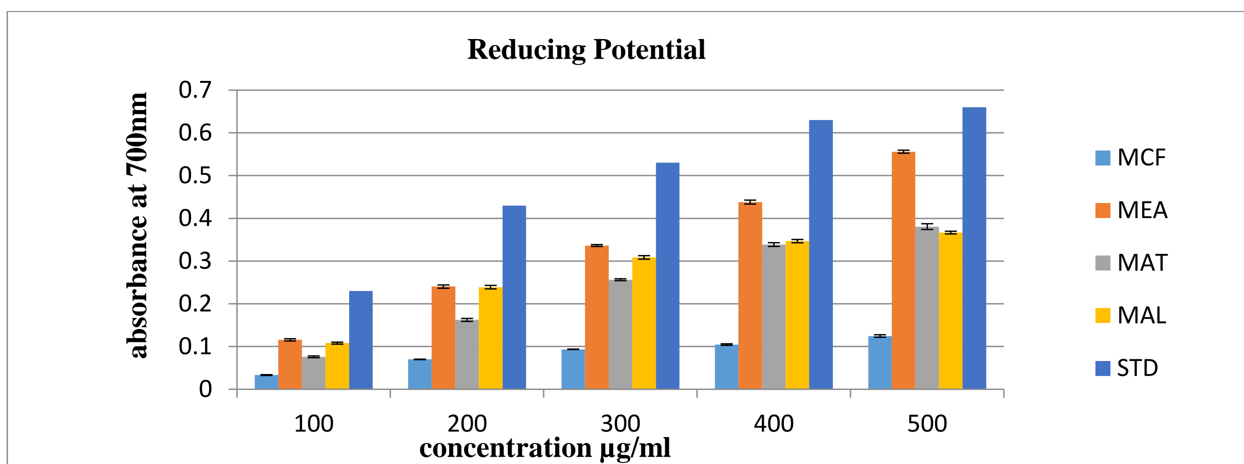

Figure-3 Reducing potential of different extracts of aerial parts of *M. longifolia.*

Results and Discussion

Total phenolic and flavonoids contents- The Phenolic substances and flavonoids have been shown to be responsible for the antioxidant activity of many of the plant species (Geetha et al., 2005) [21]. Therefore, the amount of total phenolics and total flavonoids present in the different extracts were investigated. The content of total phenolics is expressed as mg Gallic acid/100 mg of extract (GAE) and the content of total flavonoids as mg quercetin/100 mg of extract (QE) as shown in Table 1. The acetone extract (MAT) of *M. longifolia* was found to have the maximum content of total phenolics contents, 15.47 ± 0.187 mg (GAE/100 mg extract) and total flavonoids content was found in ethyl acetate extract (EA) 4.27 ± 0.086 mg QE/100 mg extract). *M.* Chloroform (MCF) extract had the lowest content of total phenolics content (10.22 ± 0.095 mg GAE/100 mg extract) as well as total flavonoids content (0.70 ± 0.015 mg QE/100 mg extract).

Reducing Power- The reducing potential of different extracts of *M. longifolia* was determine by using five different concentration of each extract and was compared with ascorbic acid as positive control. The reducing potential was found to be concentration dependent and the order of RP was found MCF<MAL<MAT<MEA. The MEA extract shows maximum reducing potential at the conc. of 500 µg/ml (**0.556 ± 0.003)** which is slightly less than the standard (0.63). Minimum reducing potential was found in MCF extracts (0.033±0.001) at the concentration of 100 µg/ml.

DPPH Radical Scavenging Activity- Five different concentrations of each extract of *M. longifolia* were used to determine the DPPH scavenging activity using ascorbic acid as positive control. The free radical scavenging activity of MAT extracts of *M. longifolia* showed maximum activity whereas MCF showed minimum activity. It was found that the free radical scavenging activity of all extracts increases with the increase in concentrations. The order of DPPH scavenging activity of different extracts was found to be MCF<MAL<<MEA<MAT.

The result of present study showed that acetone and ethyl acetate extracts shows more reducing potential and DPPH scavenging activity then the other extracts which might be due to presence of polyphenolic and flavonoid compounds in these extracts. Our previous study on different extracts of *M. longifolia* revealed positive test for polyphenolic compounds in the acetone and ethyl extracts [22]. Previous study on antioxidant activity shows that extracts rich in polyphenolic compounds showed antioxidant activity. A study by Matheus et al. (2025) on antioxidant activity of *M. oleifera* showed that the leaf and seed oil extracts exhibited high antioxidant activity due to rich concentration of polyphenolic and flavonoid compounds [23].

Polyphenolic compounds and flavonoids represent one of the largest groups of secondary metabolites in plants which play a major role in reducing the oxidative stress. The antioxidant activity of these compounds is due to their ability to neutralize the free radicals, to coordinate with pro-oxidant metals and to modulates the antioxidant enzyme systems [24]. Based on the clinical evidences, diets rich in polyphenols and flavonoids are suggested to confer protective effects against chronic diseases, particularly cancer, diabetes, inflammatory disorders, nervous system diseases and cardiovascular diseases etc. [25-26].

The present study revealed the scavenging property of *M. longifolia* may be due to the presence of polyphenolic and flavonoid compounds that can scavenge the free radicals and chelate the pro-oxidant metals. The result of present study showed that acetone and ethyl acetate extracts which shows more reducing potential and DPPH scavenging activity then the other extracts.

Conclusion

From the above study it can be concluded that all the extracts of aerial parts of *M. longifolia* exhibited antioxidant activity but the extracts which are rich in phenolic and flavonoid contents shows more activity than the other extracts.

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Disclaimer Statement

Author declares that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Phytochemical and Neuroprotective activity of *Coccinia grandis* leaves using hydroxydopamine (6-OHDA) Model

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Abstract- Cognitive impairment in neuro-degenerative disorders is closely associated with oxidative stress, neuro-inflammation, and cholinergic dysfunction. *Coccinia grandis*, a medicinal plant rich in bioactive constituents, has been explored for its potential neuroprotective effects. The present study aimed to evaluate the neuroprotective activity of hydroalcoholic fruit extract of *Coccinia grandis* and its fractions against scopolamine induced memory impairment in rats. The extract was subjected to phytochemical screening, thin-layer chromatography (TLC), and column chromatography for characterization. Neurobehavioral assessments were conducted using the Morris Water Maze (MWM) and Elevated Plus Maze (EPM) models. Biochemical parameters including acetylcholinesterase (AChE), interleukin-1β (IL-1β), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase were evaluated. Scopolamine administration significantly impaired cognitive function, as evidenced by increased escape and transfer latency and reduced retention time. Treatment with *Coccinia grandis* extract (200mg/kg) significantly improved behavioral performance, comparable to the standard drug rivastigmine. Biochemical findings showed reduced AChE, IL-1β, and MDA levels, along with enhanced anti-oxidant enzyme activity (SOD and catalase). Acute toxicity studies confirmed safety up to 1000 mg/kg. Overall, the findings suggest that *Coccinia grandis* exhibits significant neuroprotective activity via antioxidant, anti-inflammatory, and cholinergic modulation mechanisms.

Introduction

The disturbance of the normal anatomical and functional integrity of the skin or underlying tissues due to physical, chemical, or biological forces is referred to as a wound. Any damage to the skin triggers a complicated series of physiological processes intended to restore tissue integrity since the skin serves as the body's main defense against environmental dangers and microbial invasion[1]. Trauma, burns, surgery, infections, and chronic illnesses like diabetes can all cause wounds that seriously hinder the healing process[2]. Wounds are categorized as either acute or chronic based on their ability to heal; chronic wounds provide significant treatment problems because of prolonged inflammation and delayed repair[3]. Hemostasis, inflammation, proliferation, and remodeling are the four overlapping stages of the tightly controlled process of wound healing. Coordinated

interactions between immune cells, fibroblasts, keratin-ocytes, cytokines, and growth factors are necessary for these stages[4]. Any interference with these processes brought on by oxidative stress, infection, or systemic diseases might cause problems and postpone healing. Clinically, wounds are a major worldwide burden that frequently results in morbidity, higher medical expenses, and a lower standard of living[5].

The epidermis, dermis, and hypodermis make up the skin, which is essential for immunological defense, thermoregulation, and protection. When this structure is damaged, the repair process is initiated and its barrier function is compromised[6]. Due to their interference with collagen synthesis, angiogenesis, and cellular regeneration, infection, oxidative stress, nutritional deficiencies, aging, and systemic disorders like diabetes are among the many factors that affect wound healing.

In wound healing, oxidative stress has two functions. Excessive reactive oxygen species (ROS) can harm proteins, lipids, and DNA, which eventually delays tissue repair, while moderate ROS levels support cellular signaling and microbial defense[7]. Antioxidants are therefore crucial for preserving redox equilibrium and encouraging repair.

Antiseptics, antibiotics, anti-inflammatory medications, and synthetic dressings are examples of conventional wound treatments. Despite their effectiveness, these methods have drawbacks such cytotoxicity, antimicrobial resistance, high cost, and insufficient healing in long-term circumstances[1-8]. Alternative remedies are becoming more popular as a result of these difficulties.

The abundance of bioactive substances found in medicinal plants, such as flavonoids, tannins, and phenolics, which have antibacterial, anti-inflammatory, and antioxidant properties, has drawn attention[9]. These characteristics make plant-based treatments viable substitutes for wound care because they promote increased collagen synthesis, angiogenesis, and tissue regeneration[4,9].

Because they offer structurally varied molecules with substantial pharmacological potential, natural products have historically been essential to drug discovery. The relevance of plants in therapeutic development is highlighted by the fact that many contemporary medications are derived from them[10]. Since ancient systems like Ayurveda and Unani have traditionally used plant-based formulations to treat skin injuries, ethnomedical knowledge further supports the use of plants in wound healing[11].

Chronic wounds continue to be a major problem despite developments in contemporary medicine, requiring the creation of safer and more potent treatments. *Mimosa tenuiflora* has bioactive chemicals with possible therapeutic properties and is traditionally used to heal burns, wounds, and infections. Its ability to cure wounds, however, has to be systematically validated by science using experimental models.

Coccinia grandis

The perennial climbing vine *Coccinia grandis* (L.) Voigt (Cucurbitaceae) is found throughout tropical regions, including Southeast Asia, India, and Sri Lanka. It thrives in open fields and hedges and is sometimes referred to as ivy gourd (Tindora, Kovai). The plant produces white

dioecious flowers and simple, alternating leaves with three to five lobes. Fruits are cylindrical, length (2–6 cm), green when immature and scarlet when ripe, and packed with seeds. Fruits, leaves, and roots have historically been used to treat inflammation, fever, and diabetes. The fruits are eaten as vegetables as well. Along with nutritional elements like fiber and vitamin C, phytochemical ingredients include flavonoids (quercetin, kaempferol), triterpenoids (cucurbitacins), and other substances like alkaloids, glycosides, and phenolics.

Pharmacologically, the herb has antipyretic, anti-inflammatory, anti-diabetic, and antioxidant properties. Fruits are picked when they are ripe for therapeutic uses and when they are green for consumption. Formulations typically use powdered or fresh fruits (10–20 g/day)[12].

Material and Methods

Plant Material Standardization- Ash levels and moisture content were among the pharmacopoeial characteristics used to standardize the authorized plant material. To assess purity and inorganic content, total ash, water-soluble ash, and acid-insoluble ash were measured. Loss on drying using an oven technique was used to determine the moisture content. To guarantee the purity and identification of the crude medication, these procedures were performed in accordance with conventional standards[13].

Table-1 List of Chemicals

Sr .no	Chemicals	Supplied by
1	Ethanol	Solanki enterprise pune
2	H2so4	Solanki enterprise pune
3	Ferric chloride	Solanki enterprise pune
4	Iodine	Solanki enterprise pune
5	Ethyl acetate	Solanki enterprise pune
6	Chloroform	Solanki enterprise pune
7	Petroleum ether	Solanki enterprise pune

Extraction of *Coccinia grandis* - Soxhlet extraction was performed on 150 g of dried *Coccinia grandis* leaf powder using a hydroalcoholic solvent (ethanol:water, 80:20). The extraction was done for 16 hours at 30 to 40°C. A semisolid extract (~100 ml) was produced by concentrating the extracted material (~500 ml) through evaporation[14].

Phytochemicals Screening - To find the main bioactive components, the ethanolic extract was put through a qualitative phytochemical screening. Based on distinctive color changes and precipitate formation, standard tests verified the presence of carbohydrates (Molisch test), alkaloids (Hager's test), saponins (foam test), tannins (ferric chloride test), terpenoids (Salkowski test), glycosides (Keller–Killiani test), steroids, phenols, amino acids (ninhydrin test), flavonoids, and anthraquinones[15].

Thin-layer chromatography (TLC) - Using silica gel-coated plates as the stationary phase and suitable solvent systems as the mobile phase, TLC was used to separate and identify phytoconstituent. Plates were produced in a saturated chamber after samples were applied as spots. Following development, spots were

seen using detecting reagents and UV light, and Rf values were computed to describe the constituents[16].

Column Chromatography - Column chromatography packed with silica gel (60–120 mesh) was used to separate bioactive substances. After the extract was injected into the column and adsorbed onto silica, it was eluted using more polar solvents (ethyl acetate, petroleum ether, and chloroform). Fractions were gathered, condensed, and then examined further[17].

Phytochemical Analysis of Fractions- Standard qualitative assays were used to screen collected fractions for phyto-constituents. Based on distinctive color responses, the presence of carbohydrates, alkaloids, saponins, tannins, terpenoids, glycosides, steroids, phenols, amino acids, flavonoids, and anthraquinones was confirmed.

Bioactive Fraction Characterization by TLC - With an observed Rf value of 0.81, TLC profiling of bioactive fractions employing methanol:water (8:2) as the mobile phase revealed the presence of phenolic chemicals, validating successful separation and identification[18].

In-vivo Studies

Institutional Animal Ethics Committee (IAEC) Approval- All experimental procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals as prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the study.

Experimental Animals - A certified and registered laboratory animal supplier provided healthy adult Wistar albino rats of any sex, weighing between 180 and 220 g. The animals were kept in the institutional animal facility under conventional environmental conditions, which included a 12-hour light-dark cycle, a controlled temperature of 22 ± 2°C, and a relative humidity of 50–60%. To maintain hygienic conditions, rats were housed in sterile polypropylene cages with sterile rice husk bedding that was changed on a regular basis. For the duration of the study, the animals were given a regular pellet meal and unlimited access to water. To reduce stress and guarantee physiological stability, all animals were acclimated to laboratory conditions for at least seven days prior to the start of the experiment. Throughout the course of the trial, animals were handled with care and kept in uniform conditions.

Experimental Design - The experimental study was carried out using Wistar albino rats (180–220 g), randomly divided into six groups (n=6 per group). Cognitive impairment was induced using scopolamine (1 mg/kg, i.p.), and treatment was continued for 28 days.

Table-2 Grouping and Treatment

Group	Treatment	Dose / Details
Group I	Normal Control	Normal saline (p.o.)
Group II	Negative Control	Scopolamine (1 mg/kg, i.p.)
Group III	Standard	Rivastigmine (0.5 mg/kg, p.o.) + Scopolamine
Group IV	Test I (CGEE)	*Coccinia grandis* extract (200 mg/kg, p.o.) + Scopolamine
Group V	Test II (PEF)	Petroleum ether fraction (200 mg/kg, p.o.) + Scopolamine
Group VI	Test III (EAF)	Ethyl acetate fraction (200 mg/kg, p.o.) + Scopolamine

Oral Acute Toxicity - According to OECD 423, the acute toxicity of *Coccinia grandis* ethanolic extract (CGEE) and its fractions was evaluated. To determine a safe dose range, animals were given single oral doses (100–2000 mg/kg) and monitored for behavioral, neurological, physiological, and mortality changes over a 14-day period.

Behavioral Evaluation (Memory and Learning)- The Morris Water Maze (MWM) and Elevated Plus Maze (EPM) were used to assess cognitive function:

• **Elevated Plus Maze (EPM):** The main measure of memory and learning was transfer latency (TL). Increased open-arm entries and time spent demonstrated decreased anxiety and higher cognitive ability, while a decrease in TL on successive trials suggested improved memory retention.

• **Morris Water Maze (MWM):** Time spent in the target quadrant during the probe trial and escape latency (EL) during training trials were used to measure spatial learning and memory. While longer retention times in the target quadrant showed memory consolidation, lower EL across sessions suggested learning acquisition.

To determine the effectiveness of neuro-protective therapy, behavioral assessments were carried out both before and after 28 days of treatment.

Cognitive Impairment Induction- Scopolamine and chronic restraint stress (6 hours per day for 28 days) were used to generate memory impairment, which resulted in neuroinflammation and cognitive deficits.

Estimation of Biochemistry - Brain homogenates were examined for:

• Activity of acetylcholinesterase (AChE) (412 nm)

• ELISA measurement of interleukin-1β (IL-1β) levels

Neuroinflammation and cholinergic function were evaluated using these markers.

Model 6-OHDA - 6-OHDA was used to promote neurodegeneration. CGEE (200 and 400 mg/kg) and rivastigmine were given for 28 days. To ascertain neuroprotective effects, behavioral and physiological markers were assessed.

Statistical Analysis- One-way ANOVA and Dunnett's test were used to evaluate the data, which were presented as Mean ± SEM ($n = 6$). Significance was defined as $P < 0.05$.

Results

Gathering, Drying, and Verification- Fruits of *Coccinia grandis* were collected, cleaned, and washed to remove impurities. The material was shade dried or oven dried (40–50°C), powdered, and stored in airtight containers. The plant was authenticated based on morphological characteristics by a qualified authority.

Table- 3 Physicochemical Evaluation of *Coccinia grandis* Extract

Sr. No.	Evaluation parameters	Values *Coccinia grandis* extract
1	Total ash value	4.6 %
2	Acid insoluble ash	1.4 %
3	Water soluble ash	3%
4	Moisture content	7 %

Conclusion

The *Coccinia grandis* extract's physico-chemical parameters—total ash (4.6%), acid-insoluble ash (1.4%), water-soluble ash (3%), and moisture content (7%)—were all within allowable bounds, suggesting minimal impurity levels and strong ability. These results validate the extract's purity and applicability for additional pharmacological and neuroprotective research.

Extraction- The *Coccinia grandis*, extracted with various 80% ethanol and 20% water.

Table-4 Yield of various extracts obtained from the fruit

Sr. No.	Evaluation Parameters	Color	Nature	Percentage Yield (% W/W)
1.	Ethanol (80%) and water (20%)	Bright red	Smooth and soft	10

Phytochemical Screening- The extraction procedure is as described previously, using ethanol (800 mL) as the solvent and keeping the solid-to-solvent ratio at about 1:8 (w/v) for 24 hours at 90°C.

Table-5 Ethanolic extraction of *Coccinia grandis*

Sr no	Test name	Procedure	Observation	Result
1.	Alkaloid Test: B) Wagner's test:	2ml extract + 1-2 drops of wagner's reagent	No reddish brown precipitate	Alkaloid absent
2.	Glycosides Test: A) Keller Killani test:	1 ml extract + 1.5 ml glacial acetic acid + 1 drop of 5 % ferric chloride + conc.H_2SO_4	A blue coloured solution (in the layer)	Glycosides present
3.	Flavonoid Test: A) Ferric chloride test:	Extract solution + few drops 10 % ferric chloride solution	Green precipitate	Flavonoid present
4.	Tannins Test: A) Dil. HNO_3:	Few ml extract + dil. HNO_3	Yellow precipitate	Tannis absent
5.	Phenol Test: A) Ferric Chloride Test:	Extract + ferric chloride solution	dark blue or greenish black colour appeared	Phenol absent

Conclusion

Phytochemical screening revealed the absence of alkaloids but the presence of glycosides, flavonoids, tannins, and phenolic substances. These constituents indicate the presence of bioactive compounds that may contribute to the pharmacological potential of the extract, including antidiabetic activity.

Table-6 TLC for Phytoconstituent

Type of Constituent	Mobile phase	Observation	RF value found
Flavonoids	n-Hexane: Ethanol 6:4	Light green	0.67
Glycosides	Chloroform : Methanol 6:4	Light green	0.81

Conclusion

Using the proper solvent systems, thin layer chromatographic profiling of *Coccinia grandis* extract showed discrete spots corresponding to flavonoids (Rf 0.67) and glycosides (Rf 0.81). These findings indicate the extract's potential neuroprotective action and its appropriateness for additional pharmacological testing by confirming the presence of antioxidant rich phyto-constituents.

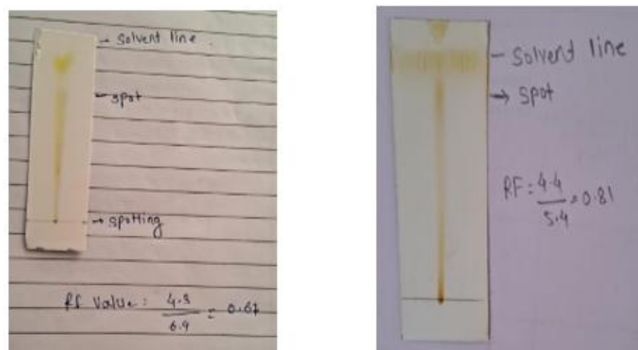


Figure-1 TLC for Flavonoids and Glycosides

Column Chromatography- The hydro-alcoholic extract's initial phytochemical examination revealed the existence of phenolic compounds, which was corroborated by TLC profiling that revealed several phytoconstituents. In light of these results, column chromatography was used to separate the extract's bioactive constituents.

Table-7 Phytoconstituents Tests of Bioactive Fractions

Sr no.	Phytoconstituent	Test performed and reagents	Ethanolic fraction	Pet ether fraction	Ethyl acetate fraction
1	Alkaloids	i)Mayer's test ii)Dragondroff's test iii)Hager's test	+ + +	+ + +	+ + +
2	Carbohydrates	i) Benidict's test ii) Fehling's test iii) Molisch's test	+ + +	+ + +	+ + +
3	Glycosides	• Keller-killani test	+	+	+
4	Tannins	i) Gelatin test • Lead acetate test	+ +	+ +	+ +
5	Saponins	• Foam test	+	+	+
6	Flavonoids	i) Ferric chloride test ii) Alkaline • reagent test	+ +	+ +	+ +
7	Steroids	1. Liebermann-Burchard' test	+	+	+
8	Terpenoids	2. Salkowski's test	+	+	+
9	Amino acid	3. Ninhydrin Test	_	_	_
10	Anthraquinones	4. Borntranger's test	_	_	_

(+ Present , - Absent)

Conclusion

While amino acids and anthraquinones were not present, phytochemical screening of ethanolic, petroleum ether, and ethyl acetate fractions revealed the presence of alkaloids, carbohydrates, glycosides, tannins, saponins, flavonoids, steroids, and terpenoids. These findings show that the plant has a variety of bioactive components that contribute to its medicinal potential.

In-vivo Study

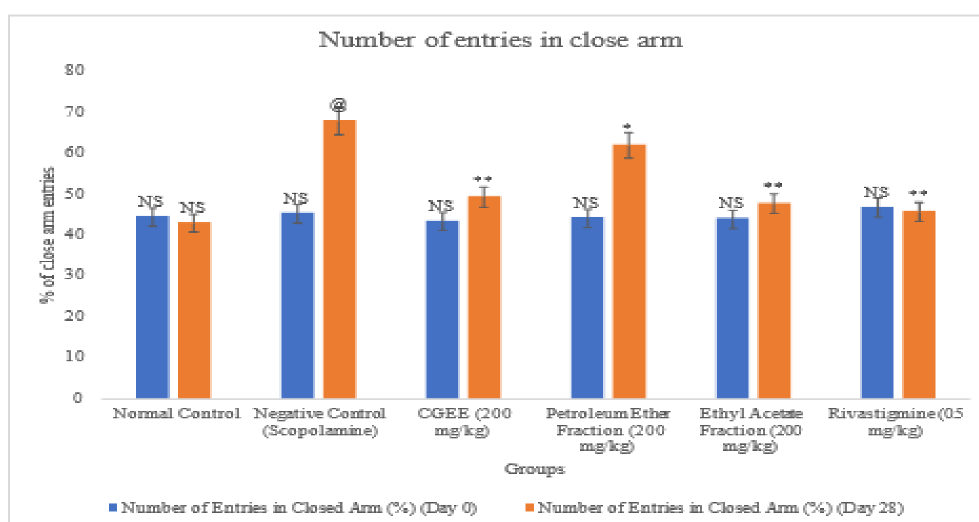
Oral Acute Toxicity- Mice were used to test the acute oral toxicity of *Coccinia grandis* fractions (PEF, CF, and EAF) over a 14-day period. Up to 1000 mg/kg, no mortality or notable behavioral changes were seen, and normal body weight, eating, and movement were maintained. Mild and temporary lethargy was observed at 2000 mg/kg without significant toxicity. These results show that all fractions are safe and have high tolerance. LD50 was predicted to be greater than 1000 mg/kg, and all fractions were safe up to 1000 mg/kg. For additional neuroprotective testing, a dosage of 200 mg/kg was chosen.

I. Estimation of Behavioural Study

A. Elevated Plus Maze (EPM) Test

Table-8 Effect of *Coccinia grandis* fraction on Number of Entries in Closed Arm in Elevated Plus Maze

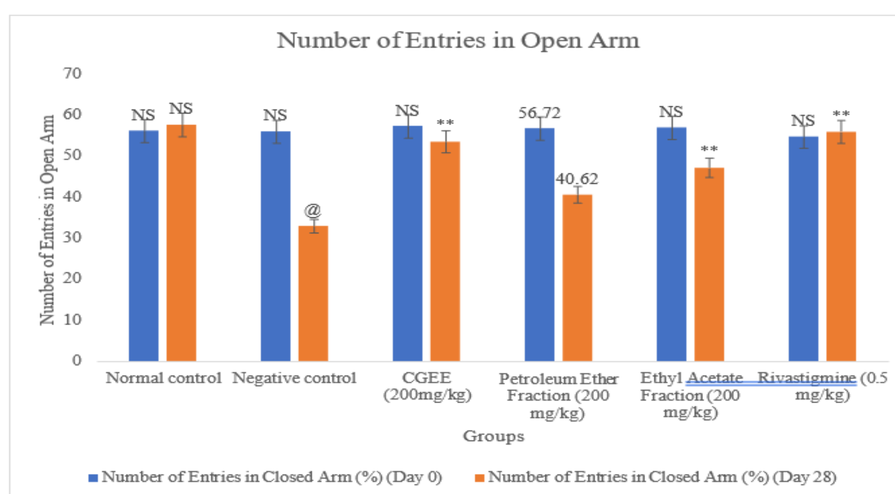
Sr. No.	Groups	Number of Entries in Closed Arm (%) (Day 0)	Number of Entries in Closed Arm (%) (Day 28)
1	Normal Control	44.62 ± 0.47	43.12 ± 0.68
2	Negative Control (Scopolamine)	45.45 ± 0.59ns	68.20 ± 1.61@
3	CGEE (200 mg/kg)	44.12 ± 0.52ns	49.84 ± 0.61**
4	Petroleum Ether Fraction (200 mg/kg)	44.25 ± 0.58ns	62.15 ± 0.56**
5	Ethyl Acetate Fraction (200 mg/kg)	44.08 ± 0.47ns	47.92 ± 0.49**
6	Rivastigmine (0.5 mg/kg)	46.92 ± 0.22ns	45.92 ± 0.50**



Graph-1 Effect of *Coccinia grandis* fraction on number of entries in close arm of rats in EPM apparatus

Table-9 Effect of *Coccinia grandis* fraction on number of entries in open arm of rats in EPM apparatus

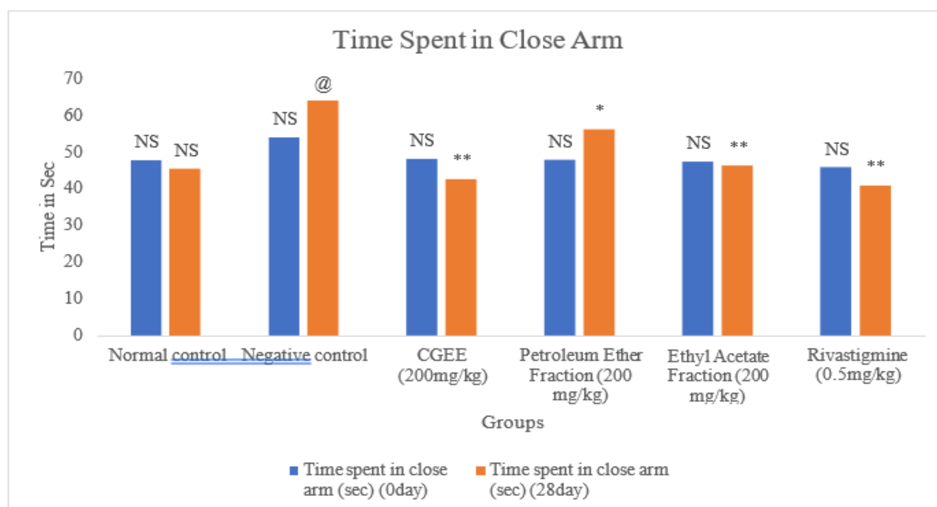
Sr. No.	Groups	Number of Entries in Open Arm (%) (Day 0)	Number of Entries in Open Arm (%) (Day 28)
1	Normal Control	56.12 ± 0.77	57.62 ± 0.51
2	Negative Control (Scopolamine)	55.95 ± 0.58ns	32.95 ± 0.66@
3	CGEE (200 mg/kg)	56.84 ± 0.42ns	53.15 ± 0.73**
4	Petroleum Ether Fraction (200 mg/kg)	56.72 ± 0.39ns	40.62 ± 0.68**
5	Ethyl Acetate Fraction (200 mg/kg)	56.91 ± 0.47ns	47.18 ± 0.55**
6	Rivastigmine (0.5 mg/kg)	54.67 ± 1.68ns	55.92 ± 0.50**



Graph-2 Effect of *Coccinia grandis* fraction on number of entries in open arm of rats in EPM apparatus

Table-10 Effect of *Coccinia grandis* fraction on time spend in close arm by rats in EPM apparatus

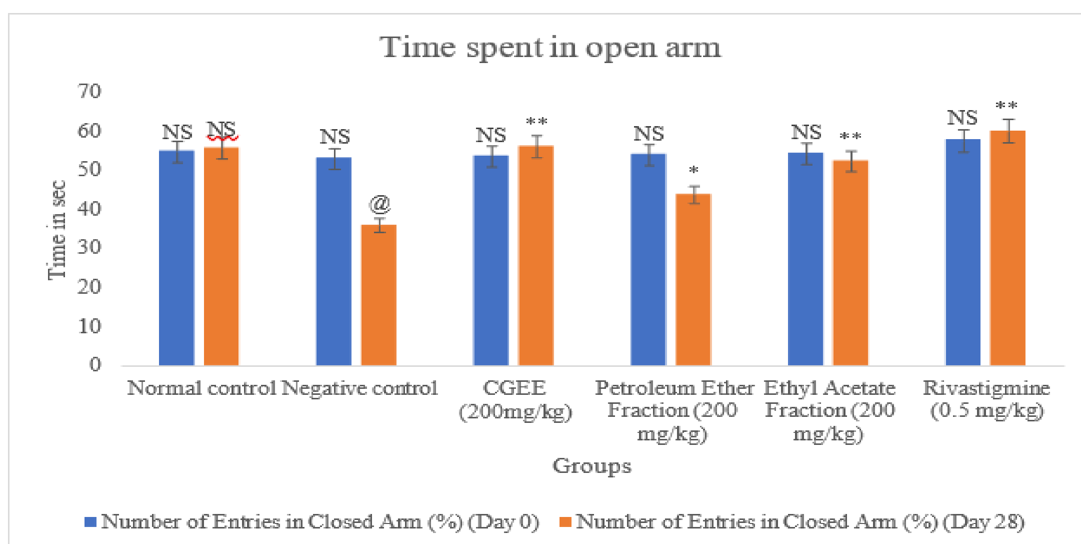
Sr. No.	Groups	Time Spent in Closed Arm (sec) (Day 0)	Time Spent in Closed Arm (sec) (Day 28)
1	Normal Control	47.75 ± 0.85	45.50 ± 0.24
2	Negative Control (Scopolamine)	53.96 ± 1.23ns	63.96 ± 1.23@
3	CGEE (200 mg/kg)	48.10 ± 1.37ns	42.32 ± 0.42**
4	Petroleum Ether Fraction (200 mg/kg)	47.88 ± 1.12ns	56.14 ± 0.51*
5	Ethyl Acetate Fraction (200 mg/kg)	47.35 ± 1.05ns	46.28 ± 0.44**
6	Rivastigmine (0.5 mg/kg)	45.85 ± 3.2	40.86 ± 0.51**



Graph-3 Effect of *Coccinia grandis* fraction on time spend in close arm by rats in EPM apparatus

Table-11 Effect of *Coccinia grandis* fraction on time spend in open arm by rats in EPM apparatus

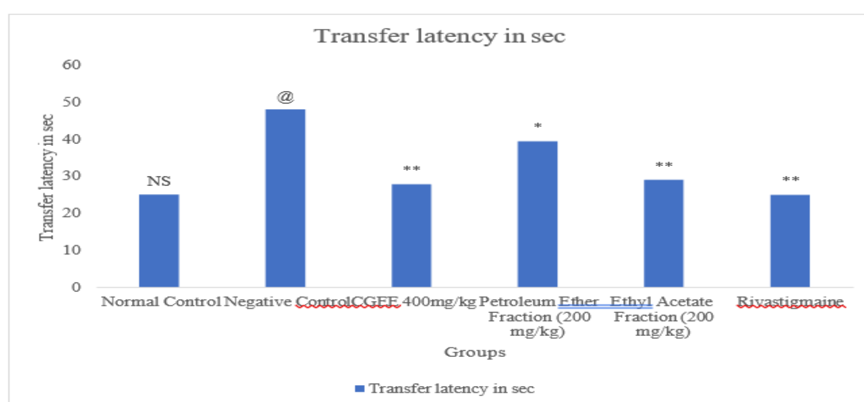
Sr. No.	Groups	Time Spent in Open Arm (sec) (Day 0)	Time Spent in Open Arm (sec) (Day 28)
1	Normal Control	54.82 ± 0.43	55.82 ± 0.66
2	Negative Control (Scopolamine)	53.07 ± 1.15ns	36.07 ± 1.83@
3	CGEE (200 mg/kg)	54.18 ± 0.61ns	56.92 ± 1.74**
4	Petroleum Ether Fraction (200 mg/kg)	54.05 ± 0.58ns	43.86 ± 1.62**
5	Ethyl Acetate Fraction (200 mg/kg)	54.36 ± 0.47ns	52.48 ± 1.51**
6	Rivastigmine (0.5 mg/kg)	57.70 ± 0.44ns	60.20 ± 2.18**



Graph-4 Effect of *Coccinia grandis* fraction on time spend in open arm by rats in EPM apparatus

Table-12 Effect of *Coccinia grandis* fraction on transfer latency of rats in EPM apparatus.

Sr. No.	Groups	Transfer Latency (sec)
1	Normal Control	25.01 ± 1.15
2	Negative Control (Scopolamine)	48.03 ± 1.16@
3	CGEE (200 mg/kg)	36.97 ± 1.13**
4	Petroleum Ether Fraction (200 mg/kg)	39.42 ± 1.08**
5	Ethyl Acetate Fraction (200 mg/kg)	28.94 ± 0.85**
6	Rivastigmine (0.5 mg/kg)	24.94 ± 1.16**

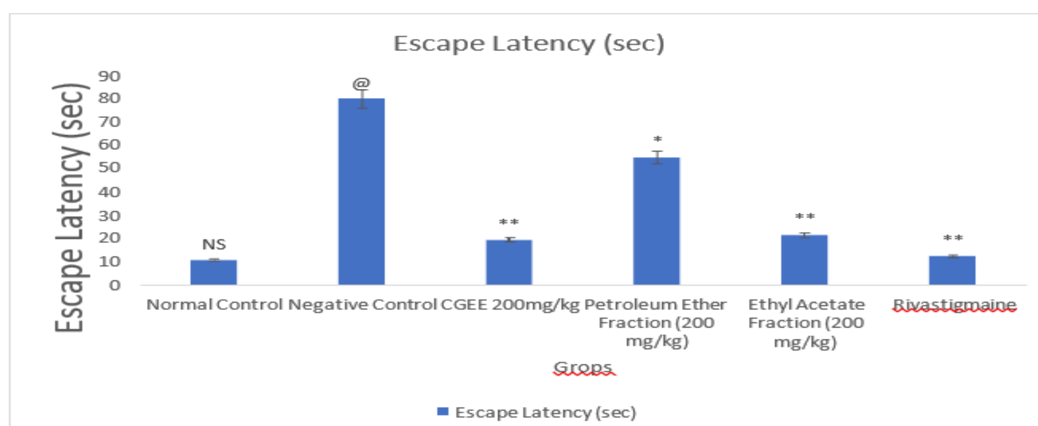


Graph-5 Effect of *Coccinia grandis* fraction on transfer latency of rats in EPM apparatus.

B. Morris Water Maze Apparatus

Table - 13 Effect of *Coccinia grandis* fraction on escape latency of rats in MWM apparatus.

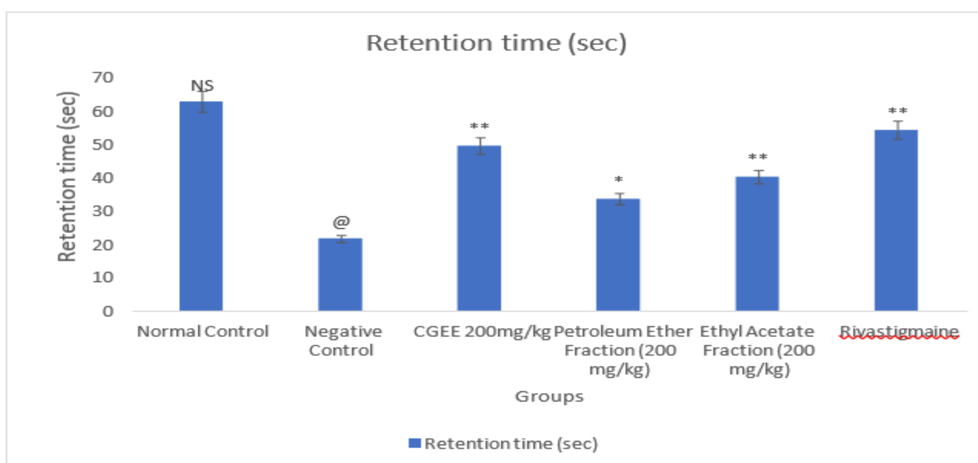
Sr. No.	Groups	Escape Latency (sec)
1	Normal Control	10.50 ± 1.29
2	Negative Control (Scopolamine)	80.25 ± 1.72@
3	CGEE (200 mg/kg)	19.14 ± 1.65**
4	Petroleum Ether Fraction (200 mg/kg)	54.76 ± 1.88**
5	Ethyl Acetate Fraction (200 mg/kg)	21.35 ± 1.54**
6	Rivastigmine (0.5 mg/kg)	12.25 ± 1.78**



Graph-6 Effect of *Coccinia grandis* fraction on escape latency of rats in MWM apparatus

Table-14 Effect of *Coccinia grandis* fraction on retention time of rats in MWM apparatus.

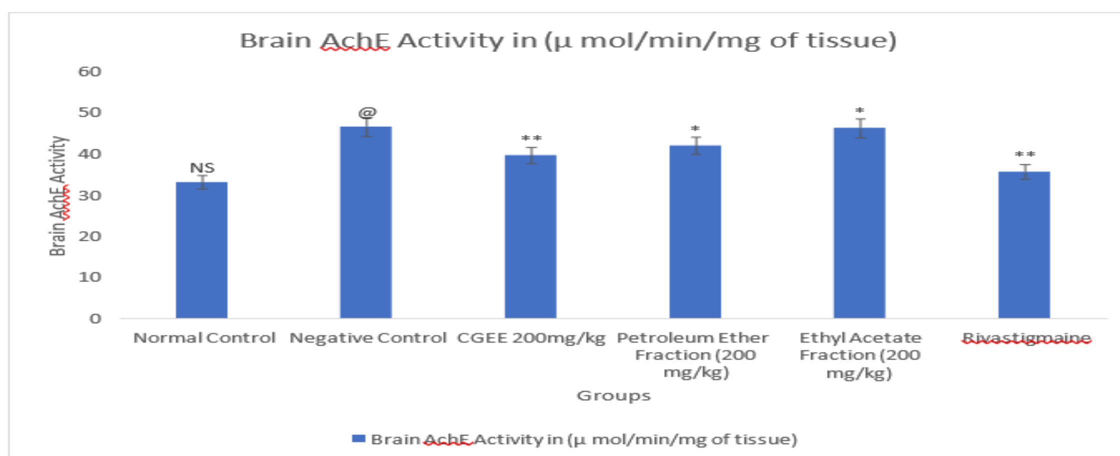
Sr. No.	Groups	Retention Time (sec)
1	Normal Control	62.75 ± 1.78
2	Negative Control (Scopolamine)	21.69 ± 1.82@
3	CGEE (200 mg/kg)	49.5 ± 2.11**
4	Petroleum Ether Fraction (200 mg/kg)	33.62 ± 1.94**
5	Ethyl Acetate Fraction (200 mg/kg)	40.18 ± 1.63**
6	Rivastigmine (0.5 mg/kg)	54.25 ± 1.71**



Graph -7 Effect of *Coccinia grandis* fraction on escape latency of rats in MWM apparatus.

II. Estimation of Biochemical Parameter

Effect of *Coccinia grandis fraction* and Rivastigmine on Brain Acetyl Cholinesterase (AChE) Activity in rats



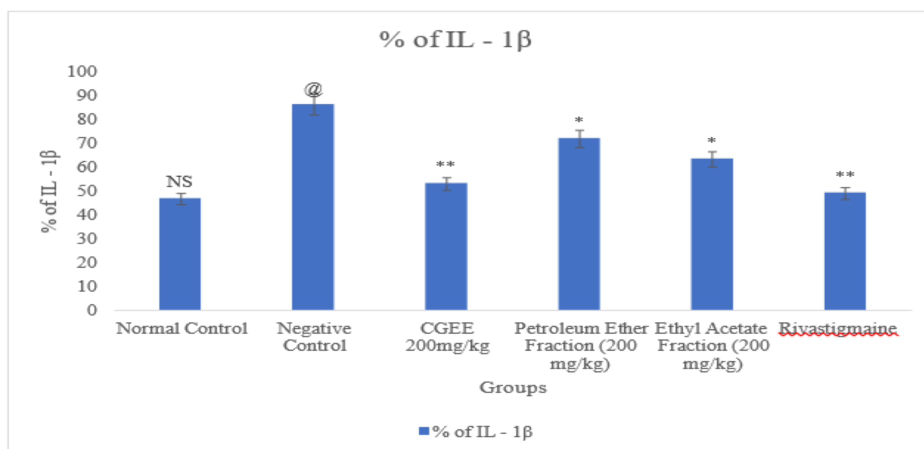
Graph-8 Effect of *Coccinia grandis* fraction on brain ache activity in rats.

A. Estimation of Interleukin -1β level.

Table-15 Effect of *Coccinia grandis* fraction on Iinterleukin -1β level in rats.

Sr. No.	Groups	IL-1β (%)
1	Normal Control	47.02 ± 0.99
2	Negative Control (Scopolamine)	86.52 ± 0.61@

3	CGEE (200 mg/kg)	53.45 ± 0.92**
4	Petroleum Ether Fraction (200 mg/kg)	72.18 ± 0.88**
5	Ethyl Acetate Fraction (200 mg/kg)	63.64 ± 0.76**
6	Rivastigmine (0.5 mg/kg)	49.32 ± 0.87**

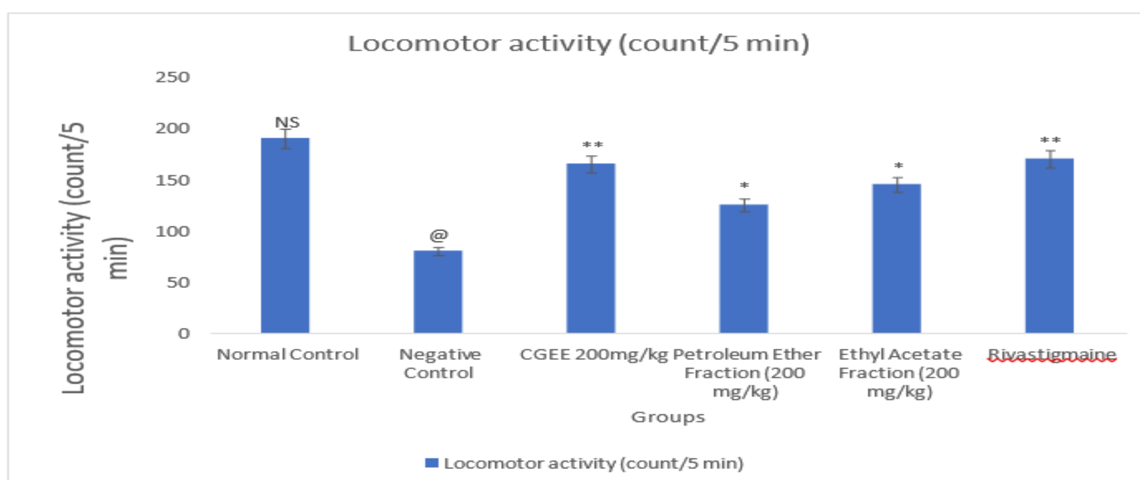


Graph-9 Effect of *Coccinia grandis* fraction on Iinterleukin -1β level in rats.

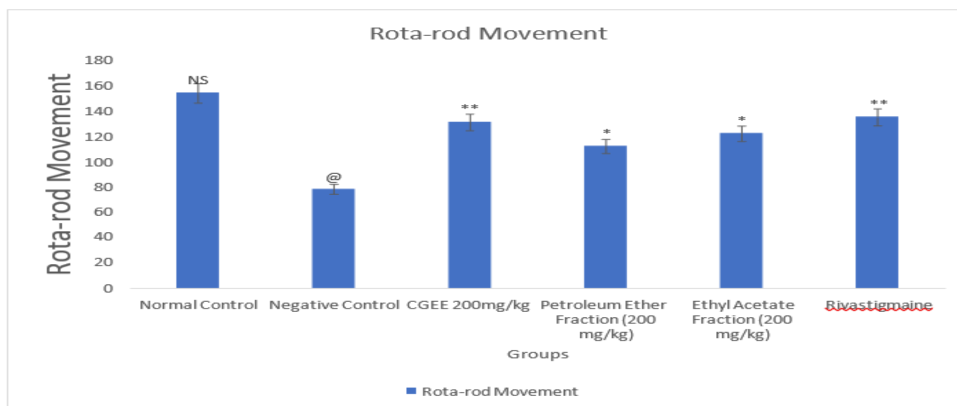
B. 6 - hydroxydopamine (6-OHDA) Model

Table-17 Effect of *Coccinia grandis* fraction on Locomotor Activity (Actophotometer)

Sr. No.	Group Name	Locomotor Activity (count/5 min)
1	Normal Control	190 ± 8
2	Negative Control (Scopolamine)	80 ± 6
3	CGEE (200 mg/kg)	165 ± 7
4	Petroleum Ether Fraction (200 mg/kg)	125 ± 6
5	Ethyl Acetate Fraction (200 mg/kg)	145 ± 6
6	Rivastigmine (0.5 mg/kg)	170 ± 5



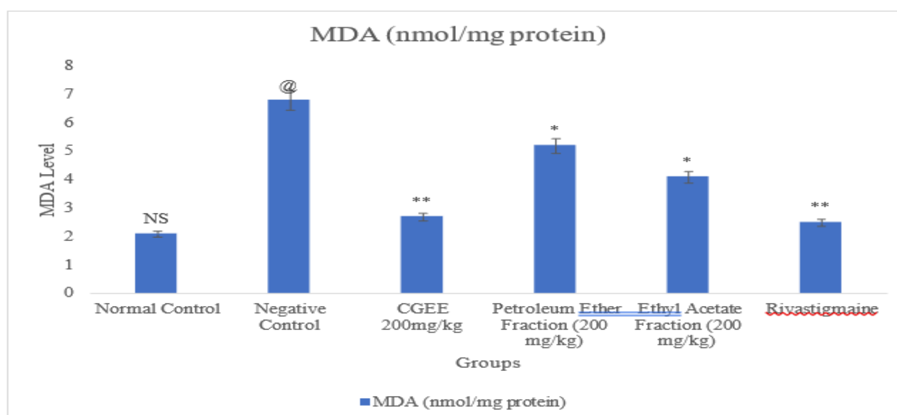
Graph-10 Effect of *Coccinia grandis* fraction – Locomotor activity (Actophotometer)



Graph-11 Effect of *Coccinia grandis* fraction on rota-rod apparatus

Table-18 Effect of *Coccinia grandis* fraction on MDA levels

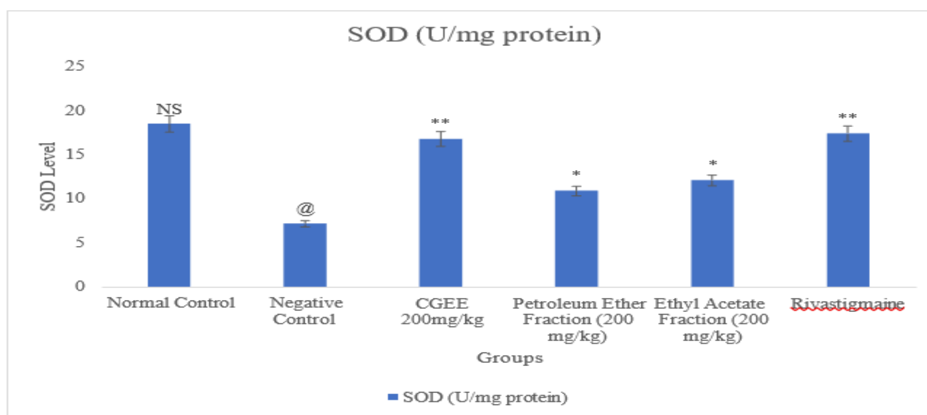
Sr. No.	Group Name	MDA (nmol/mg protein)
1	Normal Control	2.1 ± 0.2
2	Negative Control (Scopolamine)	6.8 ± 0.3
3	CGEE (200 mg/kg)	2.7 ± 0.3
4	Petroleum Ether Fraction (200 mg/kg)	5.2 ± 0.3
5	Ethyl Acetate Fraction (200 mg/kg)	4.1 ± 0.2
6	Rivastigmine (0.5 mg/kg)	2.5 ± 0.2



Graph-12 Effect of *Coccinia grandis* fraction on MDA levels

Table-19 Effect of *Coccinia grandis* fraction on SOD (Superoxide Dismutase)

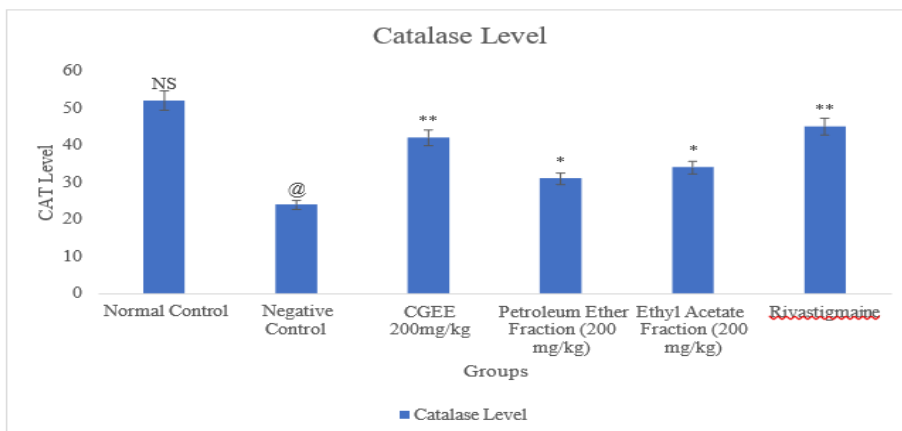
Sr. No.	Group Name	SOD (U/mg protein)
1	Normal Control	18.5 ± 0.6
2	Negative Control (Scopolamine)	7.2 ± 0.4
3	CGEE (200 mg/kg)	16.8 ± 0.5
4	Petroleum Ether Fraction (200 mg/kg)	10.9 ± 0.5
5	Ethyl Acetate Fraction (200 mg/kg)	12.1 ± 0.6
6	Rivastigmine (0.5 mg/kg)	17.4 ± 0.5



Graph-13 Effect of *Coccinia grandis* fraction on SOD (Superoxide Dismutase)

Table-20 Effect of *Coccinia grandis* fraction on Catalase Activity

Sr. No.	Group Name	Catalase Level (U/mg protein)
1	Normal Control	52 ± 0.6
2	Negative Control (Scopolamine)	24 ± 0.4
3	CGEE (200 mg/kg)	42 ± 0.5
4	Petroleum Ether Fraction (200 mg/kg)	31 ± 0.5
5	Ethyl Acetate Fraction (200 mg/kg)	34 ± 0.6
6	Rivastigmine (0.5 mg/kg)	45 ± 0.5



Conclusion

In scopolamine induced cognitive impairment, learning and memory were considerably enhanced by *Coccinia grandis* extract and its fractions. Behavioral outcomes (EPM, Morris Water Maze) showed marked improvement, with CGEE exhibiting effects comparable to rivastigmine. Biochemical findings (↓AChE, IL-1β, MDA; ↑SOD, catalase) further support its neuroprotective and antioxidant potential.

Discussion

The current study used scopolamine-induced cognitive impairment in rats to assess the neuroprotective potential of *Coccinia grandis* extract and its fractions. It is well known that scopolamine disrupts cholinergic transmission, resulting in

memory impairments akin to those seen in neurodegenerative diseases like Alzheimer's.

Scopolamine significantly increased transfer latency and decreased open arm exploration, indicating impaired learning and memory, according to behavioral tests using the Elevated Plus Maze (EPM). These effects were considerably reversed by treatment with *Coccinia grandis* extract (CGEE) and its fractions, as seen by enhanced open arm entries and time spent and reduced transfer delay. The most noticeable effect among the fractions was CGEE, which was comparable to the activity of the common medication rivastigmine (Tables 8–11).

Similarly, scopolamine-treated rats had a significant decrease in retention time and an increase in escape latency in the Morris Water Maze (MWM) test, indicating compromised spatial learning and memory. Improved memory acquisition and retention were indicated by treatment with CGEE and its fractions, which dramatically decreased escape latency and increased time spent in the target quadrant. Once more, CGEE outperformed other fractions in terms of efficacy (Tables 12–13). The behavioral results were further corroborated by biochemical studies. Acetylcholinesterase (AChE) activity was markedly boosted by scopolamine treatment, which resulted in decreased acetylcholine levels and higher oxidative stress (MDA) and inflammatory (IL-1β) indicators. AChE activity and IL-1β levels were dramatically reduced after treatment with *Coccinia grandis* extract, indicating a reduction in neuroinflammation and a restoration of cholinergic function. Furthermore, a notable decrease in MDA levels and a rise in the activity of antioxidant enzymes (SOD and catalase) were noted, suggesting that oxidative stress had been attenuated.

Coccinia grandis's rich phytochemical makeup, especially flavonoids and phenolic chemicals, which are recognized for their anti-inflammatory and antioxidant qualities, may be responsible for the plant's demonstrated neuroprotective effects. By scavenging free radicals, lowering lipid peroxidation, and adjusting neuro-transmitter levels, these substances probably help protect neuronal cells.

Overall, the results indicate that *Coccinia grandis* has strong neuroprotective action, which may be mediated by anti-inflammatory, antioxidant, and cholinergic pathways. The findings also show that the crude extract (CGEE) is more effective than specific fractions, perhaps as a result of several phytoconstituents working in concert.

Conclusion

In behavioral animals, *Coccinia grandis* extract showed notable neuroprotective efficacy against scopolamine-induced memory impairment, enhancing learning, memory, and spatial ability. Reduced oxidative stress and restoration of cholinergic function are indicated by biochemical data (MDA, AChE, SOD, catalase). Flavonoids and phenolic chemicals are probably responsible for the action, indicating a multi-targeted mechanism. All things considered, *Coccinia grandis* exhibits potential as a natural treatment for cognitive conditions like Alzheimer's.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research.

There is no conflict of interest between authors and producers of the product.

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Phytochemical and Antidiabetic Activity of *Mimosa tenuiflora* leaves

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Abstract- Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both, leading to severe microvascular and macrovascular complications. The increasing global prevalence of diabetes and the limitations associated with synthetic antidiabetic drugs have prompted the exploration of plant-based therapeutics. Mimosa tenuiflora, a medicinal plant belonging to the Fabaceae family, is rich in bioactive phytoconstituents such as flavonoids, tannins, alkaloids, and phenolic compounds, which are known to exhibit antioxidant and antidiabetic properties. *Mimosa tenuiflora* is a medicinal plant known for its pharmacological properties. This study focuses on extraction, isolation, and identification of bioactive compounds and evaluation of antidiabetic activity using in vitro and in vivo models. The results indicated significant glucose-lowering potential due to presence of flavonoids and phenolics.

The present study was undertaken to extract, isolate, and identify bioactive compounds from *Mimosa tenuiflora* and evaluate their antidiabetic potential. The plant material was subjected to hydro-alcoholic extraction followed by phytochemical screening, chromatographic separation (TLC, column chromatography), and advanced analytical techniques such as HR-LC-MS for identification of active constituents. The extract and isolated fractions were evaluated for in vitro antioxidant activity using DPPH and other free radical scavenging assays, as well as in vitro antidiabetic activity through α-amylase and α-glucosidase inhibition studies. Furthermore, in vivo antidiabetic activity was assessed using streptozotocin-induced diabetic Wistar rats by evaluating biochemical parameters including blood glucose, lipid profile, and oxidative stress markers such as SOD, GSH, and MDA.

The results demonstrated that *Mimosa tenuiflora* extract possesses significant antioxidant and antidiabetic activity, which may be attributed to the presence of polyphenolic compounds and flavornoids. The study supports the traditional use of this plant and highlights its potential as a source of novel, safe, and effective antidiabetic agents.

Keywords: Mimosa tenuiflora, Antidiabetic Activity, Phytochemicals, Extraction, Isolation, HR-LC-MS, Flavonoids, Antioxidant, Streptozotocin, Wistar Rats

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and represents a major public health challenge due to its increasing incidence, long-term complications, and associated healthcare burden. Chronic hyperglycemia leads to disturbances in carbohydrate, lipid, and protein metabolism, ultimately resulting in serious complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy. The global burden of diabetes has risen dramatically over the past few decades, with developing countries like India experiencing a particularly rapid increase in prevalence due to urbanization, sedentary lifestyle, and dietary changes.

Diabetes mellitus is broadly classified into Type-1 diabetes mellitus (T1DM), Type-2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and other specific types. Among these, Type-2 diabetes is the most common, accounting for approximately 90–95% of all cases. It is primarily associated with insulin resistance and impaired insulin secretion. Genetic predisposition, obesity, lack of physical activity, unhealthy dietary habits, and aging are major risk factors contributing to the development of the disease. In addition, chronic stress and hormonal imbalance further exacerbate metabolic dysfunction, leading to progressive deterioration of glucose homeostasis.

The pathophysiology of diabetes involves a complex interplay of metabolic, hormonal, and molecular mechanisms. Insulin resistance in peripheral tissues such as skeletal muscle, adipose tissue, and liver leads to decreased glucose uptake and increased hepatic glucose production. Simultaneously, pancreatic β-cell dysfunction results in inadequate insulin secretion. Persistent hyperglycemia triggers oxidative stress through the excessive generation of reactive oxygen species (ROS), which causes cellular damage, lipid peroxidation, protein oxidation, and DNA damage. Oxidative stress plays a crucial role in the progression of diabetes and its complications by activating inflammatory pathways and impairing cellular function.

In addition to oxidative stress, chronic low-grade inflammation is a key contributor to the development and progression of diabetes. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) interfere with insulin signaling pathways, leading to increased insulin resistance. The accumulation of advanced glycation end products (ages) further aggravates oxidative stress and inflammation, resulting in vascular damage and tissue dysfunction. These interconnected mechanisms highlight the multifactorial nature of diabetes and emphasize the need for therapeutic strategies that can target multiple pathways simultaneously.

Conventional antidiabetic therapies, including insulin and oral hypoglycemic agents such as metformin and sulfonylureas, are widely used for the management of diabetes. Although these drugs are effective in controlling blood glucose levels, they are often associated with various side effects such as hypoglycemia, gastrointestinal disturbances, weight gain, and long term complications. Furthermore, these treatments primarily focus on glycemic control and may not adequately address the underlying oxidative stress and inflammation involved in disease progression. These limitations have prompted researchers to explore alternative therapeutic approaches, particularly those derived from natural sources.

Medicinal plants have gained considerable attention as potential sources of novel antidiabetic agents due to their therapeutic efficacy, safety, and multi-target mechanisms of action. Plant-based therapies are rich in bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which exhibit a wide range of pharmacological activities. These phytoconstituents have been reported to exert antidiabetic effects through various mechanisms, including enhancement of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate digesting enzymes, and reduction of oxidative stress. Additionally, the antioxidant properties of plant-derived compounds help protect pancreatic β-cells from oxidative damage and improve overall metabolic function.

Among various medicinal plants, *Mimosa tenuiflora* has emerged as a promising candidate due to its rich phytochemical profile and traditional medicinal uses. It belongs to the family Fabaceae and is commonly known as Jurema preta or Tepezcohuite. The plant is widely distributed in tropical and semi-arid regions and has been traditionally used for the treatment of wounds, burns, inflammation, and infections. Phytochemical investigations of *Mimosa tenuiflora* have revealed the presence of tannins, flavonoids, alkaloids, and saponins, which are known to possess antioxidant, antimicrobial, and anti-inflammatory properties. These bioactive compounds are likely to contribute to its potential antidiabetic activity.

Recent studies have demonstrated that extracts of *Mimosa tenuiflora* exhibit significant antioxidant activity, which plays a crucial role in mitigating oxidative stress associated with diabetes. Antioxidants neutralize reactive oxygen species and prevent cellular damage, thereby protecting pancreatic β-cells and improving insulin function. In addition, certain phytochemicals present in the plant may inhibit key carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, leading to reduced glucose absorption and improved glycemic control. These findings suggest that *Mimosa tenuiflora* may act through multiple mechanisms to exert its antidiabetic effects.

Despite the promising therapeutic potential of Mimosa tenuiflora, there is a need for systematic scientific investigation to identify and characterize the specific bioactive compounds responsible for its pharmacological activity. Extraction, isolation, and identification of these compounds are essential steps in

understanding their mechanism of action and developing standardized herbal formulations. Advanced analytical techniques such as chromatography and mass spectrometry play a crucial role in the separation and characterization of phytoconstituents. Furthermore, evaluation of antidiabetic activity using in vitro and in vivo models provides scientific validation for the traditional use of the plant.

Therefore, the present study focuses on the extraction, isolation, and identification of bioactive compounds from *Mimosa tenuiflora* and the evaluation of their antidiabetic activity. By integrating phytochemical analysis with pharmacological evaluation, this study aims to contribute to the development of safe, effective, and plant-based therapeutic agents for the management of diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. Medicinal plants provide alternative therapies. *Mimosa tenuiflora* contains bioactive compounds like flavonoids, tannins, and alkaloids responsible for therapeutic effects.

Material and Methods

Collection of plant- Fresh leaves of *Mimosa tenuiflora* were collected from an appropriate geographical region during the optimal growing season to ensure maximum phytochemical content and biological activity. Seasonal variation plays a crucial role in the concentration of active constituents, and hence, collection at the correct time enhances the reliability and reproducibility of pharmacological studies. After collection, the plant material was carefully examined and authenticated by a qualified taxonomist to confirm its botanical identity. Proper authentication is essential to avoid misidentification, which could lead to erroneous experimental outcomes and affect the validity of the research findings.

Furthermore, a voucher specimen of the plant was prepared and deposited in an institutional herbarium. This specimen serves as a permanent reference for the plant material used in the study and allows future researchers to verify the identity of the species. The deposition of voucher specimens is considered a standard practice in pharmacognostic and phytochemical research, as it ensures transparency, traceability, and reproducibility of scientific investigations. It also aids in the standardization of herbal drugs by providing a documented source of the plant material used in experimental procedures. Therefore, proper collection, authentication, and documentation of *Mimosa tenuiflora* leaves are critical steps that contribute significantly to the credibility and scientific validity of the study.

Preparation of plant extract- The collected leaves of *Mimosa tenuiflora* were initially washed thoroughly with distilled water to eliminate dust, soil particles, and other extraneous contaminants that could interfere with subsequent extraction and analysis. The cleaned leaves were then shade dried at room temperature to preserve heat sensitive phytoconstituents such as flavonoids, phenolics, and glycosides, which might degrade under direct sunlight or high temperatures. Once completely dried, the plant material was coarsely powdered using a mechanical grinder to increase the surface area, thereby enhancing the efficiency of

solvent penetration during extraction.

The powdered material was subjected to Soxhlet extraction, a widely used continuous extraction technique that ensures exhaustive extraction of bio-active compounds. A hydroalcoholic solvent system consisting of ethanol and water was employed, as it effectively dissolves a broad range of polar and semi-polar phytochemicals. The extraction process was continued until the solvent in the siphon tube became colorless, indicating complete extraction. The obtained extract was then concentrated under reduced pressure using a rotary evaporator, which helps in removing the solvent at a lower temperature, thus preventing thermal degradation of active constituents.

Finally, the concentrated extract was transferred into an airtight container and stored at 4°C to maintain its stability and prevent microbial growth or chemical degradation. This standardized extraction procedure ensures the preservation of bioactive compounds and reproduceibility of experimental results[1].

Preliminary phytochemical screening- The prepared extract of Mimosa tenuiflora leaves was subjected to preliminary qualitative phytochemical screening to identify the presence of various bioactive constituents responsible for its therapeutic potential. This screening is an essential step in pharmacognostic studies, as it provides a preliminary understanding of the chemical composition of the plant extract and helps correlate its constituents with biological activities. Standard qualitative tests were performed to detect major classes of phytochemicals such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, and glycosides.

Specific chemical reagents were used for each class of compounds; for instance, alkaloids were identified using Dragendorff's or Mayer's reagent, which produces a characteristic precipitate. Flavonoids were detected by the Shinoda test, showing color changes upon reaction, while tannins and phenolics were confirmed using ferric chloride, resulting in a dark blue or green coloration. Saponins were identified through the frothing test, indicating their surfactant properties, and glycosides were detected using tests such as Keller–Killiani for cardiac glycosides. These qualitative assays are simple, rapid, and effective in establishing the presence or absence of key phyto-constituents.

The identification of these compounds is significant, as many of them are known to possess pharmacological activities, including antioxidant and antidiabetic effects. Thus, preliminary phyto-chemical screening serves as a found-ation for further quantitative analysis and bio-activity guided studies, ensuring scientific validation of the plant's medicinal properties[2-4].

Preparation of Hydroalcoholic Extract by Soxhlet Extraction- The hydroalcoholic extracts of *Eclipta alba* and *Ziziphus jujuba* leaves were prepared using the Soxhlet extraction method. The collected plant materials were first washed thoroughly with distilled water to remove adhering impurities and then shade-dried at room temperature. For several days. The dried leaves were coarsely powdered using a mechanical grinder and stored in airtight

containers until further use.

Approximately 100 g of the powdered plant material was placed in a Soxhlet apparatus and extracted with a hydro-alcoholic solvent consisting of ethanol and water in a suitable ratio (commonly 70:30 v/v). The extraction process was carried out for 6–8 hours until the solvent in the siphon tube became colorless, indicating complete extraction of phyto-constituents.

After completion of extraction, the extract was filtered and concentrated under reduced pressure using a rotary evaporator or by evaporating the solvent on a water bath at controlled tempe-rature. The concentrated extract was then dried to obtain a semi-solid mass. The obtained extracts were stored in airtight containers at low temperature for further pharmacological evaluation. The same procedure was followed for both *Eclipta alba* and *Ziziphus jujuba* to ensure uniformity in extraction conditions[7].

Physico-chemical Evaluation- Physi-cochemical parameters were determined for crude drug according to the proce-dures given in WHO guidelines. Ash values including acid insoluble ash, water soluble ash, total ash and extrac-tive values such as water & alcohol soluble extractive value were determined by hot extraction and cold maceration method[5,6].

The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Evaluation of Ash Values- The quality of crude medication can be effectively evaluated by ash values. Ash content signifies the inorganic substance adheres with the crude drug. Sometimes the inorganic substances can be intentionally mixed with the drug to get profit. Accor-dingly, assurance of ash value of unrefined medication gives the character and cleanliness of a medication and gives data in respect to its degradation with inorganic substances.

Acid Insoluble Ash Value- To total obtained ash 25ml of hydrochloric acid was added followed by boiling for 5 min. around five milliliters of hot water was poured on the watch glass for its complete washing and transferred to the crucible. The crucible matter was filtered on an ash less filter paper and residue so obtained was rinsed with hot water until the filtrate became neutral. This ashless filter paper comprising acid insoluble substance was placed in the crucible, dried on a hot plate to obtain the constant weight. The crucible containing acid insoluble ash was placed in desiccators and weighed. The % of acid insoluble ash [% w/w] was calculated by ratio of weight of ash to the weight of sample multiplied by 100.

Water Soluble Ash Value- To the total ash obtained as mentioned in above procedure, 25 ml of water was added and boiled for 5min. The crucible content was filtered on an ash less filter paper and the residue was washed with hot water. The filter paper comprising insoluble content was placed in the original crucible and ignited in muffle furnace at 450°C for 15 min. The weight of this residue in mg was subtracted from the weight of total ash. The percentage of water soluble ash with reference to air dried plant material was calculated.

Total Ash Value- Two-four gram plant material powder was placed in a tared silica crucible and spreaded uniformly. Then this silica crucible was placed in a muffle furnace and ignited at 500-600ºc until all the material was completely free from carbon content and converted to white color ash. It was transferred to desiccator to avoid moisture absorbance and weighed. Total ash [% w/w] was calculated by the ratio of weight of ash to the weight of sample multiplied by 100.

The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Loss on Drying- This parameter is used to determine the amount of moisture present in particular sample. The drug was powdered and the sample (10 gm) was placed on a pre-weighed evaporating dish. The evaporating dish was already dried at 105˚C for 6 hrs in an oven. The drying was sustained till two consecutive reading matches or the variation between two consecutive weightings was less than 0.25% of constant weight. The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Extractive Values- Extractive values of

crude drug determine the presence of chemical constituents in the crude drugs.

Water Soluble Extractive Value- About four gram of coarsely powdered plant material was kept in a conical flask and macerated with 100 ml water for 24 h, with occasional shaking. After completion of 24 h, the content in the flask was filtered and 25ml of filtrate was placed in a previously tared china dish for the evaporation of solvent. The residual extract obtained was heat dried at 105ºc for six hours, placed in a crucible for thirty minutes and its weight was taken. For the determination of extractive value, the results were determined in terms of mg/g of air-dried material.

Alcohol Soluble Extractive Value- 100 ml of methanol was added to glass stoppered conical flask containing 4 g coarsely, dried powdered plant material and macerated for 24 h with occasional shaking. After completion of 24 h, the content in the flask was filtered and 25ml of filtrate was placed in a previously tared flat-bottomed china dish and the solvent was allowed to evaporate. The residual extract so obtained was dried at 105ºC for 6 h and placed in a desiccator for 30 min and weighed.

The extractive value was determined in terms of mg/g of air-dried material. The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Fluorescence Analysis- The powdered drug from Carissa spinarum L. Flowers was perceived in daylight and UV light to determine its fluorescence properties. The drug was mixed with various chemical reagents to study the changes in fluorescent colour of solution.

The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Table-1 Qualitative Phytochemical Screening of Plant Extract

Sr. No	Phytochemical Constituents	Test Name	Observation	Inference
1	Alkaloids	Wagner's Test	Reddish-brown precipitate	Presence of alkaloids
		Dragendorff's Test	Orange-red precipitate	Presence of alkaloids
		Hager's Test	Yellow precipitate	Presence of alkaloids
		Mayer's Test	Cream-colored precipitate	Presence of alkaloids
		Legal's Test	Pink to red coloration	Presence of alkaloids
2	Glycosides	Keller–Killiani Test	Pale blue layer formation	Cardiac glycosides present
		Borntrager's Test	Red coloration	Anthraquinone glycosides present
		Ninhydrin Test	Blue coloration	Amino glycosides present
3	Proteins	Molisch's Test	Purple/violet ring	Presence of proteins
4	Carbohydrates & Reducing Sugars	Fehling's Test	Brick-red precipitate	Reducing sugars present
		Benedict's Test	Red precipitate	Reducing sugars present
		Lead Acetate Test	White precipitate	Carbohydrates present
5	Phenolics & Tannins	Ferric Chloride Test	Dark blue/greenish-black color	Phenolics/tannins present
		Potassium Ferricyanide Test	Red coloration	Phenolics present
		Ammonia Test	Fluorescence	Phenolics present
6	Flavonoids	Amyl alcohol Test	Yellow color (disappears with acid)	Flavonoids present
		Shinoda's Test	Cherry-red color	Flavonoids present
7	Steroids & Terpenoids	Libermann–Burchard Test	Bluish-green color	Steroids present
		Salkowski Test	Blue-green color	Steroids present
8	Saponins	Froth Test	Persistent froth	Saponins present
		Foam Test	Stable foam layer (1 cm)	Saponins present

Thin Layer Chromatography (TLC) Analysis

I. Preparation of TLC Plates- Pre-coated silica gel 60 F_{254} aluminum plates were used as the stationary phase. The plates were activated by drying in a hot air oven at 110°C for 30 minutes prior to use and allowed to cool to room temperature.

II. Preparation of Sample Solution- The hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) was dissolved in hydroalcoholic solvent (ethanol:water) to obtain a concentration of 10 mg/ml. The solution was filtered before application.

III. Mobile Phase Optimization- Different solvent systems were tried, and the optimized mobile phase was selected based on clear separation of spots.

MT-HAE =Chloroform : Methanol : Water (7:3:0.5 v/v/v)

IV. Chromatographic Development

• A line was drawn 1 cm above the lower edge of the TLC plate.

•Using a capillary tube, 5–10 µl of the extract was spotted on the plate. The plate was placed in a TLC chamber pre-

saturated with mobile phase vapor for 20 minutes.

• Development was carried out up to 8 cm from the point of application.

• The plate was removed and air-dried.

V. Detection of Spots: The developed plates were observed under:

• UV light at 254 nm

• UV light at 366 nm

For better visualization, plates were sprayed with detecting reagents such as vanillin–sulphuric acid or anisaldehyde reagent and heated at 105°C for color development.

VI. Calculation of Rf Value- Rf values were calculated for each visible spot and recorded.

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

In vitro Antioxidant Assay In vitro antioxidant study for different extracts were carried out by using some standard protocols including hydrogen-donating assay, hydrogen peroxide antioxidant assay, superoxide radicals antioxidant assay and reducing power assay.

I. Hydrogen Donating Activity- 2, 2-diphenyl-1-picryl-hydrazil stable radical (DPPH) assay was used to perform the hydrogen donating activity of extracts. Around 50 µm of DPPH was added to solution of different proportions of plant extracts and standard ascorbic acid individually. The solutions were shaken thoroughly and kept at dark for thirty minutes. Then 2ml of methanol and 2ml of DPPH was added to prepare the control soln. The absorbance of all the reaction mixtures and control solution was determined at 517 nm. The % inhibition was calculated by following equation[8].

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract or standard}}{\text{Absorbance of control}}$$

The graph was plotted between % inhibition vs different concentrations of both plant extracts and ascorbic acid. IC 50 was evaluated from the graph.

II. H_2O_2 Antioxidant Assay- H_2O_2 antioxidant assay was performed by the following method: The capability of extract to reduce hydrogen peroxide (H_2O_2) was evaluated spectro-photo-metrically at 230 nm. Varying concentrations of extract or standard were dissolved in 3.4 ml of (0.1 M) PBS at pH 7.4 and added with 0.6 ml of 40 mm H_2O_2. Absorbance at 230 nm was recorded 10 minutes later. A separated blank sample was used for each concentration, for background subtraction[9-10].

III. Superoxide Radical Antioxidant Assay- The superoxide radical antioxidant assay of extracts was evaluated by the following method: Around 0.1 ml quantity of extract were dissolved with 468 µm of 1 ml NADH in 100 mm PBS at ph 7.8 and 156 µm of 1 ml nitroblue tetrazolium solution. Further, the reaction was started with addition of 100 µl of PMS into different reaction mixtures. Then for 5 min, the mixture was kept at the temperature of 25°C and subsequently the absorbance was taken at wavelength of 560 nm by spectrophotometer. In the presence of extract, the absorbance was gradually decreased that indicated the inhibition of superoxide radicals against the control. The percentage inhibition was deter-mined and IC_{50} values for various ex-tracts were estimated. The % inhibition of superoxide free radicals was evaluated by the formula illustrated in previous

section[11,12].

IV. Reducing Power Assay- Around 10–100 µg/ml of extract was added with 2.5 ml of PBS at ph 6.6 and 2.5 ml of 1% kfecn$_3$. The solution was properly mixed and placed in an incubator for 20 min at 50ºc. After incubation, the resulting solution was cooled and 2.5 ml of 10% trichloroacetic acid was mixed with the reaction mixture, accompanied by centrifugation at a speed of 3000 rpm for ten minutes. After centrifugation, an equal volume of distilled water was mixed with 2.5 ml of supernatant and finally 0.5 ml of 0.1% fecl$_3$ was added. Then the mixture was stirred thoroughly and kept at room temperature for ten minutes. The absorbance was taken at a wavelength of 700 nm.

V. Quantitative Analysis of Carissa spinarum L. Root bark-Quantitative estimation of ethyl acetate and methanol was done for the presence of total flavonoids, alkaloids, saponins and phenols.

VI.Total Phenolic Content Estimation- Around 1.5 ml of Folin's reagent was mixed with 1 ml of each sample and standard & then incubated for five minutes at room temperature. Further, 4 ml of Na_2CO_3 and 3.5 ml of distilled water were added to the solution. This reaction mixture was kept for thirty minutes at room temperature after shaking. Then absorbance was measured at a wavelength of 765 nm. For estimation of total phenolic content from the extracts, gallic acid graph was taken as a standard and the value was expressed as mg GAE/g (gallic acid eqv/g of dry wt. of extract)[13].

VII. Total Flavonoid Content Estimation- Around 1 ml of each standard and sample solutions, 3 ml methanol, 0.2 ml alcl$_3$, 5.6 ml distilled water and 0.2 ml of CH_3CO_2K were added. The reaction mixture was kept for thirty minutes at room temperature after shaking. Then absorbance of reaction mixtures was determined at wavelength of 415 nm. For the estimation of total flavonoid content from the extracts, quercetin graph was taken as standard and the value was expressed as mg QE/g [quercetin eqv/gm of dry weight of extract].

VIII. Total Alkaloid Content Estimation- Test sample was dissolved in DMSO and then 1ml of 2 N hydrochloric acid was added and filtered. Around 5 ml of bromocresol green solution and 5 ml of PBS was mixed to the above solution. The solution was vigorously stirred with the constant addition of 1–4 ml of chloroform, then this mixture was kept in 10 ml of volumetric flask. Absorbance was determined at wavelength of 470 nm by utilizing UV spectroscopy. Standard graph of atropine was used for the determination of total alkaloid content from the extracts.

IX. Total Saponin Content Estimation-Test sample was dissolved in DMSO, then 1 ml of 2 N hydrochloric acid was added and filtered. Around 5 ml of bromocresol green solution and 5 ml of PBS was mixed with the above solution. About 1–4 ml of chloroform was constantly mixed to the solution with thorough stirring. The solution was kept in a 10 ml volumetric flask and the volume was made up with chloroform. Absorbance was determined at wavelength of 470 nm by using UV spectroscopy. Different concentrations

of diosgenin solution (50, 62.5, 75, 87.5, 100, 112.5 and 125.5 µg/ml) were made by adding diosgenin distilled water & methanol at 4:6 ratio respectively. 0.25 ml of 8% vanillin reagent and 2.5 ml of 72% sulphuric acid were blended with each sample. The samples were kept at 60 °C in water bath and incubated for ten minutes. Then absorbance of solution was determined at wavelength of 544 nm. Further, 0.1 g of extract was heated in aqueous methanol and the solution was prepared up to 0.25 ml. The total saponins present in the prepared solution were estimated at 544 nm.

Experimental animals- Healthy adult Wistar rats weighing between 180–220 g of either sex were selected for the experimental study. These animals are widely used in pharmacological and toxicological research due to their well-characterized physiology, ease of handling, and consistent response to experimental conditions. The rats were procured from a certified animal facility and maintained under standard laboratory conditions to ensure uniformity and minimize environmental stress. The animals were housed in clean polypropylene cages under controlled temperature (22 ± 2°C) and relative humidity (55 ± 5%), with a 12-hour light and dark cycle to mimic natural circadian rhythms. Proper environmental conditions are essential for maintaining normal metabolic and physiological functions, thereby ensuring the reliability of experimental outcomes. The animals were provided with a standard pellet diet and had free access to clean drinking water ad libitum.

Prior to the commencement of the experiment, all animals were acclimatized to the laboratory environment for one week. This acclimatization period is crucial as it allows the animals to adjust to new surroundings, reducing stress-induced variability in experimental results. Proper care and handling of laboratory animals in accordance with ethical guidelines are fundamental to obtaining reproducible and scientifically valid data. These standardized conditions help ensure consistency, reduce experimental bias, and improve the reproducibility of pharmacological studies.

Acute oral toxicity- The acute oral toxicity study of the *Mimosa tenuiflora* leaf extract was conducted in accordance with OECD Guideline 423, also known as the Acute Toxic Class Method. This standardized protocol is widely accepted for evaluating the safety profile of test substances and determining their approximate lethal dose. The extract was administered orally to experimental animals at graded dose levels of 100, 200, 500, 1000, and 2000 mg/kg body weight to assess dose-dependent toxic effects. Following administration, the animals were closely monitored for a period of 14 days for any signs of toxicity. Observations included changes in behavioral parameters such as locomotor activity, alertness, and grooming, as well as neurological responses like tremors, convulsions, and coordination. Addition-ally, autonomic responses such as salivation, lacrimation, respiration, and changes in food and water intake were recorded. Mortality, if any, was also noted to estimate the safety margin of the extract.

This systematic observation period is crucial for identifying both immediate and delayed toxic effects. Based on the

absence or presence of toxic symptoms and mortality, the extract's. Safety profile was established, and sui-table doses were selected for further pharmacological evaluation. Thus, the acute toxicity study provides essential baseline data to ensure the safe adminis-tration of the plant extract in subsequent experimental models[14].

Induction of experimental diabetes- Experimental diabetes was induced in Wistar rats using Streptozotocin (STZ), a well-known diabetogenic agent that selectively destroys insulin-producing β-cells of the pancreas. A single intra-peritoneal injection of STZ was adminis-tered at a dose of 50 mg/kg body weight. The compound was freshly prepared in cold citrate buffer (0.1M, pH 4.5) to maintain its stability, as STZ is highly unstable in aqueous solutions at neutral ph. Prior to administration, the animals were fasted overnight to enhance the effectiveness of STZ and ensure consis-tent induction of diabetes. Following STZ injection, the animals were provided with a 5% glucose solution for 24 hours to prevent sudden hypoglycemia, which may occur due to the acute release of insulin from dama-ged pancreatic cells. This step is critical for reducing mortality during the initial phase of diabetes induction. After 72 hrs, fasting blood glucose levels were meas-ured using a standard glucometer. Rats exhibiting fasting blood glucose levels greater than 250 mg/dl were considered diabetic and selected for further experi-mental studies. This method of diabetes induction is widely used due to its reproducibility and its ability to mimic human diabetes mellitus, particularly insulin-dependent diabetes. It provides a reliable experi-mental model for evaluating the anti-diabetic potential of plant extracts and pharmacological agents.

Experimental design- In this study, Wistar rats were randomly divided into six groups, with six animals in each group (n = 6), to evaluate the antidiabetic potential of the plant extract in a controlled and systematic manner. Such grouping ensures proper comparison between normal, diabetic, standard, and treatment groups, thereby improving the reliability of experimental outcomes.

Group I served as the normal control and received only the vehicle, maintaining physiological conditions without any treatment.

Group II acted as the diabetic control, in which diabetes was induced using Strep-tozotocin (STZ), but no treatment was administered.

Group III was treated with a standard antidiabetic drug such as Metformin or Glibenclamide to serve as a positive control for comparison.

Groups IV to VI were designated as test groups and received low, medium, and high doses of the plant extract, respect-tively. All treatments were administered orally once daily for a duration of 21 days. This duration is sufficient to observe the therapeutic effects of the extract on blood glucose levels and other biochemical parameters. The structured experimental design allows for dose-dependent evaluation and comparison with standard treatment, thereby helping to establish the efficacy of the plant extract in managing diabetes.

Table-2 Experimental Design

Group	Description	Treatment Given
Group I	Normal control	Vehicle only
Group II	Diabetic control	STZ only
Group III	Standard drug-treated	Metformin/ Glibenclamide
Group IV	Treatment(Low dose) 100 Mg/kg p.o.	Low dose of extract
Group V	Treatment(Low dose)200 Mg/kg p.o.	Medium dose of extract
Group VI	Treatment(Low dose) 400 Mg/kg p.o.	High dose of extract

This design ensures clear differentiation between control and treatment effects, enabling accurate assessment of anti-diabetic activity.

Results

Evaluation of antidiabetic activity- The antidiabetic activity of the plant extract was evaluated by monitoring key physiological and biochemical para-meters in Wistar rats throughout the study period. Fasting blood glucose levels were measured at regular intervals using a standard glucometer, which provides a rapid and reliable assessment of glucose concentration in blood. Measurements were typically taken at baseline (day 0) and at predetermined intervals (such as day 7, 14, and 21) to observe the progression or reduction of hyperglycemia in response to treatment. Regular monitoring of blood glucose is essential for determining the efficacy of the extract in controlling diabetes.

In addition to glucose levels, body weight of the animals was recorded periodically, as weight loss is a common symptom associated with diabetes due to altered metabolism and protein cata-bolism. Improvement or stabilization of body weight indicates a positive thera-peutic effect of the treatment. Further-more, general health conditions such as activity level, grooming behavior, food and water intake, and any signs of distress or illness were carefully observed and recorded. These para-meters provide supportive evidence regarding the safety and overall bene-ficial effects of the extract. Thus, the combined evaluation of blood glu-cose levels, body weight, and general health status offers a comprehensive assessment of the antidiabetic potential of the test extract over the experimental period.

These tables facilitate systematic recording and comparison of experi-mental data, ensuring clarity and accu-racy in evaluating the antidiabetic activity of the extract[15,16].

Biochemical estimation- At the end of the experimental period, blood samples were collected from Wistar rats under appropriate conditions, typically via retro-orbital puncture or cardiac punc-ture under mild anesthesia. The collected blood was allowed to clot and then centrifuged to separate the serum, which was used for various biochemical estimations. These analyses are crucial for evaluating the overall metabolic status and organ function in diabetic conditions and after treatment.

Serum glucose levels were measured to confirm the antidiabetic effect of the extract. In addition, the lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was assessed to evaluate the impact on lipid metabolism, as diabetes is often associated with dyslipidemia. Renal function markers such as Serum creatinine and blood urea nitrogen (BUN) were also estimated, since prolonged hyperglycemia can lead to kidney damage or diabetic nephropathy. All bio-chemical parameters were analyzed using standard commercially available diagnostic kits, following the manu-facturer's protocols to ensure accuracy and reproducibility. These kits are designed to provide precise quantitative measurements and are widely used in experimental and clinical studies. The combined evaluation of glucose, lipid profile, and renal markers provides a comprehensive understanding of the therapeutic efficacy and safety of the plant extract in managing diabetes and its associated complications[17,18].

Table-3 Biochemical Parameters Evaluated

Parameter	Significance
Serum Glucose	Assessment of antidiabetic activity
Total Cholesterol	Indicator of lipid metabolism
Triglycerides	Marker of dyslipidemia
HDL (High-density lipoprotein)	Protective lipid fraction
LDL (Low-density lipoprotein)	Atherogenic lipid fraction
Serum Creatinine	Indicator of kidney function
Blood Urea Nitrogen (BUN)	Marker of renal function

These biochemical estimations provide essential insights into both metabolic control and organ protection offered by the treatment.

Estimation of oxidative stress parameters- At the end of the experimental study, pancreatic tissue from Wistar rats was carefully excised, washed with ice-cold saline, and homogenized to prepare tissue samples for the assessment of oxidative stress para-meters. Tissue homogenization was carried out in an appropriate buffer under cold conditions to preserve enzyme activity and prevent degradation of antioxidant molecules. The resulting homogenate was then centrifuged, and the supernatant was used for biochemical analysis.

The estimation of antioxidant enzymes is crucial, as oxidative stress plays a significant role in the pathogenesis of diabetes and its complications. Key antioxidant parameters evaluated included superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radicals into hydrogen peroxide; catalase (CAT), which further converts hydrogen peroxide into water and oxygen; and reduced glutathione (GSH), a major intracellular antioxidant that protects cells against oxidative damage. Additionally, lipid peroxidation (LPO) levels were measured, commonly expressed as malondialdehyde (MDA), to assess the extent of oxidative damage to cellular membranes.

All these parameters were determined using standard biochemical methods, ensuring accuracy and reproducibility of results. An increase in antioxidant enzyme levels along with a decrease in lipid peroxidation indicates a protective effect of the plant extract against oxidative stress. Therefore, the evaluation of these markers provides important insights into the antioxidant potential and therapeutic efficacy of the extract in diabetic conditions[17,18].

Table-4 Oxidative Stress Parameters

Parameter	Full Form	Significance
SOD	Superoxide Dismutase	Converts superoxide radicals to H_2O_2
CAT	Catalase	Breaks down hydrogen peroxide into Water and O_2
GSH	Reduced Glutathione	Major intracellular antioxidant

Histopathological examination- Histopathological evaluation of pancreatic tissue was performed by fixing excised samples in 10% neutral buffered formalin, followed by dehydration, clearing, and paraffin embedding. Thin sections (4–5 µm) were prepared using a microtome and stained with hematoxylin and eosin (H&E) for microscopic examination. The stained sections were analyzed to assess structural changes in β-cells of the islets of Langerhans, including degeneration, necrosis, inflammation, and restoration of normal architecture. This evaluation provides visual confirmation of pancreatic damage and the protective effects of the treatment, supporting the biochemical findings.

Statistical analysis- All experimental data obtained from studies on Wistar rats were expressed as mean ± standard error of the mean (SEM), which reflects the variability and reliability of the results. This format allows easy comparison between different experimental groups and improves the interpretation of data precision. Statistical analysis was performed using one way ANOVA to evaluate differences among multiple groups. Post hoc tests such as Tukey's or Dunnett's were applied to identify specific group differences.

Standardization of Plant Material (Preliminary Pharmacognostical Studies)- The safety, quality, and therapeutic efficacy of herbal formulations depend on proper identification, authentication, and standardization of the plant material used. Therefore, preliminary pharmacognostical and physicochemical evaluation of the powdered leaves of *Mimosa tenuiflora* was carried out to establish its identity, purity, and quality prior to pharmacological investigation.

The physicochemical parameters of the powdered leaf material were determined using standard pharmacognostical procedures. The total ash value was found to be 6.8 ± 0.12%, indicating the presence of inorganic constituents such as carbonates, phosphates, and silicates. The acid-insoluble ash value was 1.1 ± 0.10%, suggesting minimal contamination with siliceous matter and confirming the purity of the crude drug.

The water-soluble ash value was observed to be 2.3 ± 0.20%, indicating the presence of water-soluble inorganic salts. The loss on drying was found to be 9.6 ± 0.75%, reflecting acceptable moisture content and suitability for storage without significant risk of microbial growth.

The extractive values were determined to estimate the amount of active phyto-constituents present in the plant material. The alcohol-soluble extractive value was found to be 15.2 ± 0.58%, while the water-soluble extractive value was 20.6 ± 0.70%, indicating the predominance of polar constituents.

Fluorescence analysis of the powdered leaf material was carried out as an additional parameter for authentication and quality evaluation. The powder exhibited characteristic color changes under daylight and ultraviolet light upon treatment with different chemical reagents. These fluorescence character-ristics serve as an important tool for identification and detection of adult-eration.

Table-5 Physicochemical Parameters of Powdered Leaves of Mimosa tenuiflora

Parameters (% w/w)	*Mimosa tenuiflora*
Total ash	7.48 ± 0.12
Acid insoluble ash	1.36 ± 0.05
Water soluble ash	3.14 ± 0.09
Loss on drying	6.72 ± 0.11
Alcohol extractive value	10.47 ± 0.13
Water extractive value	12.83 ± 0.15

Values are expressed as mean ± SEM (n = 3)

Table-6 Fluorescence Characteristics of Powdered Leaves of *Mimosa tenuiflora*

Treatment	Day Light	UV Light (254 nm)	UV Light (366 nm)
Powder as such	Brown	Dark brown	Greenish brown
Powder + Distilled water	Light brown	Dark green	Green
Powder + 1N hcl	Reddish brown	Dark brown	Orange brown
Powder + 1N naoh	Dark brown	Green	Yellowish green
Powder + Methanol	Brown	Greenish brown	Light green
Powder + Ethanol	Brown	Dark green	Yellowish green
Powder + Chloroform	Dark brown	Green	Light green
Powder + Acetic acid	Brown	Dark brown	Greenish brown

Extraction of Plant Material- Extraction of plant material was carried out to isolate the bioactive phytoconstituents present in the leaves of *Mimosa tenuiflora* for further pharmacological evaluation. The dried and powdered leaves of Mimosa tenuiflorawere subjected to Soxhlet

extraction using a hydroalcoholic solvent system. The extract obtained was concentrated and dried to obtain a semi-solid residue. The percentage yield of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was calculated based on the initial weight of the powdered drug. The percentage yield was found to be 11.2% w/w.

Qualitative Phytochemical Screening- Preliminary phytochemical screening of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was carried out to identify the presence of major bioactive constituents. The phytochemical investigation revealed the presence of secondary metabolites such as proteins, carbohydrates, flavonoids, glycosides, saponins, polyphenols, tannins, and alkaloids in the extract. Steroids were found to be present, while fats and oils were absent. The results of qualitative phytochemical screening of HAEMT are presented in Table 7.

Table-7 Phytochemical Constituents of Hydroalcoholic Extract of *Mimosa tenuiflora*

Phytoconstituents	HAEMT
Proteins	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Saponins	+
Polyphenols	+
Tannins and phenolic compounds	+
Alkaloids	+
Steroids	+
Fats and oils	-

(+ = Present, − = Absent)

Quantitative Analysis of Hydro-alcoholic Extract- Quantitative estimation of bioactive constituents was carried out for the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) following preliminary phytochemical screening. The total phenolic content (TPC) and total flavonoid content (TFC) were determined using standard procedures.

Estimation of Total Phenolic Content (TPC)- The total phenolic content of HAEMT was determined using the Folin–Ciocalteu method, with gallic acid as the standard. The calibration curve obtained from gallic acid was used to calculate the phenolic content of the extract. The total phenolic content of HAEMT was found to be 212.85 ± 0.88 mg/g extract (gallic acid equivalent).

Estimation of Total Flavonoid Content (TFC)- The total flavonoid content of HAEMT was determined using rutin as the standard. The calibration curve of rutin was used to estimate the flavonoid content in the extract. The total flavonoid content of HAEMT was found to be 76.94 ± 3.84 mg/g extract (rutin equivalent)

Estimation of Total Alkaloid Content- The total alkaloid content of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was determined using atropine as the standard. The calibration curve of atropine was used to quantify the alkaloid content in the extract. The total alkaloid content of HAEMT was found to be 27.63 ± 2.48 mg/g extract (atropine equivalent)

Estimation of Total Saponin Content- The total saponin content of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was determined using the vanillin–sulfuric acid method. Diosgenin was used as the standard reference compound, and the results were expressed as mg/g diosgenin equivalent using the standard calibration curve. The total saponin content of HAEMT was found to be 54.72 ± 1.28 mg/g extract (diosgenin equivalent).

Thin Layer Chromatography (TLC) Results of Hydroalcoholic Extract- Thin Layer Chromatography of the hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) was performed using chloroform: methanol: water (7:3:0.5 v/v/v) as the mobile phase. The developed TLC plates showed distinct and well-resolved spots indicating the presence of multiple phytoconstituents in the extract. The chromatogram was observed under UV light at 254 nm and 366 nm, followed by spraying with vanillin–sulphuric acid reagent, which resulted in the appearance of colored spots corresponding to different classes of compounds.

Table-8 TLC Profile of MT-HAE

Spot No.	Rf Value	Color (After Spraying)	Observation under UV	Probable Phytoconstituent
1	0.18	Light yellow	Faint fluorescence	Phenolic compounds
2	0.32	Yellow-orange	Bright fluorescence	Flavonoids
3	0.45	Brown	Moderate	Tannins
4	0.58	Greenish-yellow	Strong fluorescence	Polyphenols
5	0.71	Violet	Visible	Terpenoids
6	0.86	Dark brown	Weak	Glycosides

Interpretation- The TLC profile of the hydroalcoholic extract revealed the presence of multiple phytoconstituents with different polarities, as indicated by varying Rf values. The appearance of yellow, orange, and green fluorescent spots suggests the presence of flavonoids and phenolic compounds, which are known for their antioxidant activity. The brown colored spots indicate tannins, while violet coloration may correspond to terpenoid compounds. The distribution of spots across the TLC plate indicates that the hydroalcoholic extract contains a complex mixture of bioactive compounds, supporting its potential pharmacological activity.

Figure-1 TLC Analysis

In vitro Antioxidant Activity

DPPH Radical Scavenging Activity- The hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) exhibited significant hydrogen donating ability in

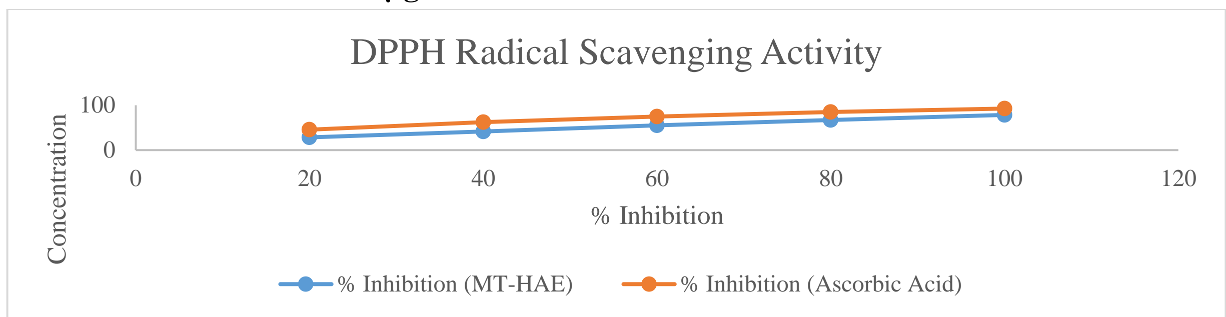
the DPPH assay. The percentage inhibition increased in a concentration-dependent manner when compared with standard ascorbic acid.

Table-9 DPPH Radical Scavenging Activity

Concentration (µg/ml)	% Inhibition (MT-HAE)	% Inhibition (Ascorbic Acid)
20	28.4 ± 1.2	45.6 ± 1.1
40	41.7 ± 1.4	62.3 ± 1.3
60	55.2 ± 1.5	74.8 ± 1.2
80	66.9 ± 1.3	85.2 ± 1.1
100	78.5 ± 1.2	92.6 ± 1.0

IC_{50} value:

- MT-HAE → **~58 µg/ml**
- Ascorbic acid → **~32 µg/ml**

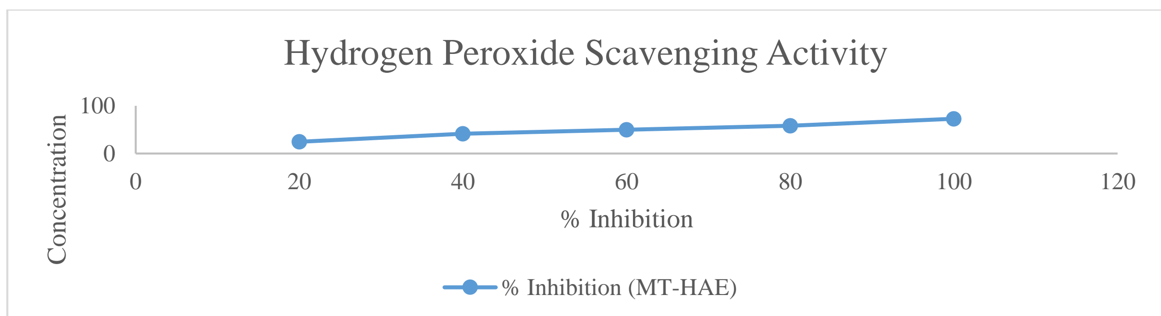


Graph-1 DPPH Radical Scavenging Activity

II. Hydrogen Peroxide Scavenging Activity-The extract demonstrated moderate to strong H_2O_2 scavenging activity, indicating its ability to neutralize reactive oxygen species.

Table-10 Hydrogen Peroxide Scavenging Activity

Concentration (µg/ml)	% Inhibition (MT-HAE)
20	25.6 ± 1.0
40	38.2 ± 1.2
60	49.7 ± 1.3
80	61.3 ± 1.4
100	72.8 ± 1.2

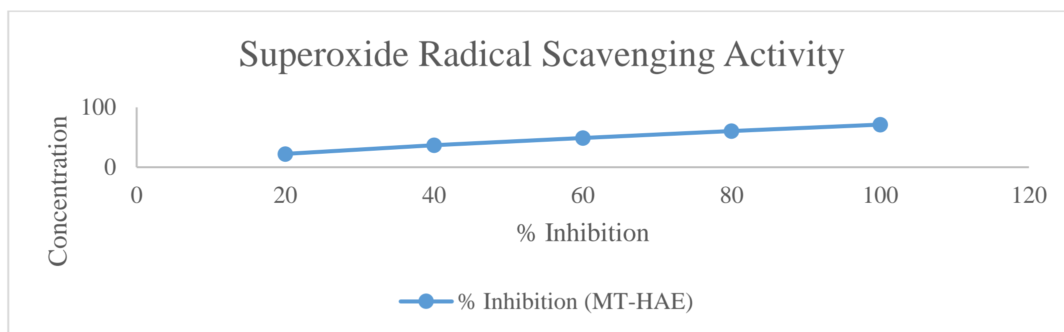


Graph- 2 Hydrogen Peroxide Scavenging Activity

III. Superoxide Radical Scavenging Activity- The extract showed effective inhibition of superoxide radicals, confirming its antioxidant potential.

Table-11 Superoxide Radical Scavenging Activity

Concentration (µg/ml)	% Inhibition (MT-HAE)
20	22.3 ± 1.1
40	36.8 ± 1.2
60	48.9 ± 1.4
80	60.5 ± 1.3
100	71.2 ± 1.1

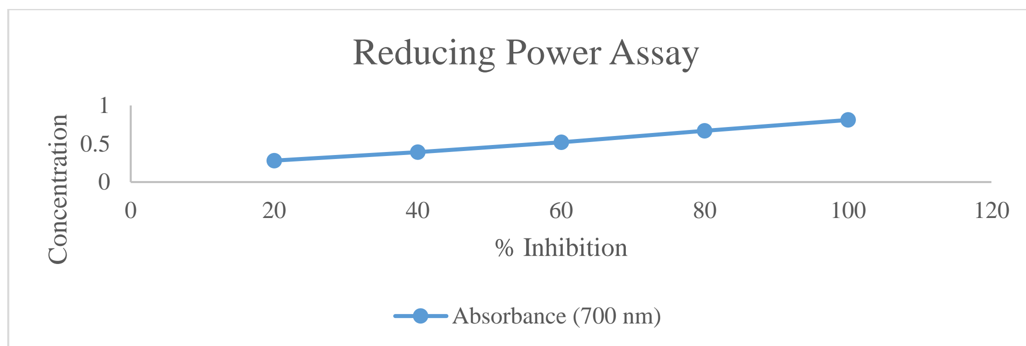


Graph- 3 Superoxide Radical Scavenging Activity

IV. Reducing Power Assay- The reducing power of MT-HAE increased with increasing concentration, indicating strong electron-donating capacity.

Table-12 Reducing Power Assay

Concentration (µg/ml)	Absorbance (700 nm)
20	0.28 ± 0.01
40	0.39 ± 0.02
60	0.52 ± 0.02
80	0.67 ± 0.01
100	0.81 ± 0.02



Graph-4 Reducing Power Assay

Quantitative Phytochemical Estimation- The quantitative phytochemical evaluation of the hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) was carried out to determine the concentration of major bioactive constituents.

These phyto-constituents are responsible for important pharmacological activities, especially antioxidant and antidiabetic effects. The results were expressed as mean ± SEM to ensure accuracy, reliability, and reproducibility.

Total Phenolic Content- The total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard. The extract showed a phenolic content of 82.5 ±2.1mg GAE/g, indicating a high abundance of phenolic compounds. This suggests strong antioxidant potential, which may help in reducing oxidative stress, particularly under diabetic conditions.

Total Flavonoid Content- The total flavonoid content was estimated using the aluminium chloride colorimetric method with quercetin as the standard. The extract exhibited 64.3 ± 1.8 mg QE/g, indicating a substantial presence of flavonoids. These compounds contribute to antioxidant, anti-inflammatory, and antidiabetic activities by improving glucose uptake and scavenging free radicals.

Total Alkaloid Content- The total alkaloid content was determined using a spectrophotometric method with atropine as the reference standard. The extract contained 21.6 ± 1.2 mg/g of alkaloids, showing a moderate level of these compounds. Although present in smaller quantities, alkaloids may contribute synergistically to the overall pharmacological effects of the extract.

Total Saponin Content- The total saponin content was estimated using standard colorimetric methods. The extract showed 18.9 ±1.0 mg/g, indicating a moderate presence of saponins. These compounds are known to enhance glucose metabolism and improve insulin sensitivity, supporting the antidiabetic potential of the extract.

In vivo Pharmacological Evaluation- The in vivo study was carried out to evaluate the pharmacological activity of the hydroalcoholic extract of *Mimosa tenuiflora* (HAEMT) in experimental animals.

Acute Oral Toxicity Study- The acute oral toxicity study of the hydroalcoholic extract of *Mimosa tenuiflora*(HAEMT) was carried out in experimental animals in accordance with OECD guideline 423.

The extract was administered orally at a limit dose of 2000 mg/kg body weight. The animals were observed continuously for the first 4 hours for any immediate signs of toxicity and periodically up to 24 hours, followed by daily observations for a period of 14 days.

No mortality was observed in any of the treated animals during the entire study period. The animals did not exhibit any signs of toxicity such as tremors, convulsions, salivation, diarrhea, lethargy, or coma. No abnormal behavioral changes were observed, and the animals remained active and healthy throughout the study.

There were no noticeable changes in skin, fur, eyes, or mucous membranes. Food and water intake were found to be normal, and no significant variation in body weight was observed during the observation period.

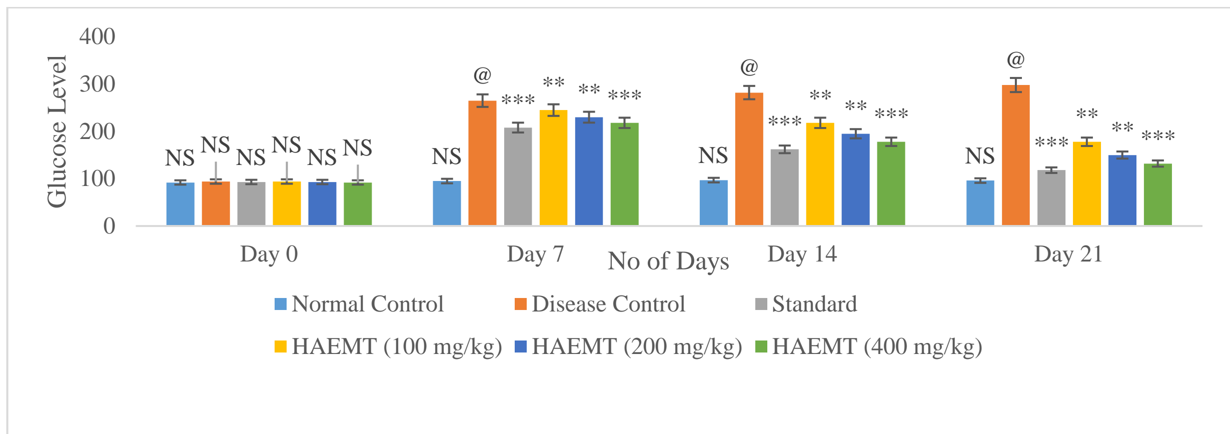
Based on these observations, the hydroalcoholic extract of *Mimosa tenuiflora* was found to be safe at the dose of 2000 mg/kg body weight, and the median lethal dose (LD_{50}) was considered to be greater than 2000 mg/kg.

Change in Blood Glucose Levels- The effect of HAEMT on blood glucose levels was evaluated over a period of 21 days. The observations are presented in Table-13.

Table-13 Effect of Extract on Blood Glucose Levels (mg/dl)

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	92 ± 3	95 ± 4	97 ± 3	96 ± 2
Disease Control	94 ± 8	265 ± 11	282 ± 12	298 ± 13
Standard	93 ± 8	208 ± 7	162 ± 6	118 ± 5
HAEMT (100 mg/kg)	94 ± 7	245 ± 9	218 ± 8	178 ± 7
HAEMT (200 mg/kg)	93 ± 7	230 ± 8	195 ± 7	150 ± 6
HAEMT (400 mg/kg)	92 ± 6	218 ± 7	178 ± 6	132 ± 5

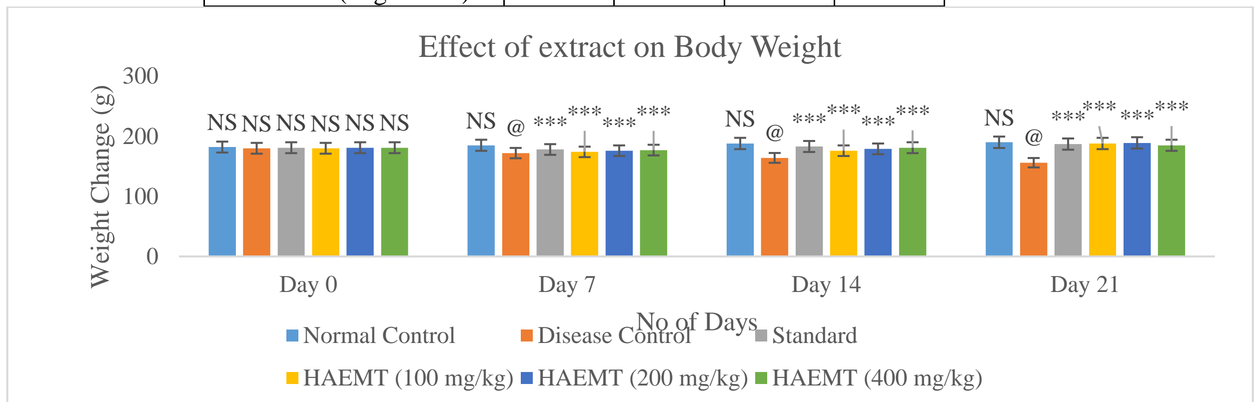
Values are expressed as mean ± SEM (n = 6)



Graph-5 Effect of Extract on Blood Glucose Levels (mg/dl)

Table-14 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Body Weight (g)

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	182 ± 5	185 ± 6	188 ± 5	190 ± 6
Disease Control	180 ± 6	172 ± 5	164 ± 6	156 ± 7
Standard	181 ± 5	178 ± 5	183 ± 5	187 ± 6
HAEMT (Low Dose)	180 ± 6	174 ± 5	176 ± 6	179 ± 5
HAEMT (Medium Dose)	181 ± 5	176 ± 5	179 ± 5	183 ± 5
HAEMT (High Dose)	181 ± 5	177 ± 5	181 ± 5	185 ± 6



Graph-6 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Body Weight (g)

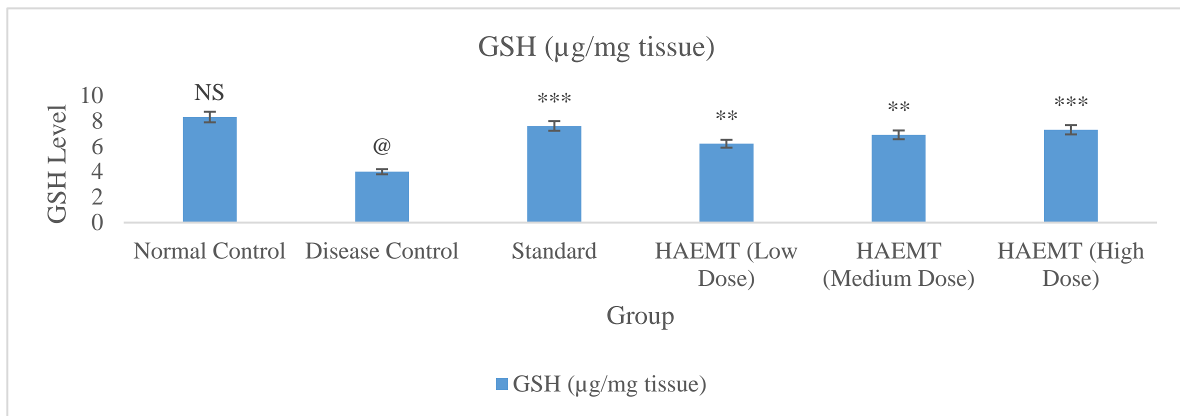
Effect on Antioxidant Parameters

Table-15 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on GSH, SOD, and MDA Levels.

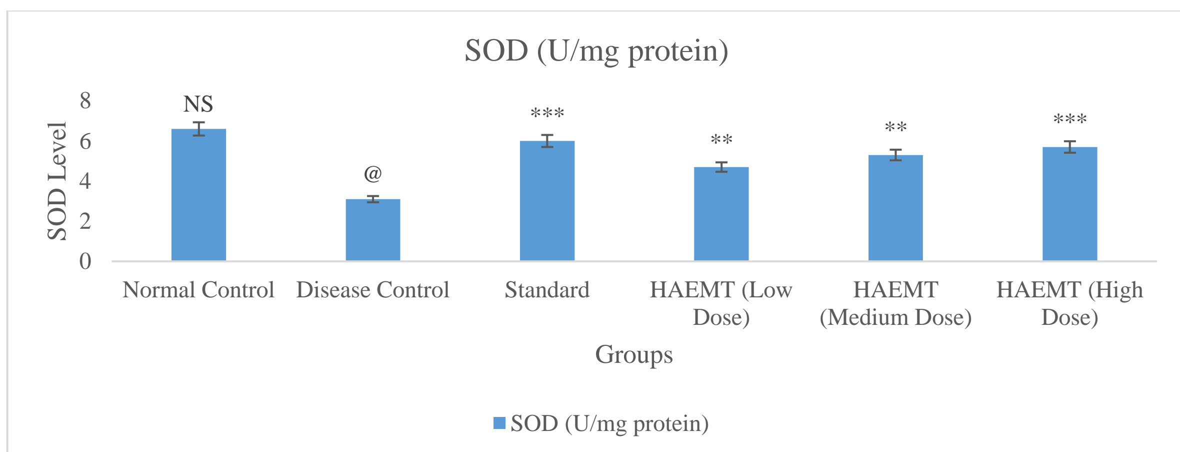
Group	GSH (µg/mg tissue)	SOD (U/mg protein)	MDA (nmol/mg tissue)
Normal Control	8.3 ± 0.4	6.6 ± 0.3	2.0 ± 0.1

Disease Control	4.0 ± 0.3	3.1 ± 0.2	5.9 ± 0.3
Standard	7.6 ± 0.3	6.0 ± 0.3	2.5 ± 0.2
HAEMT (Low Dose)	6.2 ± 0.3	4.7 ± 0.2	3.8 ± 0.2
HAEMT (Medium Dose)	6.9 ± 0.3	5.3 ± 0.2	3.3 ± 0.2
HAEMT (High Dose)	7.3 ± 0.3	5.7 ± 0.3	2.9 ± 0.2

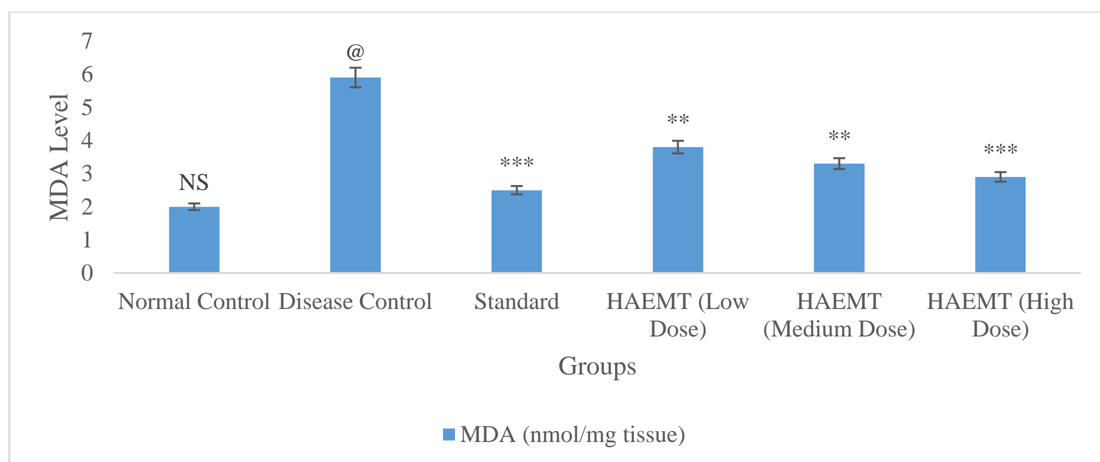
Values are expressed as mean ± SEM (n = 6)



Graph- 7 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on GSH Levels



Graph-8 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on SOD Levels

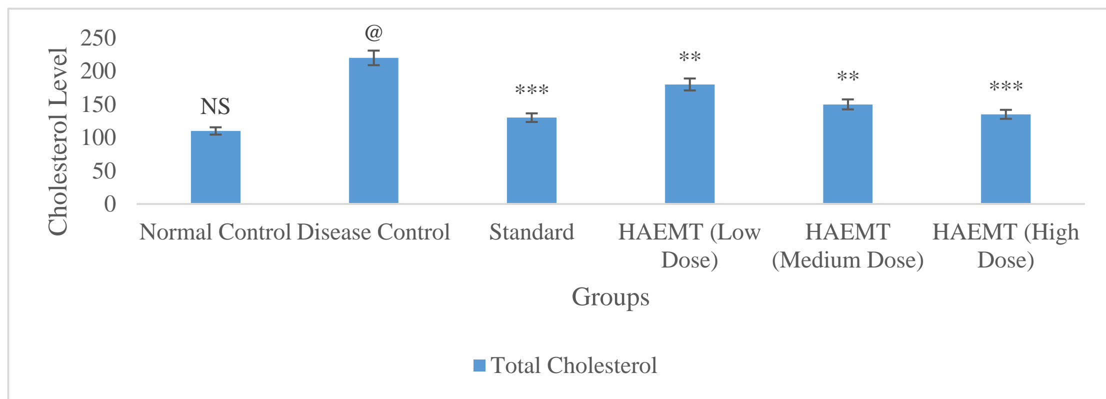


Graph-9 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on MDA Levels

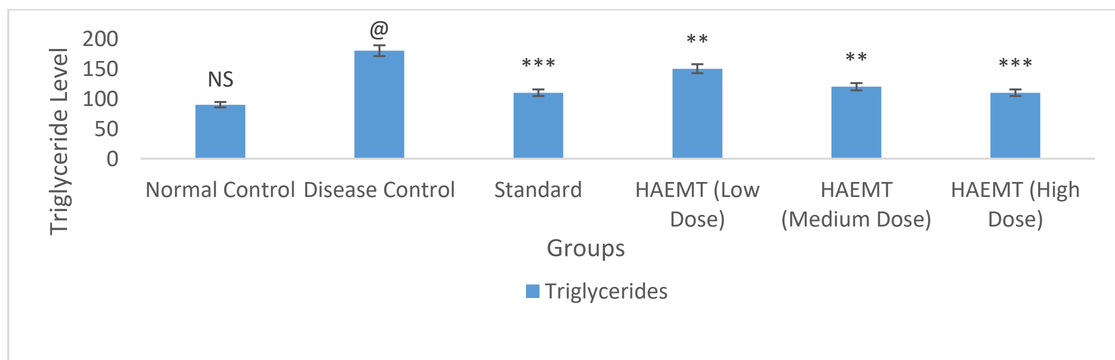
Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Lipid Profile

Table-16 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Lipid Profile

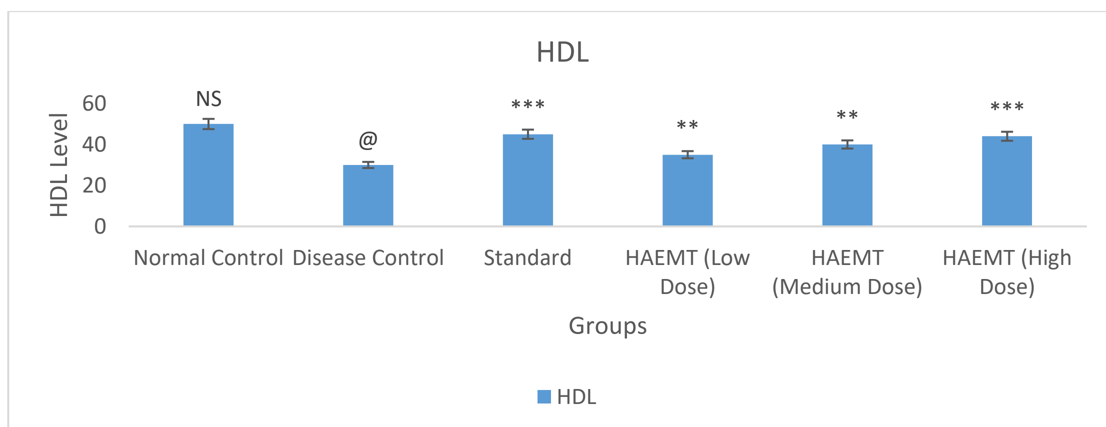
Group	Total Cholesterol	Triglycerides	HDL	LDL
Normal Control	110 ± 3	90 ± 2	50 ± 2	40 ± 2
Disease Control	220 ± 5	180 ± 4	30 ± 1	140 ± 3
Standard	130 ± 3	110 ± 3	45 ± 2	60 ± 2
HAEMT (Low Dose)	180 ± 4	150 ± 3	35 ± 2	100 ± 3
HAEMT (Medium Dose)	150 ± 3	120 ± 3	40 ± 2	80 ± 2
HAEMT (High Dose)	135 ± 3	110 ± 2	44 ± 2	65 ± 2



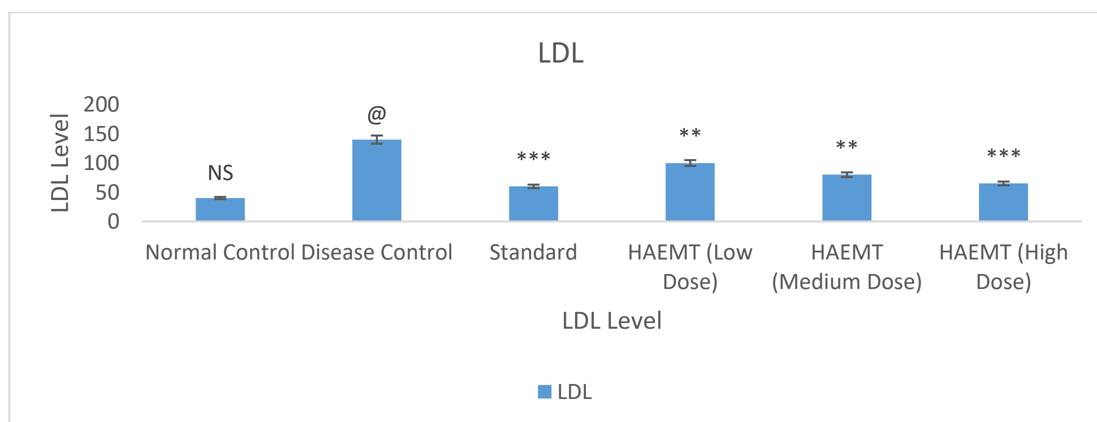
Graph -10 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on total cholesterol Level



Graph-11 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Triglycerides Level

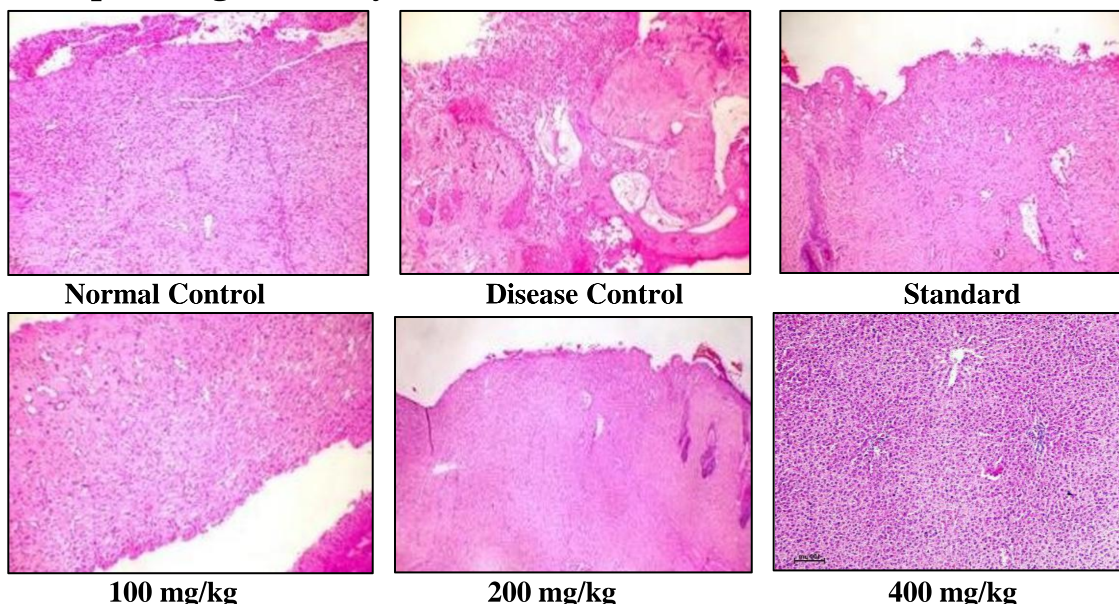


Graph-12 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on HDL Level



Graph-13 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on LDL Level

Histopathological Analysis



Phytochemicals such as flavonoids and tannins were identified. Extract showed significant α-amylase and α-glucosidase inhibition and reduced blood glucose in animal models.

Discussion

The present study was conducted to evaluate the antidiabetic potential of *Mimosa tenuiflora* through extraction, isolation, and identification of its bio-active compounds, along with pharma-cological assessment using in vitro and in vivo models. The findings of this study clearly demonstrate that the plant extract possesses significant antidiabetic and antioxidant activity, which can be attributed to its rich phytochemical composition. Diabetes mellitus is a multifactorial metabolic disorder in which chronic hyperglycemia leads to oxidative stress and inflammation, playing a crucial role in disease progression and complications. Persistent elevation of blood glucose levels results in excessive production of reactive oxygen species (RO-S), which disrupt cellular homeo-stasis and damage lipids, proteins, and DNA. This oxidative damage contributes to the

development of complications such as neuropathy, nephropathy, and cardio-vascular disorders.

In the present study, phytochemical screening revealed the presence of flavo-noids, phenolic compounds, tannins, and alkaloids, which are well known for their antioxidant and anti-diabetic properties. These compounds play a significant role in neutralizing ROS and protecting pan-creatic β-cells from oxidative damage. The antioxidant activity observed in assays such as DPPH supports the ability of the extract to scavenge free radicals and reduce oxidative stress. The anti-diabetic activity of *Mimosa tenuiflora* can be explained through multiple mec-hanisms. Firstly, flavonoids and phen-olic compounds may enhance insulin secretion and improve insulin sens-itivity in peripheral tissues. Secondly, inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase delays glucose absorption, thereby controlling postprandial hyper-glycemia. Additionally, certain phyto-constituents may reduce hepatic glucose production, contributing to overall glyc-emic control. The in vivo results obtained from strep-tozotocin-induced diabetic Wistar rats further validate the antidiabetic potential of the plant extract. A significant reduction in blood glucose levels, along with improvement in biochemical para-meters such as lipid profile and anti-oxidant enzymes (SOD, GSH, MDA), indicates the effectiveness of the extract in managing diabetes and associated oxi-dative stress. These findings are consis-tent with previous reports that highlight the role of plant-derived antioxidants in improving metabolic function and pre-venting diabetic complications. The selection of plant-based therapy in this study is justified by the limitations associated with conventional anti-diabetic drugs, which often produce adverse effects and target single pathways. In contrast, phytochemicals exhibit a multi-target mode of action, addressing various aspects of diabetes, including insulin resistance, oxidative stress, and inflammation. The synergistic interaction of multiple bioactive compounds present in the extract enhances its therapeutic efficacy and provides a holistic approach to disease management.

Overall, the results of the present study strongly support the therapeutic poten-tial of *Mimosa tenuiflora* as a natural source of antidiabetic agents. The integration of phytochemical analysis with pharma-cological evaluation provides a scientific basis for its traditional use and highlights its potential for further development into safe and effective herbal formulations.

Conclusion

The present study was carried out to investigate the extraction, isolation, and identification of bioactive compounds from *Mimosa tenuiflora* and to evaluate its antidiabetic potential using both in vitro and in vivo approaches. The findings of the study clearly indicate that *Mimosa tenuiflora* is a rich source of pharmacologically active phyto-constituents, including flavonoids, phen-olic compounds, tannins, and alkaloids, which are known to possess significant antioxidant and therapeutic properties.

Phytochemical analysis confirmed the presence of these bioactive compounds,

while chromatographic and analytical techniques facilitated their separation and identification. The antioxidant studies demonstrated that the extract possesses strong free radical scavenging activity, suggesting its ability to reduce oxidative stress, which is a key factor in the pathogenesis of diabetes mellitus. The in vitro antidiabetic assays further revealed that the extract effectively inhibits carbohydrate digesting enzymes, thereby contributing to improved glycemic control.

The in-vivo evaluation using streptozotocin-induced diabetic Wistar rats provided substantial evidence of the antidiabetic efficacy of the plant extract. A significant reduction in blood glucose levels, along with improvement in lipid profile and antioxidant parameters such as SOD, GSH, and MDA, highlights its potential in managing both hyperglycemia and associated metabolic disturbances. These effects may be attributed to multiple mechanisms, including enhancement of insulin secretion, improvement of insulin sensitivity, reduction of oxidative stress, and protection of pancreatic β-cells.

Overall, the study supports the potential of *Mimosa tenuiflora* as a promising natural source for the development of novel antidiabetic agents. Its multi-target mechanism of action and favorable safety profile make it a valuable candidate for further research. Future studies focusing on isolation of pure compounds, clinical validation, and formulation development are warranted to establish its therapeutic application in diabetes management. Mimosa tenuiflora shows promising antidiabetic activity and can be a potential source for herbal drug development.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Free Radical Scavenging Efficiency of *Celastrus paniculatus*: Comparative Analysis of Leaf-Derived Extracts and Seed Oil

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Abstract-Free radicals induce oxidative stress in the human body, which is directly linked to many chronic diseases such as cancer, heart disease, and neurological problems. Nowadays, plants are a great source of natural antioxidants because they include many bioactive phytochemicals like flavonoids, alkaloids and phenolic compounds that help neutralize free radicals. In recent years, plant-derived antioxidants have attracted significant attention due to their potential therapeutic applications and low risk of adverse effects. Among these, one is *Celastrus paniculatus*, sometimes referred to as the intellect tree or Malkangani. It is adaptable and can be used to treat a range of illnesses due to its diverse phytoconstituents. The present study aimed to evaluate and compare the antioxidant potential of leaf extracts obtained through successive solvent extraction and the seed oil of *Celastrus paniculatus*. Leaf samples were subjected to successive solvent extraction using solvents of increasing polarity, while seed oil was obtained through standard extraction methods. The antioxidant activity of the extracts and seed oil was assessed using the in vitro DPPH free radical scavenging assay, with ascorbic acid used as the standard reference, which is also used as the standard antioxidant, which has a IC_{50} value ranging approximately 10-35ppm.

The results revealed that the seed oil exhibited strong radical scavenging activity with an IC_{50} value of approximately 34 ppm, indicating the highest antioxidant potential among the tested samples. Among the leaf extracts, the methanolic extract showed good antioxidant activity with an IC_{50} value of approximately 42 ppm, followed by the other extracts, which demonstrate moderate antioxidant potential.

These findings suggest that the seed oil of *Celastrus paniculatus* possesses greater antioxidant properties and is comparable to the standard antioxidant, and represents a promising natural source of antioxidants for potential therapeutic applications.

Keywords: Oxidative stress, Antioxidants, *Celastrus paniculatus*, IC50.

Introduction

Free radicals, or reactive oxygen species (ROS), formed within the body, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, are highly reactive

and potentially damaging chemical species[1]. A natural mechanism used by all aerobic organism, including humans to deal with the process of oxidation used a series of antioxidant defence system which is naturally present in our body, such as a preventive antioxidant system that reduces the rate of free radical formation and chain breaking anti-oxidant that neutralize and stabilize the free radical these can be enzymatic or non-enzymatic[2] but if somehow they are not able to manage or control the free radical generation and they exceeds the normal range than that might lead to potential risk in the body such as cellular damage, DNA disorder, cancer, aging etc. Antioxidants are compounds that inhibit or delay the oxidation of molecules, particularly lipids, thereby preventing the formation and propagation of free radicals. Anti-oxidants can be broadly classified based on their origin and method of preparation into natural and synthetic types[3]. Synthetic antioxidants have been widely used in the food and pharmaceutical industries for many years due to their effectiveness and stability. However, growing concerns regarding their potential toxicity and adverse health effects have led to increased scrutiny and a gradual shift in preference toward natural alternatives. Natural antioxidants not only perform the essential function of preventing oxidation but also exhibit additional health-promoting properties, including anti-inflammatory, anti-microbial and cardioprotective effects. Extensive research has demonstrated that plant materials are rich sources of natural anti-oxidant phytochemicals, especially flavonoids and other polyphenolic compounds. These bioactive constituents play a crucial role in neutralizing free radicals and interrupting chain reactions, thereby reducing oxidative stress and lowering the risk of disease development.

Among such plants, Cleastrus *paniculatus* is one of the most common, which is also known as Jyotishmati or Malkangani or Black oil plant. It is a traditional climbing shrub belonging to the family Celastraceae, widely used for several medicinal activities in ayurvedic an Unani systems[4,5]. In Ayurveda, Siddha and Unani, parts of the *Celastrus paniculatus* are used to prevent various diseases like brain-related disorders, arthritis, paralysis, asthma, cancer[6], stimulant nerve tonic, rejuvenant, sedative, tranquillizer, and diuretic. It is also used in the treatment of rheumatism, gout, leprosy, leucoderma, paralysis and asthma[7].

In India, *Celastrus Paniculatus* is an evergreen endangered medicinally important plant also known as the elixir of life due to its cognitive boosting properties, which makes it interesting and also important as it will be a natural source of antioxidant where, causing no harmful effects to us. Due to this, the plant is selected for the analysis of its antioxidant potential and from those part which is most widely used.

Material and Methods

Preparation of the sample- Leaves of *Celastrus paniculatus* were collected from the local area of Tehri Garhwal, and seeds were collected from the local market of Dehradun, Uttarakhand. The seeds and leaves were dried in the sun and shade, and crushed respectively. Both of the materials were subjected to

Soxhlet extraction using different solvents, and excess solvent was removed and extracts were prepared.

Extraction of Seeds of C. *paniculatus*- The seeds were dried in the shade and used for analytical work. About ~50 gm of shade dried seeds were crushed with mortal pestle. The material was then filled in the thimble for the Soxhlet apparatus and exhaustively extracted with petroleum ether for about 9 hours. The solvent was distilled off at low temperature and concentrated on a water bath to get a thick syrup after extraction with Petroleum ether

Chemicals and instruments used - For the extraction process and evaluation, different solvents were used, with the following names: DPPH for evaluation of antioxidant potential; Methanol, Chloroform, Pet ether, Aluminium Chloride, NaOH, Sodium Nitrite for TFC and chemicals for Phytochemical screening. For the evaluation, a UV-Visible Spectrometer, Soxhlet and a distillation apparatus were used.

Qualitative Analysis of the Phytochemicals

i. **Test for Tannins**- This was done by boiling extract samples (separately) in water in a test tube and then filtering. A brownish-green or blue-black colouration was observed after adding a few drops of 0.1% ferric chloride.

ii. **Test for Saponins-** To detect the presence of saponins, the Foam Test was performed. The *Celastrus Paniculatus* extract was diluted with distilled water and heated to a boil. Once cooled, the solution was shaken vigorously. The formation of a persistent foam was taken as a positive indicator for the presence of saponins.

iii. Test for Flavonoids- a. This was determined through heating of the extract of the Cleastrus leaves sample (separately) with ethyl acetate over a steam water bath for a few min.

b. To dilute the ammonia solution, filtrate from the filtered mixture was added and shaken. The appearance of yellow colouration indicates the presence of flavonoids.

c. Ferric chloride test- To the test solution, add a few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoids.

iv. Test for Steroids- This was carried out by the addition of a little amount of acetic anhydride to each of the crude extracts with further addition of H_2S0_4. The presence of steroids was indicated by a change of colour from violet to blue or green.

v. Test for Terpenoids- This was carried out by Salkowski's test. To the extract was added chloroform, followed by the careful further addition of concentrated H_2SO_4. Appearance of red colour at the interface is an indication of a positive result for the presence of terpenoids.

vi. Test for Alkaloids- The *Celastrus* leaves extract was taken, to it was added 1% HCl, mixed, warmed and later filtered. Mayer's and Dragendorff's reagents were added to the filtrate, and the absence or presence was determined based on appearance of the turbidity or precipitate development.

Preparation of DPPH Solution - The free radical scavenging activity was determined using the in-vitro DPPH

assay. 0.7mg of DPPH was dissolved in 100 mL of Methanol for the free radical, this will be the working solution. The solution was mixed thoroughly until complete dissolution and stored in the dark at low temperature for 2 hours for further use.

Preparation of Extract Solutions- Stock solution of different extracts (Leaf-Methanol, chloroform, pet ether and Seed) of 1 mg/mL was prepared by dissolving the required quantity of each extract in the required volume of methanol. From the sample stock solutions 50, 100, 150, 200 and 250 μg / mL sol. of each extract were prepared.

Evaluation of Antioxidant Potential- Take the sample solutions of different concentrations.2 mL DPPH solution and 2 mL of the extract solution were mixed and incubated at room temperature in the dark for 2 hours. A Blank solution was also prepared, only using 2 mL of DPPH solution. Finally, the absorbance of the solutions was measured by a UV-visible spectrophotometer at 517 nm. Ascorbic acid was used as the standard. IC_{50} values, which is 50% inhibition of the free radicals of the extracts, were calculated from the graph as concentration v/s % inhibition. Radical scavenging activity was expressed as a percentage of inhibition. IC_{50} value is the concentration of the sample required to scavenge 50% of DPPH free radical. IC_{50} of the extracts indicates the corresponding concentration at which the radical scavenging potential is 50%. The IC_{50} of the extract and standards were determined graphically.

The percentage of inhibition was calculated by using the formula:

$$I\% = \frac{Acontrol - Asample}{Acontrol} \, x \, 100$$

Where,

$A_{Control}$ = absorbance of the control

A_{sample} = absorbance of the sample sol.

I% = percentage of inhibition.

The radical scavenging activities of the extracts are expressed in terms of their IC_{50} values.

Results

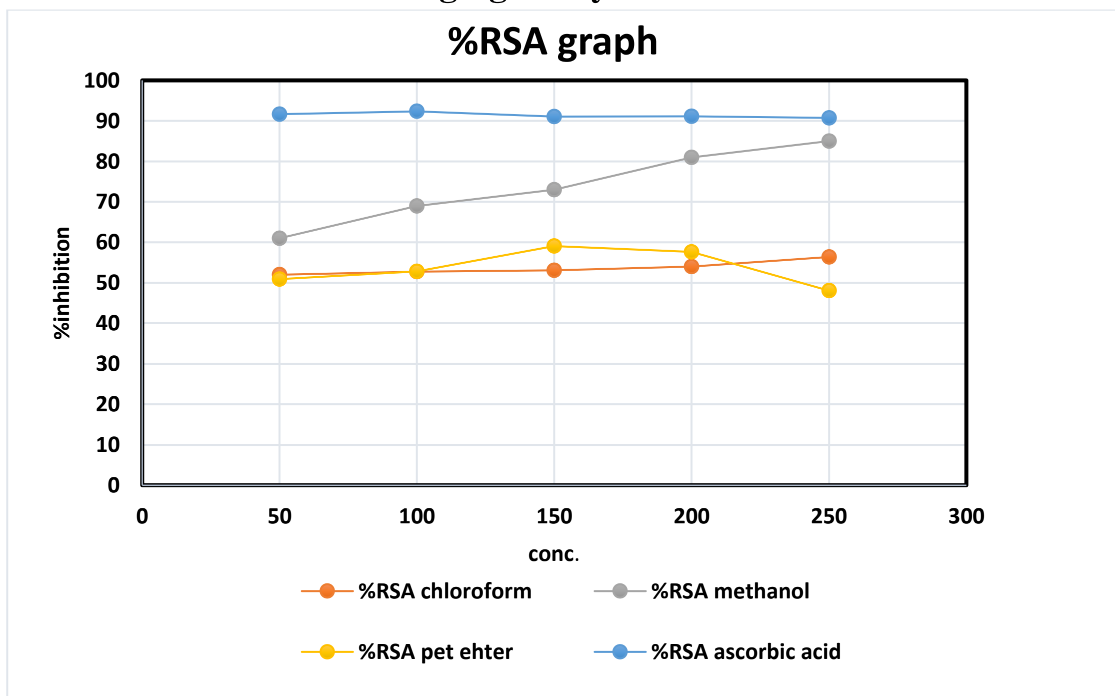
Table-1 Qualitative Analysis of Leaves of *Celastrus p.*

Phytoconstituents	Methanol	Chloroform	Pet ether
Tannins	+	-	-
Saponins	+	-	-
Steroids	+	+	+
Flavonoids	+	-	-
Terpenoids	+	+	+
Alkaloids	+	Trace	-

Table-2 Qualitative Analysis of the Seed of *Celastrus p.*

Phytoconstituents	Absence(-)/Presence(+)
Tannins	-
Saponins	-
Steroids	+
Flavonoids	-
Terpenoids	+
Alkaloids	-

DPPH Free Radical Scavenging Assay



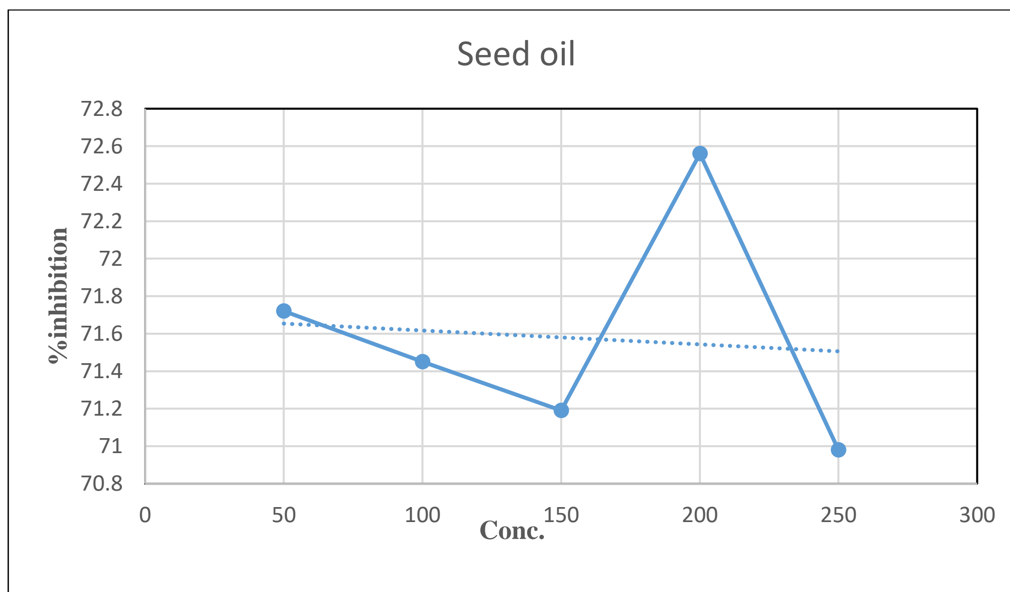
Graph- 1 showing % Inhibition of the different solvent extracts of *Celastrus paniculatus*

Radical Scavenging Activity (%RSA) of Leaf Extracts and Seed Oil- The antioxidant potential of *Celastrus paniculatus* leaf extracts was evaluated using different solvent systems: methanol, chloroform, and petroleum ether. Ascorbic acid served as the standard, maintaining a consistently high inhibition rate of approximately 90-92% across all tested concentrations (50 to 250 ppm).(Graph- 1)

Among the leaf extracts, the methanol extract exhibited the highest antioxidant activity, showing a clear concentration-dependent increase from 60.8% at 50ppm to 84.9% at 250ppm. In contrast, the chloroform and Pet Ether extract showed significantly lower activity, around 52-56% and 47-58.9%, respectively. In comparison to the methanol extract and standard, it showed lower inhibition.

Graph-2 showed the inhibition percent-tage of seed oil demonstrated a high baseline of antioxidant activity, with % inhibition ranging between 71% and 72.6%. Unlike the methanol leaf extract, the seed oil did not show a strong positive correlation with concentration. The highest inhibition (72.57%) was recorded at 200ppm, followed by a slight decrease at higher concentrations.

While there is a higher percentage of inhibition in the methanol leaf extract, overall, looking at the IC_{50} value, which is the value where the substance will show 50% of inhibition in free radical scavenging. Methanol showed a value of 42ppm, Chloroform, and Pet ether have an IC50 value, while the seed oil showed a value of 34ppm, and the standard has an IC50 value ranging from 10-35ppm.



Graph-2 Showing %Inhibition of Seed Oil of *Celastrus Paniculatu*

Discussion

This data demonstrates that in % inhibition, Methanol exhibits the highest value reaching up to 84%, from which we can say that the primary antioxidant metabolites of the leaves are polar in nature. The methanol extract contains alkaloids, flavonoids, and phenolic compounds, which are known to have antioxidant properties by donating a hydrogen atom to stabilise free radicals, whereas Chloroform and Petroleum ether have very limited antioxidant capacity due to their nonpolar constituents. The seed oil showed a good antioxidant property. At its lowest concentration, it also showed higher inhibition of free radical also it has a higher value of IC50 value also which shows the efficacy of the seed oil has higher antioxidant. The seed oil serves asa more consistent and potent source of radical scavengers.

Conclusion

The present comparative study between leaves and seeds of *Celastrus pani-culatus* highlights clear differences in both phytochemical content and anti-oxidant potential. The methanolic leaf extract exhibited higher total flavonoid content (84.09 mg QE/g) compared to the seed sample (59.01 mg QE/g), indicating that leaves are a richer source of flavonoid compounds when extracted with methanol.

In terms of antioxidant activity, a distinct variation was observed between the two plant parts. The seed oil showed the highest antioxidant potential among all tested samples, demonstrating strong free radical scavenging activity with a low IC_{50} value (~35 ppm). This suggests that seeds contain potent lipid-soluble antioxidant compounds. On the other hand, the methanolic leaf extract also showed significant antioxidant activity with a relatively low IC_{50} value (~41 ppm), indicating a strong contribution from polar phytochemicals such as flavonoids and phenolics. Therefore, overall antioxidant potential was higher in seeds (oil) than in leaves.”

Overall, the study concludes that both leaves and seeds of *Celastrus paniculatus* are valuable sources of anti-oxidant compounds but differ in their

phytochemical profiles and efficiency. Seeds are more potent in terms of overall antioxidant activity, while leaves are richer in flavonoid content. This comparative evaluation suggests that different plant parts may be utilized for specific therapeutic or nutraceutical applications depending on the desired bioactive compounds.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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GC–MS Characterization and Neurostimulatory Potential of Seasonal Rhizome Essential Oils of *Hedychium spicatum*: A Comparative Study

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Abstract- Essential oils are extremely important plant products that are made up of a complex mixture of volatile substances that are naturally present in the plant. These oils have reportedly been shown to have a variety of biological effects, giving rise to the new product category known as aromaceuticals. The prevalence of mental health conditions including depression, anxiety, sleeplessness, and stress has grown along with increasing standards of life. As a result of their widespread usage in the treatment of certain ailments, essential oils have seen an increase in popularity. Therefore, the present study evaluated the central nervous system (CNS) stimulant activity of essential oils extracted from the rhizomes of *Hedychium spicatum* collected during winter and summer seasons. Essential oils were extracted by hydro-distillation and evaluated for physicochemical parameters including specific gravity, viscosity, refractive index, acid value and saponification value. Chemical profiling was carried out using Gas Chromatography–Mass Spectrometry (GC–MS). CNS activity was assessed in rodent models and compared with the standard stimulant drug caffeine (100 mg/kg body weight). GC–MS analysis revealed the presence of several bioactive constituents. Evaluation of locomotor activity using an actophotometer demonstrated a marked increase in CNS activity. Essential oils obtained from winter season (HS-1) and summer rhizomes (HS-2) exhibited locomotor activity values of 92.14% and 93.33%, respectively, compared with 97% observed for caffeine. Acute toxicity studies indicated that both oils were safe up to the tested dose levels. The findings suggest that essential oils of wild *Hedychium spicatum* possess significant CNS stimulant activity and may serve as promising natural bioactive products for further pharmacological investigations.

Keywords: *Hedychium spicatum*, Essential oil, Physico-chemical properties, GC–MS, CNS stimulant activity, Aromaceuticals

Introduction

Essential oils are complex mixtures of volatile secondary metabolites produced by aromatic plants and are recognized for their characteristic fragrance and

diverse biological activities. These oils are rich sources of physiologically active compounds and have attracted considerable attention owing to their therapeutic applications. Previous studies have reported antimicrobial, antiviral, antioxidant, anti-inflammatory, antinociceptive and antitumor activities associated with various essential oils.

The growing prevalence of stress related disorders and increasing demand for natural health products have resulted in renewed interest in essential oils and aromaceuticals. Essential oils are increasingly incorporated into complementary healthcare systems due to their ability to influence physiological and psychological responses. The study of physicochemical characteristics provides valuable information regarding the quality, purity and commercial applicability of essential oils. Parameters such as colour, specific gravity, viscosity, refractive index, acid value and saponification value are widely employed as quality indicators and play an important role in determining the suitability of oils for pharmaceutical and industrial applications. The family Zingiberaceae is well known for its medicinally important species. *Hedychium spicatum* Sm. is a perennial rhizomatous herb belonging to the family Zingiberaceae (ginger family). It is commonly known as Kapurkachri (Indian trade name), Spiked Ginger lily and Van-haldi, (Chopra,). It is a leafy plant up to 1–2 m tall having strong aromatic horizontal rhizomes. Flowers are fragrant, off white with orange-red base, appearing in dense terminal spikes 15-25 cm at the top of stem and hence the name spiked ginger lily. Flowering season is July to August. Leaves are about 30 cm or more in length, oblong, lanceolate, very variable in breadth and glabrous[2-3]. Rhizomes are 15-20 cm long; 2.0-2.5 cm in diameter, externally yellowish-brown, but change to dark brown on storage [4]. (Honda) *H. spicatum* is found in the entire Himalayan region. It occurs throughout subtropical Himalayas in the Indian state of Assam, Arunachal Pradesh and Uttarakhand within an altitudinal range of 1800-3000 m a.s.l[5]. The plant forms clumps amongst moist rocks outside forest or on the borders of cultivated fields. The plant also found in Western and Central Himalayas at an altitude of 1000-2300 m a.s.l.[6-7].

Traditionally, the rhizomes are used as stomachic, carmi-native, tonic and stimulant[8]. The rhizomes are reported to be boiled and eaten with salt, roasted powder is used in asthma, and decoction of rhizome with sawdust is used in tuberculosis[9]. Tuberous roots are reported to be edible [10]. Fruit of the species is cooked and eaten with lentils in savory dishes[11]. The rhizomes are white and starchy within and are employed in the preparation of Abir, a fragrant colored powder used during holi festival and in religious ceremonies. They are considered to have insect repelling properties and are used for preserving clothes. They may be employed as an auxiliary in dyeing to impart a pleasant smell to fabrics. They are used with henna to produce perfumed cloth known locally as Malagiri cloth[12]. Rhizomes have bitter camphor-like taste and a strong aromatic odor, with a wide application in incense and fragrant preparations. They enter into the

preparation of cosmetic powders used for promoting hair growth. They are used in Bengal, after frying or mixing with other ingredients as chars or perfumed baits for fish. They are much used in veterinary medicine. The powdered rhizome in divided doses is used in conditions like bronchial asthma, cough, chest heaviness, sleeplessness, loss of appetite and pulmonary eosinophilia. The rhizomes are also used in dyspepsia, diarrhea, piles, liver complaints, ulcers, skin diseases and rheumatoid arthritis. The essential oil of the rhizome is used to check seed-borne diseases of crops and has mild tranquilizing activity. The rhizomes showed significant analgesic, anti-inflammatory, cytotoxic, antimalarial and pediculicidal activity. It also acts as antidote for snakebite. It is used in conditions like poor circulation due to thickening of blood vessels. It forms one of the ingredients of herbal vanishing cream[13].

Material and Methods

Plant Material- *Hedychium spicatum* rhizomes were collected in winter and summer seasons through the year from Bataghat (on the way from Mussoorie to Dhanaulti), Uttarakhand, India, at an altitude, latitude and longitude ranging from 1900 m, 30.44984º N, 78.15848º E - 2000 m 30.45014º N, 78.15867º E. The identification and authentication of targeted specie was done by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. Herbarium registration of *Hedychium spicatum* Sm. is 172345. Rhizomes were cut into small pieces and oven dried at 60–80°C for 48 hours. The dried materials were stored at room temperature until use.

Extraction of Essential Oil- Dried rhizomes collected during winter (300 g) and summer (600 g) seasons were subjected to hydro-distillation using a Clevenger-type apparatus for 6–8 hr. The volatile oil fractions obtained were collected, dried over anhydrous sodium sulfate and stored at 4–6°C until further analysis.

Physicochemical Evaluation of Essential Oils - Physicochemical characteristics provide a baseline assess-ment of essential oil quality and suita-bility. These characteristics of both oils HS-1 & HS-2 studied were colour, Specific gravity, Specific Viscosity, Refractive index, Acid number and Saponification number, ester value and glycerol percentage.

Acute Toxicity Study - This study examines the acute toxicity of five groups (n = 5) of male albino mice. Animals from all groups were fasted overnight before receiving a single dosage of samples (HS-1, HS-2) at doses of 50, 100, 150, 250, 500, 1000, 1500, and 2000 Mg/Kg body weight (P.O.) from the various groups. The behaviour of the animals was monitored for 72 hours following extract administration in a group of mice that received an identical volume of P.B.S. Animals were monitored for 14 days for any indication of toxicity and death. (**Table-4)**

GC–MS Analysis of the Extracted Essential Oils- Gas chromatography mass spectrometry (GC-MS) was used to analyse the extracted essential oils. The operational parameters listed below were standardised and applied: DB-5 ms capillary GC column split injection

mode (50:1), ultra-inert 5% Phenyl 95% dimethylarylene (30 m x 0.25 mm x 0.25 m), and helium as the carrier gas. When the analysis is complete, the injector temperature is increased to 240°C at a rate of 3°C per minute from the initial oven temperature of 50°C. Using a mass selective detector and electron impact ionisation (EID) at an energy of 70 eV, the eluted analytes were found.

Identification of Compounds: Chemical components were identified by comparing their mass spectra to those in the NIST and F&F libraries, those reported by Adams, and data from the MS literature, as well as by comparing their Kovats/Retention index with standard C7-C40 alkanes.

Neurostimulatory Potential (loco-motor activity/CNS)- The actophoto-meter, which uses photoelectric cells and a counter to work, may be used to quickly assess the Neurostimulatory activity (locomotor activity/CNS)[13] A count is made whenever a light beam strikes the photocell while it is on an animal. In an actophotometer, the animal can move in either a square or circular region. Rats and mice may both be utilised for testing this apparatus. Wistar rats of both sexes, weighing between 150 and 250 grams with a variation of ± 2.0 grams, were used. The rats were individually housed in elastic cages with wire tops prior to the start of the experiment. Before the study, all animals were fasted for at least 12 hours, with access only to water.

The rats were divided into groups of five animals each. Each rat was weighed individually and marked to distinguish one from another. The equipment used for the study was checked to ensure proper functioning, and the photocell counter was set up to record the activity of each group for a duration of 10 minutes. At the end of the counting period, each group of rats was removed from the chamber. The drugs used in the study were administered orally according to the following schedule. After administration, the rats were retested for activity scores after 60 minutes, again for a duration of 10 minutes. The drug dose used was 100 mg/kg of body weight for the rats, and the reference drug used was caffeine at a dose of 100 mg/kg of body weight. Animals were divided into 5 groups and received different drugs as Control group receiving Tween 80, Standard groups receiving Caffiene and Test groups receiving HS-1&HS-2. **Table-4**

CNS motor activity was calculated as per the following formula: -

$$\% \text{ CNS activity} = \frac{\text{Initial no. of counts} - \text{Final no. of counts} * 100}{\text{Initial no. of counts}}$$

Statistical Analysis- All data were expressed as mean SEM ± wherever applicable, the data were analysed statistically by student's t- test, using graph pad instant version 2.05a and one way ANOVA. The level of significance was $p < 0.05$ and n represents five per groups.

Results

Hydro-distillation yielded pale yellow coloured essential oils from both leaves and rhizomes. Physicochemical parameters are summarized in Table 1 Chemical composition is presented in Table-2, acute toxicity studies in Table-3, and neuro-stimulatory activity in Table-4.

Table-1 Physicochemical Characteristics of Essential Oils

Season	Yield (%)	Colour	Specfic Gravity	Specfic Viscosity	Refractive index	Saponification Value (mg/g)	Acid value (mg/g)	Ester value (mg/g)	Glycerol (%)
HS-1Winter	$1.16^{a} \pm 0.012$	Pale Yellow	.986	0.195	1.543	$54.00^{b} \pm 2.88$	$0.21^{c} \pm 0.03$	$53.79^{b} \pm 2.97$	$2.90^{b} \pm 0.17$
HS-2Summer	$1.64^{c} \pm 0.018$	Light pale yellow	.987	0.198	1.534	$22.00^{a} \pm 3.05$	$0.04^{a} \pm 0.01$	$21.95^{a} \pm 3.05$	$1.20^{a} \pm 0.29$
F-value	88.59					12.667	9.60	12.481	12.489
Sig. level (P-value)	0.000 (S)					0.002 (S)	0.005 (S)	0.002 (S)	0.002 (S)

Table-2 Chemical Composition of Essential Oils

Peak	RT	Chemical constituents		CKI	AKI	
		HS-1	HS-2			
1	6.187	1.97	0.84	α-Pinene	936	939
3	7.277		0.18	Sabinene	966	975
4	7.365	3.14	1.12	β-Pinene	969	975
5	7.812	0.11		β-Myrcene	986	990
7	9.022	14.83	21.81	1,8-Cineole	1026	1038
8	9.735	0.35	0.11	ϒ-Terpinene	1054	1059
9	10.102	0.11	0.16	cis-Sabinene hydrate	1067	1070
10	11.001	3.02	5.22	Linalool	1095	1096
11	11.359	0.07	0.11	Unidentified	1110	
12	12.97	0.38	0.44	Unidentified	1166	
13	13.135	0.81	0.83	Terpinene-4-ol	1174	1167
14	13.587	1.42	1.93	α-Terpineol	1186	1178
15	17.351	0.09	0.08	Unidentified	1325	
16	17.756	0.23	0.16	α-Cubebene	1340	1351
17	18.477	0.33	0.21	α-Copaene	1368	1376
18	18.813	0.23	0.17	β-Cubebene	1335	1326
19	18.864	0.17	0.19	β-Elemene	1383	1390
21	19.567	0.92	0.84	E- Carophyllene	1410	1417
22	19.841	0.11		β-copaene	1421	1432
23	20.158		0.12	Guaia-6,9-diene	1434	1444
24	20.251	0.12	0.14	Cadina-3,5-diene	1438	1446
25	20.325	0.24	0.29	cis-Muurolene,3-5-diene	1441	1450
26	20.465	0.81	0.73	α-Humulene	1446	1454
27	20.558	0.92	0.76	Alloaromadendrene	1450	1458
28	20.637	0.26	0.21	cis-Muurola-4(14),5-diene	1453	1466
29	20.912	0.13	0.08	trans-cadina-1(6),4-diene	1464	1476
30	20.991	0.63	0.42	ϒ-Muurolene	1467	1479
31	21.112	0.35	0.24	Germacrene-D	1472	1481
32	21.299	0.13	0.1	Unidentified	1490	
33	21.364	0.41	0.27	Unidentified	1482	
34	21.499	1.24		4-Epicubebol	1488	1493
35	21.582	0.91	0.65	α-Muurolene	1492	1500

36	21.731	0.31	0.6	BHT	1497	1514
37	21.927	2.41	1.78	ϒ-cadinene	1505	1513
38	21.983	1.35	1.02	Cubebol	1507	1515
39	22.085	4.63	3.8	δ-Cadinene	1512	1523
40	22.327		0.17	δ-Amorphene	1522	1512
41	22.378	0.47	0.18	cis-cadina-1,4-diene	1524	1533
42	22.476	0.35	0.3	α-Cadinene	1528	1538
43	22.514	0.23	0.23	α-Calacorene	1530	1545
44	22.849	7.05	10.07	α-Elemol	1544	1549
45	23.081	0.14	0.12	β-Calacorene	1554	1565
46	23.17	0.99	1.01	E-Nerolidol	1558	1563
47	23.44	4.7	4.65	Germacrene-D-4-ol	1547	1575
48	23.514	0.38	0.55	Caryophyllene oxide	1572	1583
49	23.71	0.7	0.68	Unidentified	1581	
50	23.905	0.24	0.23	Unidentified	1589	
51	24.026	0.22	0.22	Ledol	1594	1602
52	24.143	1.28	0.69	Unidentified	1599	1602
53	24.306	0.37	0.49	Unidentified	1606	
54	24.455	5.94	0.88	10-epi-ϒ-Eudesmol	1613	1622
55	24.594	0.48		Hinesol	1619	1640
56	24.706	3.91	4.17	ϒ-Eudesmol	1624	1618
58	25.037	8.06	8.27	τ-Muurolol	1638	
60	25.33	18.79	17.49	α-Cadinol	1651	1652
61	25.6	0.31		Unidentified	1664	
62	26.154		0.19	Unidentified	1689	
63	26.214	0.18		Unidentified	1691	
64	37.625	2.17	3.45	Unidentified	2284	
65	38.197		0.45	Unidentified	2418	

Table-3 Acute Toxicity Studies

Treatment	Dose (mg/kg PO)	No. of Animal	No. of Animal Dead	No. of Animal Survive	% dead animals
HS-1	50	5	0	5	0
	100	5	0	5	0
	150	5	0	5	0
	250	5	0	5	0
	500	5	0	5	0
	1000	5	0	5	0
	1500	5	0	5	0
	2000	5	0	5	0
	50	5	0	5	0
HS-2	100	5	0	5	0
	150	5	0	5	0
	250	5	0	5	0
	500	5	0	5	0
	1000	5	0	5	0
	1500	5	0	5	0
	2000	5	0	5	0

Table-4 Neuro-stimulatory Activity of Essential Oils

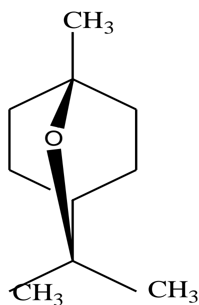
Treatment	Before	After	% Increase in Activity
Control	42.00 ± 0.21	42.33 ± 0.25	0.70
HS-1	42.00 ± 0.51	80.70 ± 1.16	92.14 ± 0.99
HS-2	42.66 ± 0.94	82.50 ± 1.66	93.33 ± 0.87
Caffeine (100 mg/kg)	42.33 ± 0.47	83.33 ± 0.78	97.00 ± 0.89

Discussion

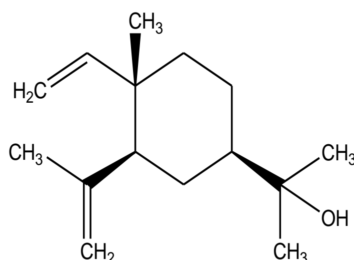
Physicochemical properties such as density, refractive index, viscosity, acid value, saponification value and chemical composition are important indicators of the quality and purity of essential oils. These parameters significantly influence stability, storage behaviour and commercial applicability.

Density and refractive index are widely accepted markers of purity, while viscosity and acid value provide useful information regarding quality and shelf stability. Understanding these properties helps distinguish authentic essential oils from adulterated products. The essential oils compositions of *H. spicatum* in winter & summer seasons HS-1 and HS-2 were analyzed by GC-MS and total 65 peaks were identified as given in table 3.

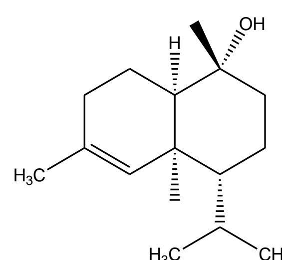
In HS-1 55 compounds were present, out of which 43 compounds were identified which constitute 93.67% area and rest 6.33% remains unidentified. Out of the total identified compounds α-cadinol (18.79%), 1, 8-cineole (14.83%), τ-muurolol (8.06%) and α-elemol (7.05%) were major. In HS-2season, 54 compounds were present, out of which 42 compounds were identified which constitute 92.82% area and rest 7.18% remain unidentified. The major chemical constituents were 1, 8-cineole (21.81%), α-cadinol (17.49%), α-elemol (10.07%) τ-muurolol (8.27%). Figure:1.Acute toxicity studies demonstrated a favourable safety profile, as neither oil produced mortality or observable toxic effects even at doses up to 2000 mg/kg body weight.



1, 8-Cineole



α-Elemol



τ-Muurolol

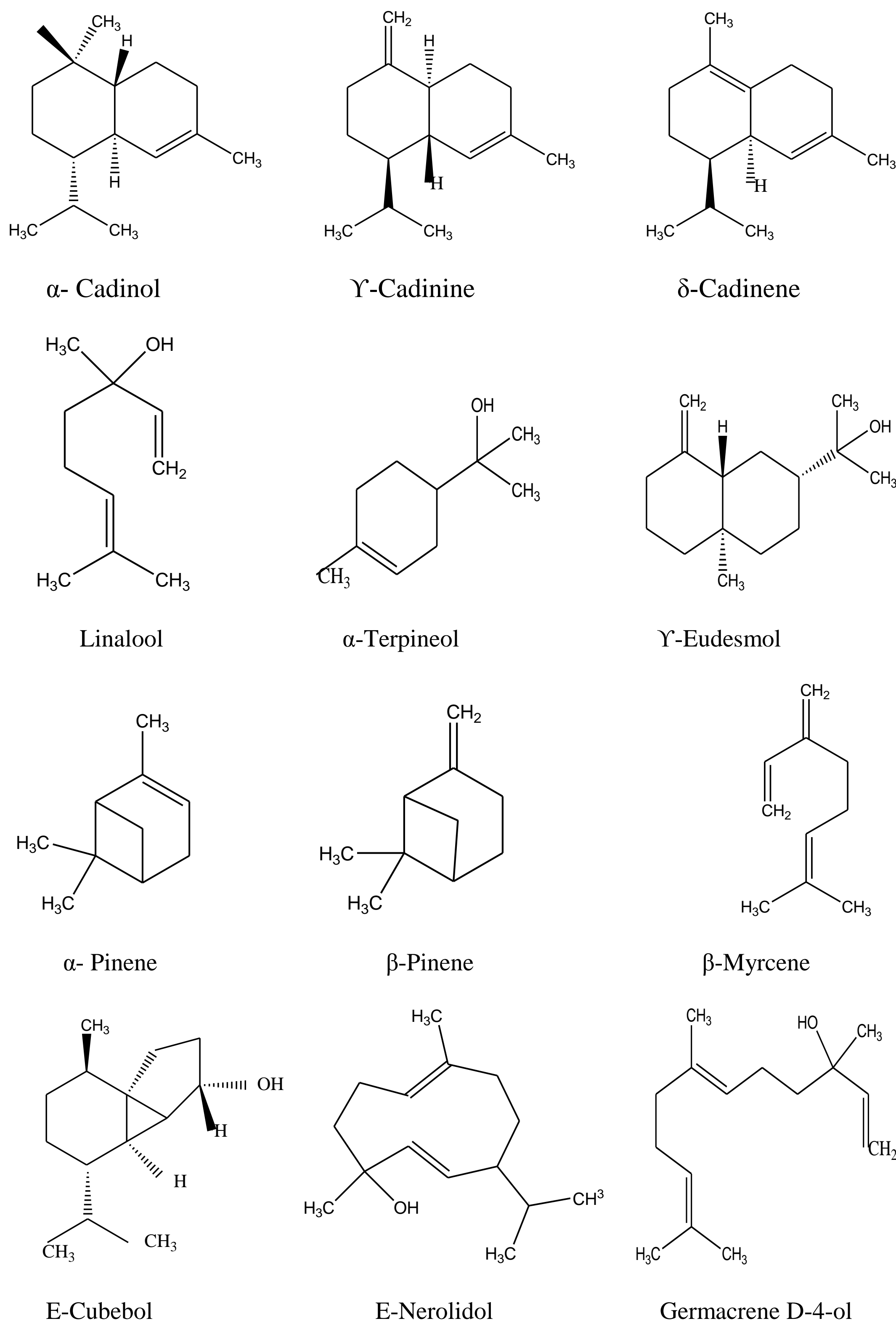


Fig.-1 Structures of major constituents identified in the rhizome essential oils of *Hedychium spicatum*.

Essential oils influence the nervous system primarily through olfactory pathways. Upon inhalation, volatile molecules stimulate olfactory receptors, transmitting signals to the olfactory bulb, limbic system and hypothalamus. This interaction can modulate neurotransmitter release and alter physiological and behavioural responses. The present study evaluated CNS activity

using locomotor behaviour as an indicator. Increased locomotor activity is generally associated with enhanced alertness and CNS stimulation, whereas decreased activity reflects CNS depression. The results clearly indicate significant CNS stimulant activity for both essential oils. HS-1 and HS-2 produced locomotor activity increases of 92.14% and 93.33%, respectively, compared with 97% for caffeine. These findings suggest that the major phytochemical constituents present in the oils may contribute to their observed neuro-stimulatory effects.

Conclusion

The present study demonstrates that the rhizome essential oils of *Hedychium spicatum* exhibit significant seasonal variations in their physicochemical characteristics and chemical composition. GC–MS analysis revealed the presence of a diverse array of bioactive constituents, with 1,8-cineole, α-cadinol, α-elemol, and τ-muurolol identified as the major compounds in both winter and summer oils. Seasonal fluctuations influenced the relative abundance of these constituents, indicating that environmental conditions play an important role in determining oil quality and composition.

The physicochemical parameters obtained in the study were within acceptable limits, confirming the purity and quality of the extracted oils. Acute toxicity evaluation further established their safety, as no mortality or adverse toxic effects were observed at doses up to 2000 mg/kg body weight. Assessment of central nervous system activity revealed that both seasonal oils significantly enhanced locomotor activity in experimental animals. The winter (HS-1) and summer (HS-2) oils produced increases in locomotor activity of 92.14% and 93.33%, respectively, which were comparable to the effect of the standard stimulant caffeine (97%). These findings suggest that the essential oils possess notable neurostimulatory properties, likely attributable to their rich content of oxygenated monoterpenes and sesquiterpenes, particularly 1,8-cineole and related constituents.

Overall, the results validate the traditional importance of *Hedychium spicatum* and highlight its potential as a valuable natural source of bioactive essential oils for aromaceutical, pharmaceutical, and wellness applications. Further investigations focusing on the isolation of active constituents, elucidation of their mechanisms of action, and clinical evaluation are warranted to fully exploit the therapeutic potential of this Himalayan medicinal plant.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Phytochemical investigation of leaves of *Hypericum oblongifolium*

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Abstract- Since primitive times, humans have been using plants for their essential requirements such as food and medicine. These plants have been used in traditional medicine in order to cure and prevent various human disorders. The important advantage for therapeutic uses of the plants includes their safety, effectiveness, economic feasibility, and ease of availability. In this paper *Hypericum oblongifolium* leaves extract was studied. For phyto-constituents five separate solvents (150 ml of each) namely methanol, ethanol, chloroform, ethyl acetate and deionized water were used to obtain extracts from plant material. The extracts were subjected to qualitative phytochemical screening using standard procedure. Phyto-chemical screening reveals the presences of flavonoids, anthraquinones, poly-phenols tannins, alkaloids, steroids saponins, glycosides and terpenoids.

Key Words: *Hypericum oblong-folium,* Phyto-constituent, Anthra-quinones, Therapeutics uses and Phytochemical Screening.

Introduction

Traditionally, medicinal plants are good sources of different active constituents. For the treatment of various diseased conditions, diverse species of medicinal plants are used due to the presence of the active chemicals. Prolonged use of the herbal plant as medicine determine its effectiveness and value for future. Various herbal products are used as medicine due to the significant effect on patient health, as it depends upon its popularity and availability[1-3]. From the time immemorial, the *Hypericum* species have been used in traditional therapy to treat various complaints. *Hypericum* , a large genus of herbs and shrubs, grows widely in temperate regions throughout the globe. These plants are the richest sources of flavones and xanthones[4]. *Hypericum ceranuum* (more accurately known as *Hypericum oblongifolium***,** belongingg to family *Hypericaceae* is an evergreen shrub native to the Himalayan region and the Khasia Hills. Leaves, flowers and stems have been reported for medicinal activity. It is traditionally found that they had been reported for treating respi-ratory disease, gastric ulcer, snake bite, wound, boils, *etc*. The plant has also been reported for various pharmaco-

logical aspects as anti-oxidant, anti-inflammatory, anti-pyretic activity. Chemo-taxonomically, it is prized for specialized metabolites like xanthones, flavonoids, and phloroglucinols, showing significant antioxidant and anti-inflammatory properties. The present paper deals with phytochemical screening of different extracts of leaves of *Hypericum oblongifolium.*

Material and methods

Collection of plant material- The plant material was collected from the forest of Adwani, Pauri Garhwal, Uttarakhand, India during September, 2025 and identified with the help of expert Taxonomist. The collected plant leaves were washed out in running tap water to remove the mud and microorganisms, shade dried for 15 days and chopped into small pieces and further dried for another 15 days. After then grounded into coarse powder using the household grinder and stored in air tight container till further use.

Chemicals and reagents- Iodine crystals (99%), Chloroform (99%), Sulphuric acid (98%), Hydrochloric acid (35%) (Himedia), Methanol (99%), Ethlye acetate (99%), Ethanol (99%) (MERK), Potassium iodide (99%), Ammonia solution (25%), Ferric chloride (98%), Lead acetate (99%) (Fisher scientific), Deionized distilled water(DI water) (milli Q), Benedict's quantitative reagent (Himedia), freshly prepared Wagner's reagent obtained by dissolving 6 gms of potassium iodide and 2 gms of iodine crystals. Similarly, the Ferric chloride solution was prepared by dissolving 2 gm of Ferric chloride in 50 ml DI water.

Preparation of root extracts- 5 gm of prepared powder was extracted via five separate solvents (150 ml of each) namely Methanol, Ethanol, Chloroform, Ethyl acetate and DI water. Each of the extract was accomplished through magnetic stirring at the temperature 60 - 75° C for 1 hour. Further the extracts were filtered with the help of Whatman filter paper no. 42 (Whatman International). All extracts were preserved in refrigerator till further use.

Phytochemical screening- Each of the prepared extract was treated as per the standard procedures to identify phytochemical components.

1) **Screeningof the tannins (Ferric chloride test)-** In a test tube holding 2 ml of the prepared extract was treated with 4-5 drops of freshly prepared ferric chloride solution. Brownish green layer confirmed the presence of tannins[5].
2) **Screening of the alkaloids (Wagner's test)-** A test tube filled with 2 ml of extract, 5-6 drops of Wagner's reagent were added slowly. The reddish-brown accelerate confirmed the acquaint of alkaloids[6].
3) **Screening of the steroids (Salkowski's Test)-** 2 ml of chloroform was mixed in 2 ml of extract; further similar volume of concentrated H_2SO_4 was added. The top layer revolve red and bottom layer revolve yellow with green glare, confirmed the presence of steroids[7].
4) **Screening of the saponins-** To identify the existence of saponins, 2ml of extract liquefyin 2 ml of Benedict's reagent. Blue –black

acquaint indicates the presence of saponins[8].

5) **Screening of the cardiac glycosides-** To identify the presence of cardiac glycosides, 2 ml of extract liquified with 2ml of chloroform in a test tube, after that added 1 ml of sulphuric acid. Appearance of deep reddish brown colour confirmed the presence of cardiac glycosides[9].

6) **Screening of the flavonoids (Lead acetate solution Test)-** 2 ml of extract liquefied with 2 ml of 10 percent lead acetate. Yellowish green colour confirmed the presence of flavonoids[10].

7) **Screening of the anthraquinones-** Firstly, 1 ml of extract simmers with10 percent of HCL for few moments by water bath process. Upon cooling to the room temperature, the same amount of chloroform and few drops of Ammonia solution (10%) were added in it. A rose pink colour confirmed the presence of anthraquinones[11].

8) **Phenol screening (ferric chloride test)-** To identify the existence of phenols, 1 ml of extract and 2 ml Millipore water combined in a test tube than few drops of 10 percent of ferric chloride in it. Appearance of blue or green colour indicates presence of phenols[12].

9) **Examination for Terpenoids (Salkowski's Test)-** 2ml of extract is liquefied with 2ml of chloroform after that few drops of sulphuric acid consciously added to it. Presence of reddish brown colour indicated the presence of terpenoids[13].

Table-1 Phytochemical examination of *Berberis asiatica* Roots

Phytochemicals	Methanol extract	Ethanol extract	Chloroform extract	Ethyl acetate extract	Aqueous extract
Tannins	+	+	+	-	+
Alkaloids	+	_	_	_	+
Steroids	_	_	_	_	_
Saponins	+	+	_	_	_
Cardiac glycosides	_	_	_	_	_
Flavonoids	_	_	_	+	_
Anthraquinones	_	_	_	_	_
Phenols	+	+	+	+	+
Terpenoids	_	_	_	_	_

+ component is present ; - component is absent

Results and Discussion

The screening was accomplished with five types of extraction solvents on *Berberis asiatica* root powder. The presence of vital components having significant therapeutic values has been confirmed. The outcome is summarized in **Table-1.** The screening process shows dissimilar results in different types of extraction solvents. The tannins are absent only in ethyl acetate extract. Alkaloids present in methanol and water extracts. Saponins presence was found in methanol and ethanol extracts. Flavonoids are present only in ethyl acetate extract. Phenols are present in all selected solvents. Steroids, cardiac

glycosides and anthraquinones were absent in all the extracts and terpenoids were present in both MeOH and EtOH extracts.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Comparative Phytochemical and Antioxidant Activity of *Ashtavarga* herbs with their *Bhavprakasha Nighantu* Substitute Herbs

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Abstract- *Ashtavarga* is a group of eight herbs which are considered very good *rasayana* (potent tonic) to rejuvenating, health promoting, strengthening the immune system, antioxidant and have immense cell regeneration capacity and are commonly used in promoting body health, healing fractures, seminal weakness, fever, abnormal thirst, diabetic conditions and also as a cure for Tri-doshas in human health. *Ashtavarga* herbs are commonly used in different dosage forms, e.g. as Avaleha (Chyavanprasha), Tonic, *Taila* (medicated oil), *Ghritam* (medicated clarified butter), *Churana* (powder) etc. In this research paper we have tried to find out the antioxidant activity and phyto-chemical screening of Ashtavargra herbs in comparison to substitute herbs recommended by the renowned Ayurvedic Text "*Bhavprakasha Nighantu*". The results of this research study shows that the substitute herbs recommended by *Bhavprakasha Nighantu* show greater antioxidant activity as compare to *Ashtavarga* herbs. *Ashtavarga* herbs shows maximum antioxidant activity of 59.82% at the concentration of 700mcg/ml whereas substitute herbs show 89.09% at only 180 mcg/ml which is very less as compared to the *Ashtavarga* herbs concentration. In case of phytochemical screening we find that the maximum total tannins and total bitters found in Varahikand (*Dioscorea bulbifera L.*) and Jeevak (*Microstylis musifera*) respectively. Similarly, minimum Total Tannins and Total Bitters found in Riddhi (*Habenaria intermedia)* and *Shatavari* ((*Asparagus Racemosus)* respectively.

Keywords: *Ashtavarga* , Antioxidant activity, Tannins, Bitters, DPPH.

Introduction

The *Ayurveda* is the ancient science of life. During starting development phase of *Ayurveda*, *Ashwani Kumars*, a reputed *Ayurvedic* wonder healers saw the old and sleazy body of *Chayavan Rishi*. They decided to formulate a medication to rejuvenate the body of their sage Chayavan Rishi. *Ashwani Kumars made* a unique preparation with *Ashtavarga herbs* (a group of eight medicinal plants) to rejuvenating the body of *Rishi Chayavan* and they successfully restored his body. Therefore, due to the name of *Rishi Chayavan* the preparation was called as *Chayavanprash*. These medicinal plants started fading away over the several centuries. All *Ashtavarga* herbs have their natural habitats in the North- West Himalaya. *Ashtavarga* herbs are considered as a very good *rasayana* to rejuvenating, health promoting, streng-

then the immune system, antioxidant and have immense cell regeneration capacity and are commonly used in promoting body health, healing fractures, seminal weakness, fever, abnormal thirst, diabetic conditions and also as a cure for *vata*, *pitta*, *rakta doshas*. *Ashtavarga* herbs are commonly used in different dosage forms, e.g. *Chyavanprasha*, Tonic, *Taila* (oil*)*, *Ghritam* (medicated clarified butter), *Churana* (powder). The *Dhanvantri Nighantu* (ancient text) has the description of highest number of names and pharmacological properties for *Ashtavarga* herbs. *Bhava Prakash Nighantu*, *Shaligram Nighantu* and *Rajnighantu* also have *Ashtavarga* herbs names and their therapeutic properties[1-11]. Due to difficulty to arrange *Ashtavarga* herbs, B*hav Prakash Nighantu* had described their substitute herbs given in **Table-1**. Therapeutic application of *Ashtavarga* herbs and their substitutes as per *Bhavprakash nighantu* is given in **Table-2**.

Table-1 *Ashtavarga* **herbs and their** *Bhavprakasha Nighantu* **recommended substitute herbs**

SN	*Ashtavarga* **Plants**	**Substitute Plants**
01.	*Meda (Polygonatum airrhifolium Royle & other species)*	Shatavari ((*AsparagusRacemosus)*
02.	*Maha Meda (Polygonatum airrhifolium Royle & other species)*	
03.	Jeevak (Microstylis musifera Ridley; Lipasis rostrata Rehd)*)*	Vidarikand (*Pueraria tuberosa*)
04.	*Rishbhak* (Microstylis wallichii, Linn; Lipasis rostrata Rehd)	
05.	Kakoli *(Roscoea procera Wall; Lillium polyphyllum D. Don))*	Aswagandha (*Withania somnifera*)
06.	Kshirakakoli *(Roscoea procera Wall; Lillium polyphyllum D. Don))*	
07.	Riddhi (*Habenaria intermedia D. Don; H. edgeworthii Hook, H. acuminate Thw; H. goodyeroides D. Don)*	Varahikand (*Dioscorea bulbifera*)
08.	*Vriddhi* (*Habenaria intermedia D. Don; H. acuminate Thw; H. goodyeroides D. Don)*	

Table-2 Therapeutic applications of *Ashtavarga* herbs **and their** *Bhavprakasha Nighantu*

SN	*Ashtavarga* **Plants**	**Therapeutic applications**
01.	*Meda (Polygonatum airrhifolium*	Veerya vardhak, Dugdh tatha Kaf ko badhane wale, Dhatu vardhak, Sheetal, Pitt, Raktsambandhi dosh, Vayu tatha juvar ko door karne wala.
02.	*Maha Meda (Polygonatum airrhifolium)*	
03.	Jeevak (Microstylis musifera	Balkarak, Sheetvirya, Guru, Shukra tatha kaf ke vardhak, Madhur rasyukt, Pitt, Dah, Raktdosh, Krushta, Vaat evam Kshe rog.
04.	*Rishbhak* (Microstylis wallichii)	
05.	Kakoli *(Roscoea procera)*	Sheetal, Shukra vardhak, Madhur, Guru, Dhatu vardhak, Vaat, Dah, Raktpit, Sosh evam jevar nashak.
06.	Kshirakakoli *(Roscoea procera)*	
07.	Riddhi (*Habenaria intermedia)*	Balkarak, Tridosh, Shukrvardhak, Madhur ras yukt, Pak me guru, aur pranprad,

		aishvaryajanak, Murchha avam Raktpit ka nash karne wali
08.	*Vriddhi* (*Habenaria intermedia*)	Garbhprad, Sheetal, Dhatuvardhak, Madhur ras yukt, Veerya vardhak, Raktpit, kshat, Kash tatha Ksha ko door karne wali.
Ashtavarga **Substituted herbs**		
01.	Shatavari ((*AsparagusRacemosus*)	Madhur, Sheet, Guru, Snehan, Stanyajanan, Mutrajanan, Shukrajanan, Balya, Virashya, Vayasthapan, Chakushya, Agnivardhak, Alpsangrahak avam Tridoshghan.
02.	Vidarikand (*Pueraria tuberosa*)	Stanyajanan, Mutrajanan, Poshtik, Balya, Dugdh vardhak, Shoth par peeskar bandhte hai,
03.	Aswagandha (Withania somnifera)	Ushna, Madhur, Bhraney, Balya, Rasayan, Vrashya, Shothhar, Ksha, Balshos, Sukhandi, Vardhakya, Poshtik, Ishtiryo ke Katishool avam Shwed pradar me.
04.	Varahikand (*Dioscorea bulbifera*)	Raktsanghrahak, Grahi, Charm rog.

Material and Methods

Ethyl acetate, Methanol, Potassium ferricyanide, Ferric Chloride, Tannic acid, 1,1-diphenyl-2-picrylhydrazyl (DP PH) (Sigma-Aldrich, Germany), and all reagents are analytical grade.

Sample Preparation- all herbs are collected from approved vendors and authenticated by Dr. Mayaram uniyal. Clean all herbs with running tap water, dry them at 30 °C in hot air oven after drying make powder with grinder. This powder without any sieving will take for all analysis.

Antioxidant Activity

DPPH radical scavenging activity Using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), Brand Williams et al methodology slightly modifies is utilized to evaluate the actions of DPPH scavenging with methanol extract of *Ashtavarga* herbs and their substitutes. The different concentration of *Ashtavarga* herbs and their substitutes has prepared (30 mcg/ml to 700 mcg/ml). Each concentration was measured in triplicate. The 3.0ml of each sample solution is mixed with 1.0ml of 0.1mM DPPH in methanol. After shaking keep the solution at room temperature for exactly 30 min. in a light protected area. The absorbance was taken at 517nm with UV 1800 Made by Shimadzu. The difference of absorbance obtained from the sample and control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radicals. The following formula was utilized to compute the degree of DPPH radical scavenging activity.

Inhibition % = Ac-As/Ac×100

Where, Ac is the absorbance of the control,

As is the absorbance of the sample.

Total Bitter Determination-Weigh about 3 g of sample powder into round bottom flask add 100 ml methanol and reflux on water bath at 80 ± 2°C for 1 hour, remove the flask and allow to cool, filter the supernatant into 250 ml beaker through whatman filter paper number 1. The residue will again reflux with 50 ml methanol, this process will continue till the extract become colorless, collect all the filtrate in the same beaker. Beaker now keep on water bath for evaporation

to get thick paste, remove the beaker and add 50 ml hot water dissolve the paste and transfer into the separating funnel and add 100 ml ethyl acetate, shake gently and allowed to separate the ethyl acetate layer. Transfer the ethyl acetate layer into pre-weighed beaker with the help of whatman paper 1. The aqueous layer will again reflux with 50 ml ethyl acetate, this process will continue till the ethyl acetate layer become colorless. Transfer all ethyl acetate layers intosame beaker, keep it on water bath for evaporation and finally keep in oven at 105 °C for 2 hours take the weigt and calculate the total bitter as per below formulae.

% Total bitter = (Weight of beaker after drying- weight of empty beaker)/weight of sample *100

Total Tannins Determination

Sample preparation- Weigh about 100 mg sample powder into 250 ml round bottom flask and 100 ml water and reflux on water bath at 100 °C for 1 hour, remove the flask and allow to cool and filter into 100 ml volumetric flask, make up the volume up to the mark with water, shake and filter through whatman filter paper (working sample solution) this filtrate will use for sample preparation for taking absorbance from UV spectroscopy.

Standard preparation- weigh 100 mg Tannic acid into 100 ml volumetric flask and add 50 ml water, sonicate for 5 minutes make up the volume with water (Stock Standard). Take 1 ml stock standard into 100 ml volumetric flask and add water to make the volume up to the mark (working standard solution), this standard solution is used for taking absorbance from UV spectroscopy[12-16].

Reagent Preparation

1. **1%Potassium Ferricyanide solution-** Take 1 g of potassium ferricyanide into 100 ml volumetric flask add about 50 ml of water dissolve by sonication for 10 minutes and finally make up the volume up to the mark and filter through whatman filter paper 1.
2. **1% Ferric chloride solution-** Take 1 g of ferric chloride into 100 ml volumetric flask add about 50 ml of water dissolve by sonication for 10 minutes and finally make up the volume up to the mark and filter through whatman filter paper 1.

Preparation for UV Spectro-photometer reading

Reagent Preparation- add 1 ml of 1% potassium ferricyanide and 1 ml of ferric chloride reagents into 10 ml volumetric flask and make up the volume up to the mark with water.

Standard preparation- take 1 ml of working standard solution into 10 ml of volumetric flask add 1 ml of potassium ferriccyanide and 1 ml of ferric chloride solution and make up the volume with water. After 30 minutes of reagent addition take the reading against reagent at 720 nm.

Test preparation- take 0.2 ml of working sample solution into 10 ml of volumetric flask add 1 ml of potassium ferriccyanide and 1 ml of ferric chloride reagent and make up the volume with water. After 30 minutes of reagent addition take the reading against reagent at 720 nm.

Test Blank preparation- take 0.2 ml of working sample solution into 10 ml of volumetric flask, make up the volume

with water. After 30 minutes take the reading against water at 720 nm.

Calculate the total tannins as per given formula-

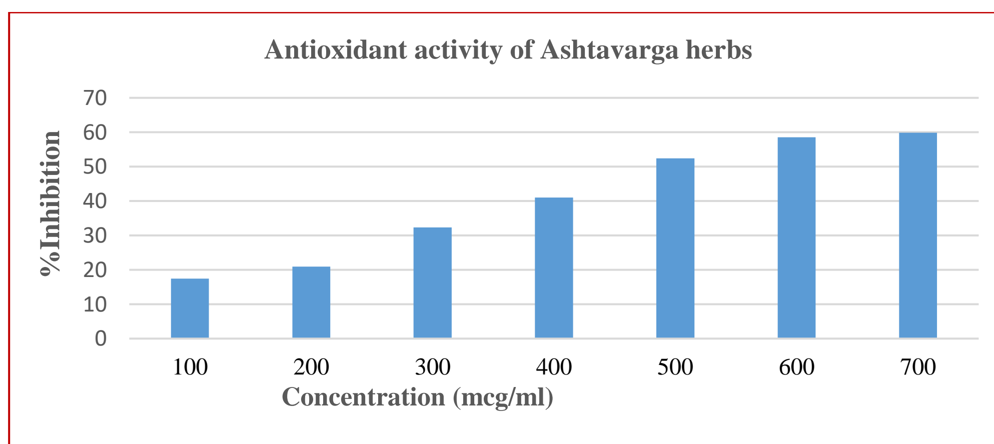
% Total Tannins =

Absorbance of sample - absorbance of sample blank	Weight of standard	1	volume of std taken	total volume of sample	% Purity of std
Absorbance of std	100	100	volume of test taken	weight of sample	

Result and Discussion

Table-3 Antioxidant activity of *Ashtavarga* **herbs**

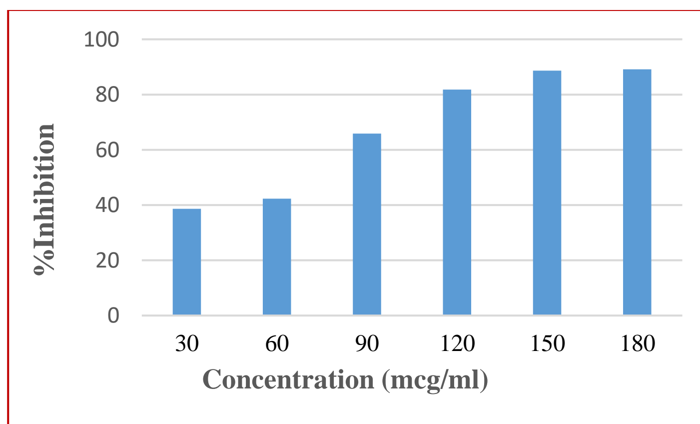
SNo.	Concentration	Plant extract (% Inhibition)
1	100 mcg/ml	17.46
2	200 mcg/ml	20.96
3	300 mcg/ml	32.31
4	400 mcg/ml	41.04
5	500 mcg/ml	52.40
6	600 mcg/ml	58.51
7	700 mcg/ml	59.82



Graph-1 Antioxidant activity of *Ashtavarga* **herbs**

Table-4 Antioxidant activity of *Bhavprakash nighantu* *Ashtavarga* **substitute herbs**

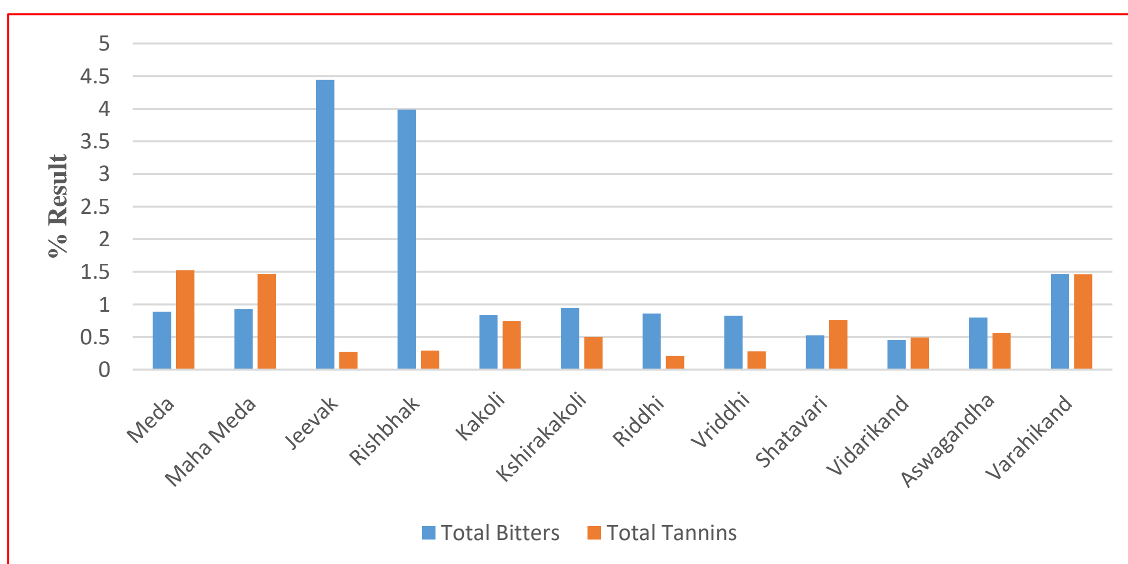
SN	Concentration	Plant extract (% Inhibition)
1	30 mcg/ml	38.63
2	60 mcg/ml	42.27
3	90 mcg/ml	65.90
4	120 mcg/ml	81.81
5	150 mcg/ml	88.63
6	180 mcg/ml	89.09



Graph-2 Antioxidant activity of Substitute herbs

Table-5 Phytochemical screening of *Ashtavarga* herbs and their substitute

SN	*Ashtavarga* **Plants**	**% Total Bitter**	**% Total Tannins**
01	*Meda (Polygonatum airrhifolium*	0.889	1.42
02	*Maha Meda (Polygonatum airrhifolium)*	0.924	1.45
03	Jeevak (Microstylis musifera	4.444	0.27
04	*Rishbhak* (Microstylis wallichii)	3.985	0.29
05	Kakoli *(Roscoea procera)*	0.841	0.74
06	Kshirakakoli *(Roscoea procera)*	0.945	0.50
07	Riddhi (*Habenaria intermedia)*	0.860	0.21
08	*Vriddhi* (*Habenaria intermedia*)	0.825	0.28
Substitute Plants			
01	Shatavari ((*AsparagusRacemosus)*	0.526	0.76
02	Vidarikand (*Pueraria tuberosa*)	0.451	0.49
03	Aswagandha (Withania somnifera)	0.797	0.56
04	Varahikand (*Dioscorea bulbifera L.*)	1.47	1.46



Graph-3 Phytochemical Screening of *Ashtavarga* Plants and their substitutes

Discussion

From the above tables and graphs, the *Ashtavarga* substitute herbs recommended by *Bhavprakasha Nighantu* shows greater antioxidant activity as compare to *Ashtavarga* herbs. Ashtavarga herbs shows maximum antioxidant activity of 59.82% at the concentration of 700mcg/ml whereas substituted herbs shows 89.09% at 180 mcg/ml which is very less as compare to *Ashtavarga* herbs concentration. In case of phytochemical screening we analysed individual herbs and find that the maximum Total Tannins and Total Bitters found in Varahikand (*Dioscorea bulbifera*) and Jeevak (Microstylis musifera) respectively. The minimum Total Tannins and Total Bitters found in Riddhi (*Habenaria intermedia)* and Shatavari ((*Asparagus Racemosus)* respectively.

Conclusion

This study concludes that the substitute herbs of *Ashtavarga* recommended by *Bhavprakash nighantu* is comparable with *Ashtavarga* herbs in their phytochemical screening. In case of antioxidant activity the substitute herbs are more effective as compared to *Ashtavarga* herbs and hence using of these herbs in polyherbal formulation like Chavyanprash is scientifically justified.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Analysis of trace metal through modern techniques and Anti-microbial Activity by well diffusion method of *Nepeta hindostana* and *Ricinus communis.*

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Abstract- Nowadays, the focus has been shifted towards the development of non-antibiotic substances such as plant or plant derived Phytochemicals molecules. The principle phytochemical constituents of medicinal plants have shown good fighting potential against drug resistant pathogens. Scientifically these phytochemical constituents have been proven extremely potent antimicrobial agents in comparison to antibiotics. In this research article we have studied about phytochemical screening, heavy metal analysis and antimicrobial activity. The result shows that Both *N. hindostana* and *R. communis* possess significant antimicrobial properties, supporting their role as viable alternatives to synthetic antibiotics. The heavy metals in both herbs are within the prescribed limit as per WHO. Phytochemical qualitative screening show that both herbs contain essential phytochemicals responsible for their antimicrobial and antioxidant activity and make them more suitable herbs for making herbal preparation.

Key words: Heavy metal, ICP-MS, Digester, Anti-microbial activity, Antibiotics.

Introduction

Natural plants have always been valued as a mode of treatment of variety of disease in folk cultures from ancient times and have played a very important role in discovering the western medicines. Although the efficacy of medicinal plants for curative purposes is often accounted for in terms of their phytochemical constituents like tannins, bitters, flavonoids, terpenoids, saponins, essential oils, vitamins, glycolsides, etc. Now, study shows that the over dose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different trace elements which causes various health problems. So, it is mandatory to evaluate elemental contents of the medicinal plants and their preparation to control their quality**.** In recent years, several authors like Wong et al., 1993 in China; Sharma et al., 2009 in India; Sheded et al., 2006 in Egypt; Koe and Sari, 2009, Basgel and Erdemoglu, 2006 in Turkey; Ajasa et al., 2004 in Nigeria; Kanias and Loukis, 1987 in Greece and many more all across the world, reported many studies on the importance of elemental constituents of the herbal drug plants which enhanced the awareness about

trace elements in these plants Most of these studies concluded that essential metals can also produce toxic effects when the metal intake is in high concentrations, whereas non-essential metals are toxic even in very low concentrations for human health. Herbal medicines are more common and most uses for treatment of diseases in India. Overseas reports are scanty with respect to elemental constituents of endemic herbal plants of India. The present study was carried out in Himalaya Wellness Company, Faridabad Haryana. Rapid Industrialization and urbanization, are threat to the medicinal flora in context of heavy metals Therefore, it is important to have a look on good quality control of medicinal herbs in order to protect consumers from contamination. In recent years, considerable efforts have been made to control the spread of pathogens with various strategies, including the use of alternative anti-microbial compounds. As the pipeline of development for newer antibiotics look bleak, therefore the focus has been shifted towards the development of non-antibiotic substances such as plant or plant-derived Phytochemicals. The principle phytochemical constituents of medicinal plants have shown good fighting potential against drug resistant pathogens. Scientifically these phytochemical constituents have been proven extremely potent antimicrobial agents in comparison to antibiotics. Due to their antimicrobial activity, these natural ingredients appear as a viable and healthy alternative to synthetic antibiotics for the treatment of different diseases. Chemical analysis of medicinal plants revealed the presence of several ingredients, most of which possess important antioxidant and anti-microbial properties. The primary aim of this study is to establish the trace metals like Pb, Cd, As, Li & Hg levels two herbs *Nepeta hindostana* and *Ricinus communis* by ICP-MS instrument along with Antimicrobial study of the same herbs against *Escherichia coli* (ATCC-8739), *Salmonella* (NCTC6017), *Pseudomonas aeruginosa*(ATCC-9027), *Staphylococcus aureus* (ATCC-6538), *Candida albicans* (ATCC-10231)[1-15].

Material and Methods

Sample collection- The plant materials were collected from the approved vendors of Himalaya Wellness Company, Faridabad, Haryana.

Sample preparation- Plant samples were washed with deionized water and oven dried at 40°C for 2 days and then subjected to grinding for powder formation.

Phytochemical screening

Steroids- Three ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H2SO4. Purple color thus formed changes into blue or green color indicating the presence of steroids.

Triterpenoids- A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

Carbohydrate (Molisch's Test)- Add a few drops of alcoholic α-naphthol to the plant extract, followed by concentrated sulfuric acid (H_2SO_4) carefully poured down the side of the test tube. A purple or reddish-violet ring at the junction of the two liquids indicates the presence of carbohydrates.

Alkaloids- A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Phenols- A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Flavonoids- A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

Saponins- A 3 ml of test solution was foamy lather indicates the presence of Saponins.

Tannins- A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

Amino Acids- A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

Digestion of sample for trace metal analysis-Weigh about 250 mg powder of each plant sample into vessel and add 0.5 ml of gold solution 10 ppm, add 8 ml trace metal Nitric acid, swirl the vessel content and allowed to keep for 5 minutes to complete any reaction, seal the vessel which are then put into cavity of digester in a manner prescribed by manufacturer. Set the method by clicking on touch screen of microwave digester, the common points are temperature ramp and temperature hold as we worked on Anton Paar microwave digester model number Multiwave 3001 50 HZ. This model is temperature control and we have set temperature ramp of 180 ° C for 20 minutes and temperature hold of 180 °C for 20 minutes. After completion of digestion cycle, remove the sample from the cavity and filter through whatman filter paper 1 into 50 ml plastic tubes and make up the volume with Milli Q water, care should be taken that all the digested sample should be clear free from any residue. The filtered sample is ready for ICP-MS analysis.

Standard preparation

1. **Diluent-**2% Trace metal Nitric Acid solution in Milli Q water.
2. **Gold solution (10 ppm)-** transfer 0.5 ml of gold standard stock sol (1000 ppm) into 50 ml plastic tube and makeup the volume with diluent.
3. **Stock Standard solution A (Mercury 10 ppm):** Transfer 0.5 ml of 1000 ppm Mercury standard solution and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube, makeup the volume with diluent.
4. **Stock Standard solution B (Mercury 100 ppb, multi element 1000 ppb :** Transfer 0.5 ml of 100 ppm Multi element standard solution, 0.5 ml of standard stock solution A and 0.5 ml of gold solution 10 ppm into 50 ml plastic tube, makeup the volume with diluent.

Working Standard solution:

1. **Standard Blank:** transfer 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.

2. **Standard 1 (Mercury 0.1 ppb, Multi element 1 ppb):** transfer 0.05 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
3. **Standard 2 (Mercury 0.5 ppb, Multi element 5 ppb):** transfer 0.25 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
4. **Standard 3 (Mercury 1 ppb, Multi element 10 ppb):** transfer 0.5 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
5. **Standard 4 (Mercury 2.5 ppb, Multi element 25 ppb):** transfer 1.25 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
6. **Standard 5 (Mercury 5 ppb, Multi element 50 ppb):** transfer 2.5 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
7. **Standard 6 (Mercury 7.5 ppb, Multi element 75 ppb):** transfer 3.75 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
8. **Standard 7 (Mercury 10 ppb, Multi element 100 ppb):** transfer 5 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the vol. with diluent.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)- is a highly sensitive analytical technique used to detect and quantify trace-to-ultratrace elements (metals and non-metals) down to parts-per-trillion (ppt) levels. It operates by ionizing samples in a high-temperature argon plasma and measuring the ions using a mass spectrometer. For this study we use Perkin Elmer NexION 1100 model. The parameters we have select for the analysis are: Sweeps/Reading 40, Replicates3, Peak Hoping, Standard mode, Dwell time per AMU 50, Integration time 2000, Mass 207.977 for Pb, 110. 904 for Cadmium, 74.9216 for As, 201. 971 for Hg and 7.016 for Li. Curve type is Linear through zero, peristaltic pump speed for sample flush -48 rpm and time is 75 seconds, read delay speed -20 rpm for 15 minutes and for washing speed -48rpm and time is 75 seconds. Reporting is select as Quant Summary+ QC rop.

Culture medium and inoculum preparation-The test organisms were sub-cultured onto fresh plates of Soybean Casein Digest Agar (Hi Media laboratories) and Sabouraud Dextrose Agar (Hi Media laboratories) for 24 hours at 37 °C and 25-30 °C for 3-5 days respectively. Colonies from these plates were suspended in Soybean Casein Digest Broth and Sabouraud Dextrose Broth. The media used for antimicrobial assays were Soybean Casein Digest Agar for bacteria and Sabouraud Dextrose Agar for fungi. All were incubated appropriately as specified for each organism for a period of 18–24 h (Burt 2004).

Antimicrobial Study

Agar Well Diffusion Method- The antimicrobial activities of the selected *Nepeta hindostana and Ricinus communis methanolic aquous and Acetone* by Agar well diffusion method. In this method, 100µl of standardized inoculum of each test bacterium were spread onto sterile Soybean Casein Digest Agar and Sabouraud Dextrose Agar for bacteria and fungus respectively. 6 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 100 µl of *Nepeta hindostana and Ricinus communis*. The plates were kept at room temperature for 1 h to allow proper diffusion of the plant extracts into agar and then incubated at 37°C for 24 h and 25-30°C for 3–5 days respectively. Triplicates were prepared for each sample. The plant extracts having antimicrobial activity inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimeters. The percentage activities of essential oils were calculated against standard drugs which was considered as higher.

Strains of tested organisms- The bacterial strains used in the study were obtained from the Microbiology Lab., Department of Quality Control and Quality Assurance Himalaya Wellness Company Faridabad Haryana. *Escherichia coli* (ATCC-8739), *Salmonella* (NCTC6017) *Pseudomonas aeruginosa* (ATCC-9027), *Staphylococcus aureus* (ATCC-6538) and *Candida albicans* (ATCC-10231) use for this study[16-26].

Results and Discussion

Table-1 Qualitative phytochemical constituents of *Nepeta hindostana and Ricinus communis* **in methanolic extract.**

Phytochemical Constituents	***Nepeta hindostana***	***Ricinus communis***
Carbohydrates	-	+
Protein	-	+
Alkaloids	-	+
Steroids	-	-
Phenolic compound	+	+
Terpenoids	-	+
Flavonoids	+	-
Saponins	-	-
Tannins	-	+

+ = Present, - = Absent

Table-2 Concentration of trace metals in *Nepeta hindostana and Ricinus communis*

Sample Name	Lead (pb)	Cadmium (cd)	Arsenic (As)	Mercury (Hg)	Lithium (Li)
Nepeta hindostana	0.849 ppm	0.072 ppm	0.322 ppm	0.010 ppm	1.264 ppm
Ricinus communis	1.137 ppm	0.076 ppm	0.107 ppm	0.012 ppm	0.281 ppm

Table-3 Antimicrobial activity of the Methanolic, Aqueous and Acetone extracts of selected medicinal plants

Sr. No	Test Microorganisms	Zone of Inhibition (mm)							
		Nepeta hindostana			*Ricinus communis*			Positive Control	
	Microbial Strains	100 µL Meth.	100 µL Aqu.	100 µL Acet.	100 µL Meth.	100 µL Aqu.	100 µL Acet.	100 µL Amox.	100 µL Itrac.
1	*Escherichia coli* (ATCC-8739)	20	18	14	17	14	12	26	-
2	*Salmonella* (NCTC6017)	18	16	12	16	14	11	26	-
3	*Pseudomonas aeruginosa* (ATCC-9027)	17	15	13	15	13	11	27	-
4	*Staphylococcus aureus* (ATCC-6538)	23	20	16	20	18	14	28	-
5	*Candida albicans* (ATCC-10231)	20	17	15	18	17	13	-	26

Using antibiotic: Bacteria – Amoxicillin (10mcg/ml); Fungi – Itraconazole (10mcg/ml), Meth. = Methanol, Aqu. =aqueous and Acet. = Acetone

Discussion

Phytochemical analysis revealed the presence of phenols and flavonoids in N. hindostana, while R. communis tested positive for carbohydrates, proteins, alkaloids, phenols, terpenoids, and tannins. ICP-MS results showed that trace metal concentrations were within the limits as per WHO, with R. communis exhibiting higher Lead (1.137 ppm) and N. hindostana showing higher Lithium (1.264 ppm) levels. In anti-microbial assays, the methanolic extracts of both plants demonstrated the highest potency. N. hindostana showed superior inhibitory activity, particularly against S.aureus (23 mm zone of inhibition) and E. coli (20 mm), compared to R. communis.

Conclusion

Both *N. hindostana* and *R. communis* possess significant antimicrobial properties, supporting their role as viable alternatives to synthetic antibiotics. The study highlights the importance of ICP-MS in quality control to ensure that elemental concentrations remain within safe limits for human consumption, protecting consumers from potential heavy metal toxicity.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Phytochemical Profiling, Antioxidant and Antibacterial Activity of *Hibiscus rosa-sinensis* against Pathogenic bacteria

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Abstract- Plants have been a valuable resource of natural products for human health. *Hibiscus rosa-sinensis* (Gurhal) is cultivated ornamental and medicinal plant widely used in traditional healthcare systems. The present study focuses on the phytochemical composition and antioxidant and antibacterial activity from the flowers of Gurhal. Phytochemical screening revealed the presence of important bioactive constituents including flavonoids, saponins, glycosides, phenolic compounds, alkaloids, tannins and terpenoids. These secondary metabolites are responsible for the therapeutic and pharmacological properties of the plant. Antioxidant activity of the extracts was evaluated using standard free radical scavenging assays, which demonstrated significant antioxidant potential due to the high content of phenolic and flavonoid compounds. In addition, antibacterial studies showed effective inhibitory activity against *staphylococcus aureus*. Among the tested extracts, the acetone and methanol extracts exhibited comparatively higher biological activity than the other extracts. The finding suggests that *Hibiscus rosa-sinensis* can serve as a valuable source of natural antioxidants and antibacterial agents for pharmaceutical and medicinal and therapeutic applications.

Key words: *Hibiscus rosa-sinensis*, Phytochemical screening, Antioxidant & Antibacterial Activity.

Introduction

Hibiscus rosa-sinensis, commonly known as China rose and gurhal, belongs to the family Malvaceae and is widely cultivated. A highly potential functional and valuable medicinal plant has been reported in the ancient medicinal literature with beneficial effects in various disorders of humans[1].

Medicinal plants are considered an important source of natural therapeutic agents because of their phytochemical constituents. *Hibiscus rosa-sinensis* is rich in secondary metabolites such as flavonoids, phenolic compounds, alkaloids, tannins, saponins, glycosides and terpenoids. These phytochemicals play a vital role in the plant's biological and pharmacological activities. The flower petals, in particular, contain anthocyanins and flavonoids that contribute to their antioxidant properties. They can function as sources for nutritional supplements or serve as natural antioxidants, which help maintain food quality and prolong shelf-life[2-3].

The natural antioxidants may have free-radical scavengers, reducing agents,

potential complexes of pro-oxidant metals, quenches of singlet oxygen [4]. Recently, interest has increased considerably in finding natural occurring antioxidants for use in medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [5]. Crude alcoholic extracts of *Hibiscus rosa-sinensis (H. rosa-sinensis)* flower petals and leaves are used *in vivo* for their antioxidant properties in some studies [6].

Rising antimicrobial resistance is forcing a search for new, natural medicine sources. *Staphylococcus aureus* is a Gram-positive coccus, and is the common cause of staph infection. About 20% of human population if carries *Staphylococcus aureus* can cause gor long term illness of skin infection, such as pimples, impetigo, and cellulitis folli-cutis. Extracts from *Hibiscus rosa-sinensis* flowers successfully inhibit *staphylococcus aureus* making them ideal candidates for herbal remedies. This therapeutic power relies on the synergistic effects of its natural plant compounds. While *H. rosa-sinensis* and *H. acetosella* are highly valuable, *H. sabdariffa* stands out globally for its culinary appeal and proven antioxidant, anti-inflammatory, and antimicrobial health benefits[7]. Antioxidant prevent occurrence of these diseases by inhibiting the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress[8].

Therefore, the present study aims to investigate the phytochemical profile, antioxidant potential, and antibacterial activity of *Hibiscus rosa-sinensis*. These findings highlight its importance as a valuable medicinal plant with promising pharmaceutical applications.

Material and Methods

Fresh flowers of *Hibiscus rosa-sinensis* were collected from healthy plants growing in the local area. The plant material was washed thoroughly with distilled water to remove dust and other impurities and then shade dried at room temperature for one week. The dried sample was powdered using mechanical grinder and stored in airtight containers for further analysis.

Preparation of plant extract- The powdered plant material was extracted using different solvents such as methanol, ethanol, acetone, hydro-alcoholic, hexane and distilled water by maceration method. 20 g of powdered sample was mixed with 200 ml of solvent and kept for 24- 48 hours with occasional shaking. The extracts were filtered with whatman no.1 filter paper and concentrated using water bath.

Phytochemical Screening- Phytochemical analysis of the extracts was carried out using standard qualitative methods to identify the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpeniods.[9]

1) **Flavonoids** (Shinoda Tests) - Took 2-3 ml of the extract solution in a test tube. Added a few magnesium turnings, few drops of conc. HCl was added drop-wise. Pink to red coloration develops.

2) **Alkaloids** (Mayer's test)- Extracts were treated with Mayer's reagent (potassium mercuric chloride). Formation of a yellow colored precipitate indicates the presence of alkaloids.

3. Tannins- To the extracts, a few drops of 10% ferric chloride solution were added. Appearance of a green or blue colour indicates the presence of tannins.

4) Phenolic compound (Ferric chloride test)- Two ml of diluted extracts were treated with dil. $FeCl_3$ solution. Appearance of a violet color indicated the presence of phenol like compounds.

5) Saponins (Foam test)- The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicated the presence of saponins.

6) Terpenoids (Salkowski Test)- Five ml of the extract were mixed with 2ml of chloroform and 3ml of conc. H_2SO_4 solution. A reddish-brown colour at the interphase indicated the presence of terpenoids.

Antibacterial Activity- The antibacterial activity of the extracts was evaluated against selected bacterial strain (*Staphylococcus aureus - ATCC 6538*) using the agar well diffusion method. Sterile nutrient agar plates were inculcated with microbial cultures, and wells were made using a sterile cook borer. The known concentration of plant extracts were added into the well, while standard antibiotics were used as positive controls. The plates were incubated at 37^0C for 24 hours for bacterial cultures. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around each well[10].

Antioxidant Activity- The radical scavenging activity (antioxidant activity) was determined according to a previously reported method[11] with slight modifycations. A stock solution of the sample (50mg/ml) was diluted for different concentrations. The following concentrations of extracts were prepared 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL, 100µg/mL and 120µg/mL. Each concentration was tested in duplicate. The 3.0ml of sample solution was mixed with 1.0ml of 0.1mM 1, 1-Diphenyl-2-2picrylhydrazyl (DPPH, in methanol) and allowed to stand at room temperature for 30 minute under light protection. The absorbance was measured at 517nm. The scavenging activity of the samples at corresponded intensity of quenching DPPH. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Inhibition % = Ac-As/Ac×100
Where, Ac is the absorbance of the control
As is the absorbance of the sample

Result and Discussion

The present study was undertaken to evaluate the phytochemical constituent, antibacterial activity, and antioxidant activity of flower extract of *Hibiscus rosa-sinensis*. The extracts were subjected to phytochemical screening, antibacterial assessment against *staphylococcus aureus*, and antioxidant evaluation using the DPPH radical scavenging assay.

The preliminary phytochemical screening of different extracts of *Hibiscus rosa-sinensis* flowers revealed the presence of several bioactive constituents shown in Table -1. Alkaloids, tannins, saponins, terpenoids, flavonoids, and phenolic compounds were detected in most of the extracts. The aqueous extracts showed a comparatively higher abundance of alkaloids, indicating that water was more

effective in extracting polar phytoconstituents. The presence of phenols tannins, flavonoids and terpenoids is significant because these compounds are known to possess antioxidant and antibacterial properties.

Table-1 Phytochemical Screening of *Hibiscus rosa-sinensis.*

Sr.No.	Chemical Test	Ethanol	Aqueous	Methanol	*Hexane*	*Acetone*	*Hydro-Alcoholic*
1	*Alkaloids*	+	++	+	+	+	+
2	*Flavonoid*	-	+	-	-	-	+
3	*Tannins*	+	+	+	-	+	+
4	*Saponins*	+	+	+	-	+	+
5	*Terpenoids*	+	+	+	-	+	+
6	*Phenol*	+	+	+	-	+	+

The antibacterial activity of different flower extracts of *Hibiscus rosa-sinensis* against *staphylococcus aureus* is presented in table-2. Among all extracts, the acetone extract exhibited the highest antibacterial activity 20mm, followed by methanol extract 17mm. the antibacterial effect observed in acetone and methanol extract may be attributed to the presence of phenolic compounds, tannins, and terpenoids, which are known to disrupt bacterial cell membranes and inhibit microbial growth. Hydro-alcoholic, aqueous, and ethanol extracts showed moderate antibacterial activity with inhibition zones of 14mm, 13mm and 13mm respectively. No antibacterial activity was observed in the hexane extract. The positive control (ofloxacin) produced a zone of inhibition of 36mm, which was significantly higher than all plant extracts.

Table-2 Antibacterial activity of *Hibiscus rosa-sinensis* **against**
(*Staphylococcus aureus* **- *ATCC 6538***)

Name of the plant extract (Flower)	**Zone of inhibition against *S.aureus (mm)***
Methanol	**17**
Hexane	**ND**
Acetone	**20**
Hydro-Alcoholic	**14**
Aqueous	**13**
Ethanol	**13**
Positive Control (ofloxacin)	**36**
Negative Control	**ND**

Note: ND= Not Detected

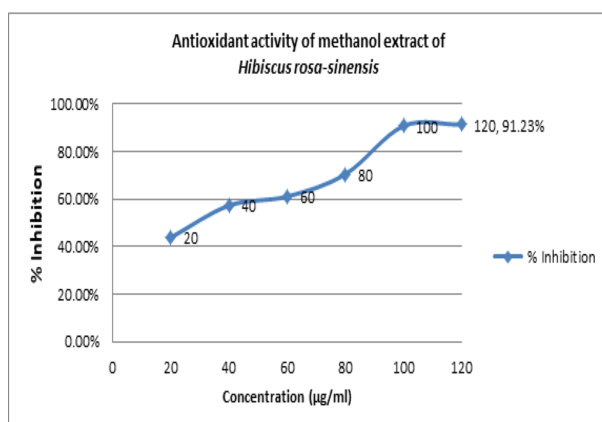
The DPPH free radical scavenging activity of the *Hibiscus rosa-sinensis* flower extract is presented in table-3. The extract showed 43.83% inhibition at 20µg/ml, which gradually increased to 91.23% at 120µg/ml. The concentration required to inhibit 50% of DPPH radicals (IC_{50}), was calculated to be approximately 29.18µg/ml for *Hibiscus rosa-sinensis* and 5.19 µg/ml for Ascorbic acid, indicating potent antioxidant activity.

The increase in antioxidant activity with concentration suggests that the methanol extract contains significant amounts of phenolic and flavonoid compounds capable of donating hydrogen atoms or electrons to neutralize free radicals.

The strong antioxidant activity observed in the methanol extract correlates with the phytochemical screening results, which confirmed the presence of phenols, tannins, terpenoids, and other secondary metabolites. These compounds are well able to scavenge reactive oxygen species.

Table-3 Antioxidant activity of methanol extract of *Hibiscus rosa-sinensis*

Concentration (µg/ml)	Plant extract (%) inhibition
20	43.83%
40	57.26%
60	61.09%
80	70.41%
100	90.68%
120	91.23%



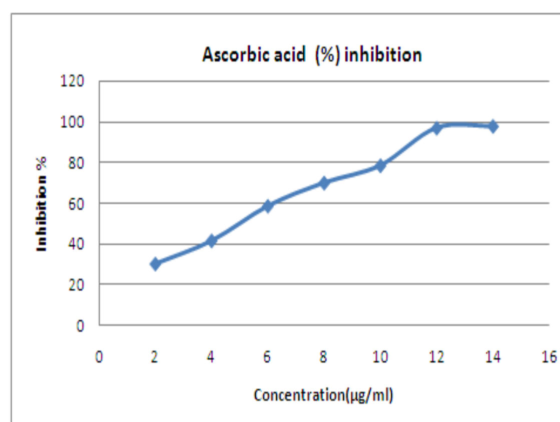


Figure-1 Antioxidant activity of Methanol extract of *Hibiscus rosa-sinensis* and Ascorbic acid

Conclusion

The present study demonstrated that *Hibiscus rosa-sinensis* flower extracts possess considerable phytochemical, antibacterial and anti-oxidant properties. The phytochemical analysis confirmed the presence of several bioactive metabolites that may contribute to the observed biological activities. Among the tested extracts, the acetone extract showed the strongest antibacterial activity against *Staphylococcus aureus.* The methanol extract exhibited remarkable antioxidant potential with an IC_{50} value 29.18 µg/ml.

These findings suggest that *Hibiscus rosa-sinensis* is a promising natural source of bioactive compounds with potential pharmaceutical and nutraceutical applications.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Molecular profiling of *Punica granatum* rind: Phenolic composition with emphasis on Tannins and Catechins and *in-vitro* antimicrobial efficacy and interaction of Rind extracts with and without antibiotics against drug resistant bacteria

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Abstract- The rind (peel) of *Punica granatum* (pomegranate) is a rich source of bioactive phytochemicals, including phenolics, flavonoids, and tannins, which contribute to their strong antioxidant, antimicrobial, and anticancer properties. Today new sources of antimicrobial compounds need to be identified and improved strategy should be developed to combat multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating anti-microbial action needs to be exploited for their synergistic action between extracts and with antibiotics to exploit it in modern chemotherapy and combinational therapy. In the present study ethanol extract of *Punica granatum* rind was screened for their antimicrobial efficacy against a wide variety of drug resistant bacteria and yeast. The extracts showed promising action against one or more drug resistant bacteria as well as against *Candida albicans*. The extract of *Punica granatum* rind exhibited synergy with antibiotics, tetracycline, chloramphenicol, ampicillin and gentamicin against methicillin resistant *S. aureus* which has indicated their potential to be exploited in combination drug therapy after careful evaluation *in vivo* model. Phytochemical investigations included qualitative detection of phytochemicals including phenols and tannins, flavonoids, Total phenolic and tannin and catechin content was determined quantitatively. Ethanolic pomegranate rind extracts showed highest amount of phenolic and flavonoid content. The presence of catechin in pomegranate rind was determined by HPTLC. The extracts of pomegranate rind was found effective in inhibiting the growth of a number of bacteria.

Key words: Antimicrobial activity, MDR bacteria, MIC, synergistic activity antibiotics

Introduction

Pomegranate is an ancient fruit native to Persia which has been cultivated in the Mediterranean region through years (Viuda Martos 2010; Silvaj et al,2013). This fruit, which belongs to the family of Punicaceae, is botanically named *Punica granatum* (Chandra et al 2010). Pomegranate trees are considered as shrubs or small trees which grow 5 to 10 meters high (Silvaj et al,2013). Its flowers are red and can occur either as single blossoms or clusters of several blossoms (Baliga et al 2013 & Stover, 2007). Pomegranate is rich in bioactive

molecules, it has shown myriad medicinal properties due to its high phenolic content (Viuda Martos 2010; Akhtar et al,2015). Pomegranate, as a fruit rich in antioxidants, can intensively and positively contribute in humans' health. Pomegranates strong historical, cultural and religious significances, besides the researches determining the phytochemicals present in pomegranate triggered analysing and evaluating pomegranate rind.

The use of herbal and other natural substances is part of the fabric of traditional medicine in different part of the world. Medicinal plants have been found good source of therapeutic and novel compounds. Targeted screening of a large diversity of medicinal plants is expected to yield novel biological activities including problematic group of multidrug resistant bacterial pathogens.

Bacteria which have evolved numerous defences against antimicrobial agents and drug resistant pathogens are on the rise and such bacteria have become a global health problem. Nearly twenty years ago over 90% *S. aureus* strains were reported β-lactamase positive. Strains of β-lacatam resistant *Staphylococcus aureus* including MRSA now pose a serious problem to hospitalized patients and their care providers (Liu, et al., 2000). The production of β-lactamase is recognized as one of the main mechanisms of bacterial-resistance to β-lactamase antibiotics. Numerous compounds have been included in the list of β-lactamase inhibitors and some of these have shown potential clinical usefulness based on their synergistic-effects when they are combined with β-lactamase-labile antibiotics. Many β-lactamase were found to be resistant to β-lactamase inhibitors. Similarly multidrug resistant problem is common in members of family, Enterobacteriaceae specially *E.coli*, *Salmonella*, *Shigella* and several other humans and animal pathogen like *Haemophilus influenza*, *Campylobacter*, *Pseudomonas* aeruginosa, *Mycobacterium tuberculosis* both in developing and developed countries (Eldelstein *et al.,* 2001; Tonkic *et al.,* 2005;).

The world health organization (WHO) reported that 80% of the world's population rely chiefly on traditional medicines and major part of the traditional therapies involve the use of plant extracts or their active constituents (WHO, 1993).

Many plant metabolites are now used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angiosperms have been investigated chemically and that 74% of pharmacologically active plants derived components were discovered after following upon ethanobotanical use of plants (Eloff, 1998). The plants are valuable in the three basic ways:

(1) they are used as source of direct therapeutic agent.
(2) As a source of new bioactive metabolites including antimicrobial, antihelminthic and antiprotozoan etc.
(3) they serve as raw material base for elaboration of more complex semi-synthetic chemical compounds.

According to a report published in the 'Journal of the American medical

association', more than 630 million visit are made to alternative practitioners each year in the U.S. also more than 15 million adults take herbal remedies while taking other medication (Hoffman, 2004). Concerted efforts have been made all over the world to explore the various biological and specific pharmacological activities and their active compounds all over the world. However, targeted screening with improved strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy.

It is expected that plant extract showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.,* 1998). In the recent years, plants have been screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Salmonella paratyphi, Escherichia coli, Shigella dysentriae* and *Candida albicans*. The selection of medicinal plant was based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et al.,* 1999).

The recent development in the phytopharmacology is development of multicombinational drug against multidrug resistant bacteria. This has been possible due to interaction among plant extracts (Phytocompounds) and with other chemotherapeutic agents that may be synergistic or additive in their interaction. The development of these drugs has grown a new future in the area of phytopharmacology and medical practices.

At present multi drug therapy or combinational antibiotic therapy is in use. However its efficacy may be severely hindered against several MDR bacteria. Therefore, there is an increased request to develop novel drugs against multi drug resistant bacteria. One possible approach is to screen unexplored Indian medicinal bioactive plant extracts for their potential to be used against multi drug resistant bacteria.

Considering the vast potential of Indian medicinal plants as an anti-infective agent, we have selected *Punica granatum* on the basis of their traditional uses, ethanopharmocoloical data and local availability. The present study has been planned to identify the active phytochemicals tannins and catechins and antimicrobial activity against drug resistant microbial pathogens and to assess synergy with antibiotics *in vitro.*

Material and methods

Plants material- Fresh pomegranate fruit was bought from the local market and washed thoroughly using water. The rind of the pomegranates was separated, washed with distilled water and dried. The dried rind was powdered using commercial grinder. Samples were kept in sealed plastic bags and stored at low temperature in dark, until use.

The authentic plant material was obtained from the Himalaya WellnessCompany, Dehradun, India. The identification of the samples was further confirmed by the plant taxonomist in the Department of Pharmacognosy, HWC Dehradun. The voucher specimen has been deposited in the Department of Pharmacognosy, HWC, Dehradun shown in Table-2.

Drug resistant and sensitive bacterial strains used in the screening programme- The Standard strains were obtained from different National and International Culture Collection Centers/ Collection of individual scientist and clinical isolates were collected from Department of Microbiology, HWC Dehradun Uttarakhand. Multidrug resistant bacteria include the strains of *Staphylococci* including methicillin resistant *Staphylococcus aureus* (MRSA), *Psuedomonas aeruginosa* and R-plasmid harbouring strains of *E. coli.* MRSA and some other Gram positive and Gram negative bacteria were also used in our laboratory. The details of the test strains and their relevant characteristics are mentioned in Table-1.

Chemicals and Antibiotics- All the antibiotic discs were purchased from Hi-Media Lab Pvt Ltd, Mumbai, India. The indicator dye p-iodonitro tetrazolium violet were purchased from Sigma Chemical Co., USA. MMS and Sodium azide were purchased from Sisco Reseach Laboratory, India. All the other media/chemicals used were of analytical grade.

Bacterial cultures- Bacterial isolates were obtained from different sources (Table-1) and were subjected to antibiotic sensitivity by disc diffusion, method (Bauer et al., 1966).

ß-lactamase production- The method described earlier (Ahmad et al., 2008) was used for detection of proudtcion of ß-lactamase.

Culture Media and Inoculum preparation- Nutrient broth/ Agar and Muller–Hinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 ^{0}C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 –10^6 CFU/ml.

Preparation of plant extracts and its fractionation- Plant extracts were prepared as described earlier (Ahmad and Beg 2001) with a little modification. 800 gram of dry, plant powder was soaked in 2.5 liter of 70% ethanol, for 8–10 days and stirred after every 10 hr using a sterilised glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). This alcoholic filtrate was concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO, to have a stock solution of 100 mg/ml concentration.

Extraction for HPTLC: Accurately weigh 1 g of dried pomegranate rind powder and extract using 10 mL of analytical-grade methanol. Sonicate for 15-20 minutes.

HPTLC method development of pomegranate peels – Used TLC silica gel G 60 F254 (10 × 10) plates are used with solvent system **chloroform: ethyl acetate: formic acid (4:3:3 v/v/v) as a solvent system.** The sample was spotted on precoated TLC plates using Linomat 5 applicator, the ascending mode is being used for the development of thin-layer chromatography, TLC plates were developed from solvent front position 85.00 mm. The plates were air-dried and then scanned at 254 nm and 366 nm. (Pooja Gadkari and Sanjay Daharwal, 2022).

Antimicrobial assay- The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Beg 2001) was used. 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm

diameter were punched into the agar medium and filled with 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

Minimum inhibitory concentration of plant extracts- Minimum inhibitory concentration of plant extracts against test bacterial strains was determined by tube broth dilution method, using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth (Eloff 1998). 2 ml of the plant extract was mixed with 2 ml of Muller-Hinton broth (Hi-Media Ltd., Mumbai, India) and serially diluted into the next tube and so on. 2 ml of an actively growing culture of different test strains was added before incubating for over night, at 37 °C. After examining turbidity visually, 0.8 ml of 0.02 mg/ml indicator dye (p-iodonitro tetrazolium violet) was added to each tube and incubated at 37 °C. The tubes were examined for the colour development, after 30 min. Absence of growth was also confirmed by spreading 0.1 ml of broth from such test tube on normal nutrient agar plate.

Synergistic interaction of plant extracts with antibiotics- Synergistic interaction between antibiotics, like ampicillin, tetracycline and chloramphenicol with crude plant extracts was studied by agar well diffusion method. For determining the synergistic effects of plant extract with antibiotic, the wells were punched at a predetermined distance so that their inhibitory circles touch each other only tangentially without influencing each other as recommended by Ahmad et al. (2008). The wells were inoculated with plant extract and antibiotic separately. Plates were then incubated at 37 °C, for 18 hours. Enlargement of inhibition zones indicates a positive interaction (synergism).

Phytochemical analysis of plant extracts- Major phytocompounds, in the crude extracts of plants, were detected by standard colour tests and thin layer chromatography, as described by(Ahmad and Beg 2001).

Results and Discussion

Antimicrobial activity of plant extracts against drug resistance pathogenic bacteria

Multiple drug resistance in pathogenic bacteria has emerged as important problem in many countries of the world. There are now increasing case reports documenting the development of clinical resistance to newer and broad spectrum antibacterial drugs like fluroquinolone (norfloxacin, Cipro-floxacin, oflaxacin etc.) in many pathogenic bacteria. In the present study, clinical isolates of *S. aureus P. aeruginosa, E. coli,* and *Candida albicans* were used. These microbial strains are found to be resistant to one or more antibiotics, showing the common occurrence of drug resistance. These finding are in agreement with the reports of previous workers as these strains have been previously tested for their sensitivity to antibiotics (Ahmad and Arina, 2001; Aqil et al., 2005, Aqil and Ahmad, 2007; Jafri et al., 2014). Further these test isolates of bacteria were also tested for the production of β-lactamases **(Table-1).**

In the present study, *Punica granatum*

was selected on the basis of their traditional uses in treatment of different disease in India and worldwide. Only alcoholic extracts of plant material have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff,1998; Ahmad et al.,1998). Antibacterial activity of crude extracts of *Punica granatum* against Gram positive bacteria (7 distinct isolates of *S. aureus*) and Gram- negative bacteria (*E.coli*, *P. aeruginosa*) and a yeast (*C. albicans*) is presented in **(Table-1)**. Activity of ethanolic crude extracts against Gram positive bacteria and Gram negative MDR bacteria and *Candida albicans* showed broad spectrum **(Table-3)**.

Table-1 Antibiotics resistant pattern and β-lactamase production by test strains

Name of bacteria	Strains code	β-lactamase hydrolyzing β-lactam antibiotics		Resistant pattern of used strains against antibiotics
		Ampicillin	Benzyl penicillin	
Staphylococcus aureus	SA-03	+	+	Cx, M, A. Pn, Cf, Do, Sm, Na
Staphylococcus aureus	SA-08	–	–	Cx, M, A, Pn, Cf, Sm,
Staphylococcus aureus	SA-11	+	+	Pn, Am, M, S, T, Do, Na, Cu,
Staphylococcus aureus	SA-21	+	+	Cx, M, A, Pn, Cf, Do, Sm,
Staphylococcus aureus	SA-22	+	+	Sensitive to all drugs
Staphylococcus aureus	SA-28	+	+	Pn, Am, Cx, Cf, M, Pc, Kt, T, S,
Staphylococcus aureus	SA-29	+	+	Cx, M, A, P,
E.coli	UP-2556	–	–	Pn, A, Cx, Do,
E.coli	EC-14	+	+	Pn, A, Cx, M, Ce, Cfx, Cep, Cu,
E.coli	EC-20	+	+	Pn, A, Cx, M, Ce, Cfx, Cu, Va, T, E,
P. aeruginosa	*P.aeruginosa*	NT	NT	A,C,T,Na, Co, Cx, Am, M

Pn, Penicillin, A, Ampicillin; Cx, Cloxacillin, Ce, Cephotaxime; Cu, Cefuroxime; Cfx, Cefixime, Cefpodoxime; M, Methicillin; Va, Vancomycin; Nf, Nitrofurantoin, Nx, Norfloxacin, Nv, Novobiocin Co, Co-trimoxazole; Na, Nalidixic acid; T, Tetracyline; C, Chloramphenicol; Do, Doxycycline; E, Erthromycin.

Table-2 Antibacterial activity of plant extracts against Gram positive bacteria

S. No	Scientific Name (Family)	Percent Yield	Antimicrobial activity (Radius in mm) ± SD						
			SA-03	SA-08	SA-11	SA-21	SA-22	SA-28	SA-29
1.	*Punica granatum*	6.25	–	12.2 ± .25	9.33± 0.41	8.5±0 .24	18.2± 0.2	10.16± 0.2	11.33± 0.2

SD – Standard deviation

Table-3 Antibacterial activity of plant extracts against Gram negative bacteria

S. No	Scientific Name (Family)	Antimicrobial activity (Zone in mm) ± SD*							
		SM-06	SM-07	SM-08	UP 2566	EC-14	EC-20	P	CA
	Punica granatum	12.1± 0.6	15.2±0.4	9.2± 0.1	13.0 ±0.4	8.2± 0.2	8.2± 0.2	6.4±0.2	18.3± 0.1

***SD – Standard deviation**

Similar findings have been reported by Mehmood et al., 1999 and few other workers (Aqil and Ahmad, 2007). *Punica granatum* peel extracts were also evaluated for their potency in terms of Minimum Inhibitory concentration against a variety of MDR bacteria as shown in **(Table-4)**. MIC values of varied greatly from 0.41 mg/ml to 4.81 mg/ml against test bacteria. Variation in MIC values might be due to difference in cell well composition and intrinsic tolerance of the test isolates, nature and composition of phytoconstituents.

Table- 4 Activity profile of crude plant extracts of Punica granatum in terms of Minimum inhibitory concentration (MIC)

S. No	Plant Extract	Yield in mg/100 gm of dry powder	Minimum inhibitory concentration against test microorganisms (mg/ml)										
			SA						EC				
			SA-03	SA-08	SA-11	SA-21	SA-28	SA-29	EC-M	EC-14	EC-20	P	CA
1	*Punica granatum*	10.25	3.5	3.62	4.62	0.41	1.38	4.16	4.11	2.03	2.03	4.81	1.30

NT - Not tested, Organisms key : SA – ***Staphylococcus aureus*****, , EC -** ***E.coli,*** **P –** ***Pseudomonas aeruginosa*****, CA-** ***Candida albicans*** .

Phytochemcial analysis of *Punica granatum* extract was made for the presence of major phytocompounds like alkaloids, flavonoides phenols & tannins as depicted in **(Table-5)**. The presence of Catechin and epicatechin was detected in the crude extracts by HTLC as given in **(Plate- 1 and 2)**. The differences in phytocompounds might be responsible for varied activity & MIC values. These observations were supported by many workers (Nakamura et al., 2002; Shanab et al., 2004). Thus our antimicrobial screening results also justify the traditional uses of these plants in ailments and localized skin infections caused by *S.aureus, E.coli, P.aeruginosa,* and *Candida albicans*.

Table 5 Phytochemical analysis of active plant extracts for major bioactive compounds

S. no.	Plant name	Part used	Phytocompounds detected					
			Alkaloids	Flavonoids	Glycosides	Phenols	Tannins	
							Epi/ gallo	Condensed salts
1.	*Punica granatum*	Rind	+	+	–	+	+	–

HPTLC Chromatograms

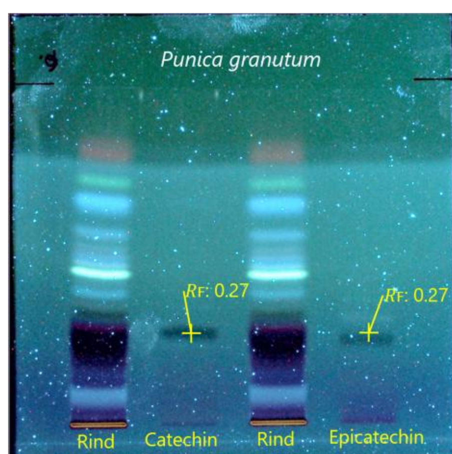

PLATE-1

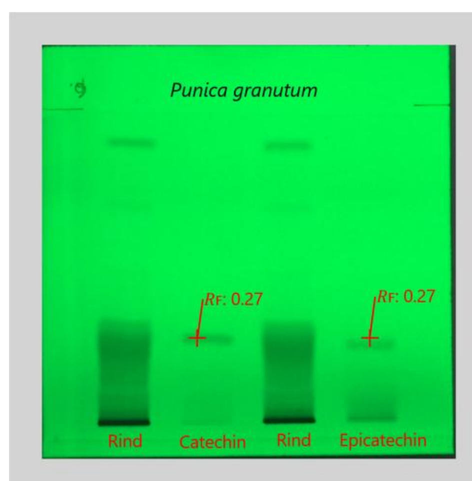

PLATE-2

Synergism in plant extracts- In the traditional system of medicine (Ayurveda and Unani-Tibbiya) formulation of herbal drugs are prepared as a mixture of many crude extracts in different preparations. It is commonly believed that various active phyto-constituents of plant extracts posses additive or synergistic activity.

Multiple antibiotic therapy is now considered an effective way to control infectious diseases caused by drug resistant bacteria. Phytocompounds which may have strong activity against antibiotic resistant bacteria is expected to give strong synergistic and additive effect with antibiotics. Considering this known fact we have tried to see the possible synergistic effect between plant extracts *Punica granatum* and antibiotics(Table-6). *The* extract showed synergistic interaction with tetracycline, chloramphenicol, ampicillin and gentamycin against mutlidrug resistant *S. aureus* (MRSA) strain. The above findings show that synergistic interactions are specific and the possible reason may be found in the interaction of different phyto-constituents with antibiotics. This result agrees with the observation of synergistic interactions of medicinal plants with chloramphenicol as reported by Lee (1998) and Aqil et al., (2005).

Table 6 Synergistic interaction between *P.granatum* extract and antibiotics

Strains Used	Plant extract (P)	r_P (in mm)	Antibiotic (A)	r_A (in mm)	Combined radius (r_P + r_A) in mm	Enlargement of Zone-size (in mm)	Synergism
SA-03	*P.granatum*	6.0 ± 0.4	C T Gm Am NA	16.8 ± 0.7 17.1 ± 0.8 14.0 ± 0.7 14.0 ± 0.2 –	25.1 ± 0.4 23.4 ± 0.5 24.5 ± 0.5 23.0 ± 0.6 –	3 – 4 3 –	+ – + + –
SA-08	*P.granatum*	7.0 ± 0.8	C T Gm Am	11.0 ± 5 12.0 ± 0.5 12.0 ± 0.5	23.1 ± 0.5 19.1 ± 0.3 22.1 ± 0.4	5 – 3 –	+ – + –

			Na Cf	10.1 ± 1.0 10.0 ± 0.3 16.2 ± 0.6	17.0 ± 0.5 17.8 ± 1.5 23.8 ± 0.6	– –	– –
SM-07	*P.granatum*	5.5 ± 0.2	C T Am Na Cf	11.3± 0.17 5.1± 0.1 8.4 ± 0.2 – 8.2 ± 0.5	16.3 ± 0.4 10.0 ± 0.3 13.6 ± 0.3 – 13.4 ± 0.7	– – – – –	– – – – –
EC-20	*P.granatum*	8.3 ± 0.3	C T Gm Am Na Cf	14.3± 0.2 16.3 ± 0.1 15.2 ± 0.3 12.1 ± 0.1 11.4 ± 0.1 20.4 ± 0.1	22.6 ± 0.5 24.4 ± 0.2 27.3 ± 0.3 20.4 ± 0.2 19.6 ± 0.2 28.4 ± 0.2	– – – – – –	– – – – – –

Conclusion

This preliminary investigation indicated that plant extracts of *Punica granatum(peel)* showing broad spectrum antimicrobial activity and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of various plants may also be exploited in preparation of herbal formulation of improved efficacy and quality.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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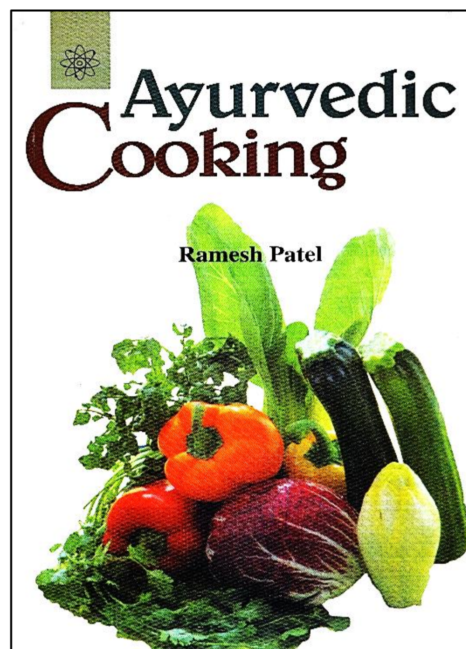
Book Review



Ayurvedic Cooking

Ramesh Patel

This books gives interesting descriptions on preparation of different dishes like carrot halva, chana coconut chutney, dahi vada, dosa, malpuhe, masalapuri raita, samosa, srikhand, vegetable biryani, lapsi, curry, idli, coconut chutney, potato onion curry, urad dal, thepla and other whose description cover 50 pages.

The author has provided easily workable method of cooking of different dishes based on Ayurveda concept.

The book act as a good guide for preparing healthy and tasteful cooking methodology of eatables to keep one healthy and happy.

Dr. I. P. Saxena
Editor (UJPAH)

About flowers on the cover page

1. *Hibiscus rosa-sinensis* (Gurhal)

Hibiscus rosa-sinensis **-Gurhal** commonly known as Chinese hibiscus or China rose, is a tropical, evergreen shrub famously cultivated for its large, showy flowers. It is a complex hybrid species that first gained botanical classification in 1753 by Carl Linnaeus in *Species Plantarum*.

Taxonomic Hierarchy

- **Kingdom:** Plantae (Plants)
- **Clade:** Tracheophyta (Vascular plants)
- **Clade:** Angiospermae (Flowering plants)
- **Clade:** Eudicots
- **Clade:** Rosids
- **Order:** Malvales
- **Family:** Malvaceae (Mallow family)
- **Subfamily:** Malvoideae
- **Tribe:** Hibisceae
- **Genus:** *Hibiscus*
- **Species:** *H. rosa-sinensis*

The specific epithet *rosa-sinensis* translates literally to "rose of China", though the plant is not a true rose and its exact native origin is believed to be the South Pacific or Vanuatu.It possesses a trait called *polyploidy*—having more than two complete sets of chromosomes. This high genetic variability allows for the random expression of ancestral traits, which is why there are thousands of cultivated, differently colored hybrids. The blooms are solitary and axillary, featuring five petals. Its defining reproductive feature is a long, prominent staminal tube where the stamens and style are fused together, with five capitate stigmas at the tip.

Hibiscus rosa-sinensis (China rose) is a tropical shrub celebrated in traditional medicine for its antioxidant, anti-inflammatory, and antimicrobial properties. Different parts of the plant, primarily the flowers and leaves, are used to manage conditions ranging from hair loss and skin ailments to cardiovascular issues and diabetes.

2. *Oxalis dehradunensis* (Dehradun wood sorrel)

Oxalis dehradunensis -(**commonly known as Dehradun Wood Sorrel**) is a bulbous, stemless perennial herb belonging to the wood sorrel family. Native to the Caribbean and widely naturalized in India (like Uttarakhand's Dehradun region) and Indonesia, it is primarily recognized by its 3-foliate leaves and pinkish-purple flowers.

Taxonomic Hierarchy

- **Kingdom:** Plantae (Plants)
- **Clade :** Tracheophytes, Angiosperms, Eudicots, Rosids
- **Order :** Oxalidales
- **Family :** Oxalidaceae (Wood sorrel family)
- **Genus :** *Oxalis* L. (Wood sorrels)
- **Species :** *Oxalis dehradunensis* Raizada

Oxalis dehradunensis Raizada—a wild mountain clover—possesses significant medicinal properties primarily driven by its rich concentration of **flavonoids**, **polyphenols**, and **tannins**. Scientific studies have validated several key pharmacological benefits:

Pain Relief & Fever Reduction (Analgesic & Antipyretic): The leaf extracts have been shown to significantly increase reaction latency and reduce elevated body temperatures, verifying traditional uses for pain and fever.

Anti-inflammatory: Studies demonstrate that ethanolic leaf extracts exhibit substantial anti-inflammatory effects.

Antioxidant & Anti-aging: The plant effectively neutralizes free radicals. When formulated into **anti-aging creams**, it significantly increases skin moisture, reduces wrinkles, and shrink’s pores.

3. *Coccinia grandis* (Kundru)

Coccinia grandis -Kundru(widely known as ivy gourd, scarlet gourd, or *tindora*) is a tropical, perennial vine belonging to the economically important **Cucurbitaceae** (gourd) family.

Taxonomic classification

- **Kingdom** : Plantae
- **Division** : Tracheophyta
- **Class** : Magnoliopsida
- **Order** : Cucurbitales
- **Family** : Cucurbitaceae
- **Genus** : *Coccinia*
- **Species** : *Coccinia grandis* (L.) Voigt

General Health Benefits

Coccinia grandis (commonly known as Ivy Gourd or *Tindora*) is a traditional medicinal plant used across tropical Asia. It is highly valued for its **anti-diabetic, anti-inflammatory, and antimicrobial properties**. Various parts of the plant, including leaves, fruits, and roots, offer multiple health benefits.

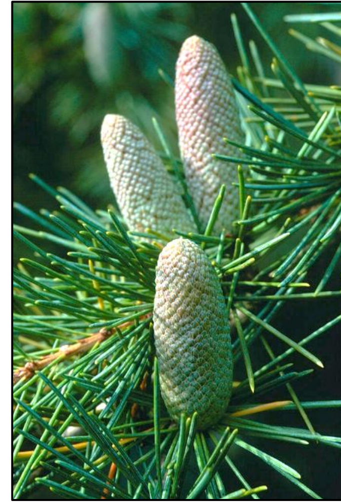
Blood Sugar Regulation (Anti-diabetic)

- **Hypoglycemic Effects:** Studies show that *Coccinia grandis* can help lower blood sugar levels. Compounds in the plant help regulate metabolic enzymes and enhance glucose uptake.]
- **Insulin Support:** It has been shown in preclinical studies to stimulate insulin secretion and promote the regeneration of pancreatic β-cells.
- **Antioxidant Power:** The plant is rich in flavonoids and phenolics, which neutralize free radicals, reduce oxidative stress, and protect against cell damage.
- **Anti-inflammatory:** Traditional use and research support its efficacy in reducing inflammation and bringing down fever (antipyretic).
- **Antimicrobial:** Leaf and fruit extracts possess antifungal and antibacterial properties, which are sometimes used topically for minor skin eruptions and wound healing.

4. *Cedrus deodara* (Deodar)

***Cedrus deodara*,-Deodar** commonly known as the **Deodar cedar** or **Himalayan cedar**, is a large, evergreen coniferous tree native to the western Himalayas. It belongs to the pine family, and is widely cultivated for its ornamental beauty and highly valued timber.

Taxonomic classification



- **Kingdom** : Plantae (Plants)
- **Subkingdom** : Tracheobionta (Vascular plants)
- **Superdivision** : Spermatophyta (Seed-bearing plants)
- **Division / Phylum** : Pinophyta / Coniferophyta (Conifers)
- **Class** : Pinopsida (Conifers)
- **Order** : Pinales
- **Family** : Pinaceae (Pine family)
- **Genus** : *Cedrus* (True cedars)
- **Species** : *Cedrus deodara*

Cedrus deodara, commonly known as Himalayan Cedar or Devadaru, is a revered medicinal tree with deep roots in Ayurvedic medicine. Its various parts—including heartwood, bark, leaves, and oil—are packed with terpenoids and flavonoids that provide potent **anti-inflammatory**, **analgesic**, **antimicrobial**, and **antioxidant** properties.

The medicinal properties of *Cedrus deodara* span a variety of health applications:

Pain & Joint Management: Its **analgesic** and **anti-inflammatory** actions make it highly effective for treating arthritis, rheumatism, and joint stiffness. The extracted oil is frequently used topically to relieve sore muscles, swellings, and inflammation.

Respiratory Relief: Deodar acts as a natural **expectorant** and **antispasmodic**. A decoction of the wood or bark is traditionally used to clear mucus, ease breathing, and treat bronchitis, asthma, and common cold symptoms.

Skin Health: The bark paste and essential oil possess **antiseptic** and **antifungal** properties, making them valuable in managing eczema, itching, psoriasis, and speeding up the healing of wounds and ulcers.

Metabolic Support: Early studies and traditional use suggest it has potential **anti-hyperglycemic** effects, which can aid in the stimulation of insulin secretion and blood sugar regulation.

General Wellness: Deodar contains compounds that act as sedatives, helping to calm the mind and manage insomnia. Its diaphoretic (sweat-inducing) nature is utilized in oils to help manage fevers.

Forthcoming events

1. **The Future of Phytochemistry – A Young Scientist Meeting (PSE), Leicester, United Kingdom**
   June 22–24, 2026
2. **International Conference on Traditional & Alternative Medicine, Chennai, India**
   June 26–27, 2026
3. **Ethnopharmacology and Pharmacognosy Conference (ICEP), Nicosia, Cyprus.**
   June 29–30, 2026
4. **2026 American Society of Pharmacognosy (ASP) Annual Meeting, Denver, Colorado, USA**
   July 26–29, 2026
5. **Ayurveda Mahasangam 2026, King George's Medical University (KGMU), Lucknow, India**
   August 21–23, 2026
6. **74th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA), Reims, France**
   August 30 – September 2, 2026
7. **Annual Meeting of the Phytochemical Society of Europe (PSE 2026), Caserta, Italy**
   September 15–18, 2026
8. **International Conference on Pharmacognosy, Phytochemistry and Natural Products, London, UK.**
   September 21–22, 2026
9. **International Conference on Pharmacognosy, Phytochemistry and Natural Products Amsterdam, Netherlands**
   November 4–5, 2026
10. **11th World Ayurveda Congress & Arogya Expo 2026, Bhubaneswar, Odisha, India**
    December 10–13, 2026

Instruction to Contributors

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3. Figures should be double spaced on separate pages, numbers consecutively in Roman numerals and placed at the end of manuscript.

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6. It is emphasized that the manuscripts should be neatly typed, double spaced throughout including tables on pages of uniform size with at least 2.5 cms margin on all sides. Author(s) should not break or hyphenate the words. Authors are required to include:
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   - iii. Complete formulation details of all crude drug mixtures.
   - iv. The herbarium specimen number of plant(s) studied in case of less well known plants be cited using the collector and collection number (eg. Doe 123) as well as indicating the names of the herbarium institution where it has been deposited.

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Prof. Durgesh Pant
Director General
UCOST, Dehardun

I am delighted to extend my warm greetings to the editorial team, contributors, and readers of the Universities' Journal of Phytochemistry and Ayurvedic Height. This journal represents an important scholarly platform dedicated to advancing research, innovation, and dialogue in the fields of phytochemistry and Ayurveda two domains that hold immense significance for scientific progress, healthcare, and sustainable development.

Uttarakhand, with its rich biodiversity and centuries-old traditions of herbal knowledge, offers unparalleled opportunities for research on medicinal plants, natural compounds, and traditional healing systems. I am pleased to note that this journal is actively contributing to the integration of modern scientific inquiry with the wisdom of Ayurveda, there by encouraging evidence-based understanding and wider applications of natural products for human well- being.

UCOST remains committed to supporting and promoting high-quality research that strengthens India's leadership in phytochemical and Ayurvedic sciences. Initiatives such as this journal help nurture young researchers, disseminate valuable scientific findings, and foster collaborations across universities, research institutions, and industry.

I commend the editorial board, reviewers, and authors for their dedication in maintaining academic rigor and for their efforts in bringing out this publication. I am confident that the Universities' Journal of Phytochemistry nd Ayurvedic Height will continue to grow as a respected academic resource and inspire further innovations in these vital fields.

I extend my best wishes for the continued success of the journal.

(Prof. Durgesh Pant)
Director General