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EDITORIAL

The authentication of herbal species is of great concern for the quality control of herbal products.

Herbal authentication is a quality assurance process that ensures the correct herbal species and plant parts are used as raw materials for herbal products. Herbal medicines and plant-based supplements are widely used across the world for their therapeutic benefits. However, the authenticity of herbal raw materials has become a major concern due to increasing cases of adulteration, substitution, and contamination. Ensuring correct species identification is essential for safety, efficacy, and quality control. Traditional identification methods often fall short, if standardized with Macroscopic (organoleptic tests), Microscopic evaluation, Chemical finger printing, Chromatographic evaluation through (HPTLC, HPLC, GC &LC-MS etc) especially when herbs are dried, powdered, or processed. Now DNA barcoding has emerged as a powerful molecular tool to address these challenges.

DNA Barcoding: A Modern Approach

DNA barcoding uses short, standardized DNA sequences to identify plant species accurately.

DNA barcoding has become an essential tool for ensuring the authenticity and safety of herbal products. By complementing traditional identification methods, it provides a reliable, scientific approach to species verification. As global demand for herbal medicines continues to rise, integrating DNA barcoding into routine quality control will strengthen consumer confidence and promote sustainable use of medicinal plant resources. This issue contains DNA Bar coding of *Moringa oleifera* (Curry patta). AI is revolutionizing drug discovery in herbal pharmaceutical by enhancing the efficiency and speed of the processes involved in creating novel herbal drugs.

I am grateful to the UJPAH board members to make this issue a memorable for science fraternity of the Uttarakhand and to all those scientists, research scholars, students and teachers who contributed for bringing out this issue and the people of science at large. Before concluding, I express my special gratitude to our chief guests, special and other guest present and the management who have provided all the guidance. May almighty bless you.

Dr S Farooq
Chief Editor

Contents

Comparative Account of the Antioxidant Property between Mature and Young Leaves of *Murraya Koenigii*

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Abstract- In this study, a comparative analysis of antioxidant content and radical scavenging activity between young and mature leaves of *Murraya koenigii* was conducted. The findings clearly indicate that maturity significantly influences the phyto-chemical profile of the leaves. Mature leaves were found to contain a higher total antioxidant content compared to young leaves, suggesting a more developed secondary metabolite profile with age. Furthermore, radical scavenging assays demonstrated that mature leaves exhibited superior free radical neutralizing ability, confirming their potential as a more effective natural antioxidant source. These results highlight the importance of leaf maturity in determining the medicinal and nutraceutical value of *Murraya koenigii*. The enhanced antioxidant properties in mature leaves could be leveraged in the development of plant-based health supplements and functional foods. Future studies may further explore the specific compounds responsible for this variation and assess the bioavailability and efficacy of extracts in vivo.

Key words: Young, Mature, Antioxidant, Neutraceuticals and Scavenging.

Introduction

Murraya koenigii, commonly known as **curry leaf** or **karipatta**, belongs to the family **Rutaceae**, is a small, tropical to sub-tropical tree or shrub that typically grows to 6-15' feet tall and is noted for its pungent, aromatic, curry leaves which are an important flavoring used in Indian/Asian cuisine. Yellow curry powder (developed by the British during the time of their colonial rule in India) is a blend of many different Indian spices, one of which is sometimes (but not always) curry leaf[1-4]. People generally use the fresh leaves, dried leaf powder and essential oil for flavouring soups, curries, fish and meat dishes, egg dishes, traditional curry powder blends etc. The aromatherapy industry uses the essential oil in the making of soaps and cosmetics[5-7]. For natural hair tone and hair growth, one can use the blanked residue of boiled curry leaves along with coconut oil[8-10]. It can be used as antihelmetics, it also acts as febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain and vomiting [11-15]. *Murraya koenigii* is used as a stimulant and anti-dysenteric. It is also effective against diabetes Mellitus[16-19]. Leaves are applied externally to bruises and eruption[20].

The leaves and roots are bitter in taste analgesic, cure inflammation and itching[21-22]. It is also useful in leucoderma and blood disorders and also cures diseases like piles[23-24]. It can be also used to stop vomiting[25-26] by infusion of the toasted leaves. If someone is bitten by poisonous animals, local application of the leave paste is effective[27-29]. The essential oil from *M. koenigii* leaves showed antibacterial effect against *B. subtilis, Staph. aureus, C. pyogenes, P. vulgaris* and *Pasteurella multicida*[30-31]. Acetone extract of *M. koenigii* is active against *Aspergillus niger*, benzene extract is most active against *Penicillum notatum*. The literature showed the antioxidative properties of the extract of *M. koenigii* leaves were done using different solvents. Alkaloid Koenoline isolated from the root bark of *M.koenigii* is found to exibit cytotoxic activity against KB cell culture system. The alcohol extract of stem bark (1 gm/kg body weight) is effective against carrageenan-induced inflammation. Crude root extract also showed anti-inflammatory activity[32]. Bioactive alkaloids, kurryam and koenimbine obtained from fractionated n-hexane extract of the seeds of *M. koenigii* were found to exhibit inhibitory activity against castor oil-induced diarrhoea and prostaglandin[33, 34].

Aim and Objectives

To do the comparatively analyse the antioxidant levels in mature and young leaves of the curry plant (*Murraya koenigii*), thereby determining the variations in antioxidant content during different stages of leaf development.

Objectives include: (i)To extract and quantify the antioxidant compounds present in both mature and young leaves of the curry plant.

(ii) Successive extraction and qualitative analysis.

(iii) **Characterization**: Evaluation of the influence of leaf maturity on the antioxidant potential will be processed using in vitro biochemical assays such as DPPH.

Material and Methodology

Collection of Plant Material

Two different types of leaves i.e., mature and young leaves of *Murraya koenigi* were collected from the locality of Balawala, Dehradun, Uttarakhand in the month of January 2025 and were identified and authenticated by the Botanical Survey of India, Dehradun. The leaves were then shade dried for few days until there is no moisture left in them.

Chemicals

1,1-diphenyl-2-picrylhhydrazyl (DPPH), Ascorbic acid (standard), Petroleum Ether, Chloroform, Methanol and Water. All the reagent and solvent sused were of AR grade.

Extraction

Curry leaves (50g) were crushed and were extracted again and again via Soxhlet method using 250 ml of Petroleum Ether, Chloroform, and Methanol. The leaves were then macerated in 500ml water for 48 hours and then sample was put into orbital shaker at room temperature and 150 RPM for 48 hours. Afterwards the sample was extracted in Rotary Vacuum Evaporator. The extracts after removal of solvents were stored at 4°C until used for antioxidant assay. The efficacy of

extracts was quantified based on the dry weight of the whole extract per volume of assay solution.

Fig 1 : Soxhlet Apparatus

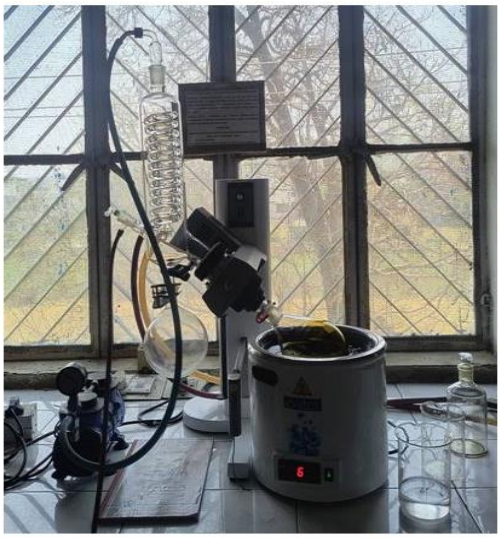

Fig 2 : Rota evaporator

Distillation

The Solvents Petroleum Ether, Chloroform and Methanol for extraction of Curry Leaves were recycled using Distillation unit for further laboratory use.

Fig 3 :Distillation Unit

Fig 4 : Orbital Shaker

DPPH Spectrophotometric

Each extract stock solution (1mg/10ml) was diluted to final concentration ranging from (20-100µg/ml) in methanol 2 ml of DPPH solution was added to 1ml of extract solutions of different concentrations and allowed to

react at room temperature in dark condition. After 30 min., the absorbance was measured at 517 nm and the percentage scavenging capacity was calculated[34].

Fig 5 : DPPH Stock Solution and Micropipette

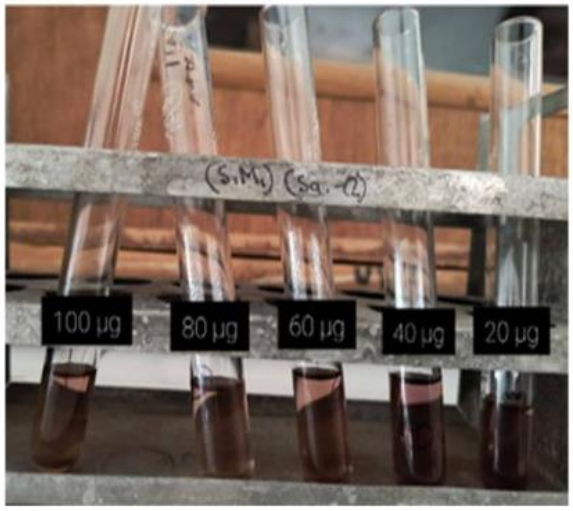

Fig 6 : DPPH Plant Extracts

Estimation of free radical scavenging activity

The ability to scavenge 1, 1-diphenyl 1-2- picrylhydrazyl (DPPH) radical by curry leaves extracts was estimated by the method Negi and Jayaprakasha[35- 36].

Yield- Maximum yield was obtained by Soxhlet extraction with Methanol and lowest yield was with Petroleum Ether in both mature and young leaves.

Result and Discussion

Table-1 Yield of Extracts

Extract	Yield Percentage	
	Mature	Young
Petroleum Ether	0.716%	4.9%
Chloroform	1.8%	5.6%
Methanol	13.4%	25%
Water	10.13%	14.27%

Table-2 In Vitro Anti-oxidation Activity

Ascorbic acid (Dilution)	% RSA
1µg/ml	2.86
5 µg/ml	12.86
10 µg/ml	25.71
15 µg/ml	40.00
20 µg/ml	50.00

Table-3 % RSA of crude Extract of Mature leaves

Dilution	%RSA of Extracts			
	Petroleum Ether	Chloroform	Methanol	Water
20 µg/ml	6.40	12..0	49.60	35.24
40 µg/ml	11.60	18.5	55.34	42.45
60 µg/ml	14.20	27.0	61.73	50.84
80 µg/ml	22.00	35.5	67.89	59.23
100 µg/ml	29.00	47.0	73.44	66.4
150 µg/ml	37.00	62.0	88.66	82.04
200 µg/ml	46.00	82.5	104.03	97.85

250 µg/ml	58.00	93.0	119.9	113.09

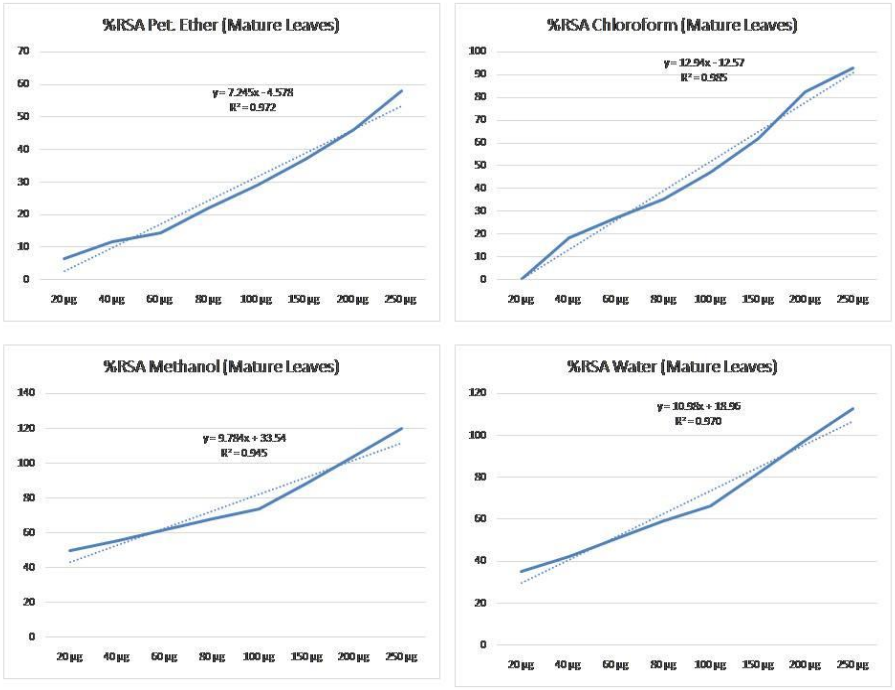

Figure-7% RSA Graphs of Various Extract of Mature Leaves of *Murraya koenigii*

Table-4 Absorbance of Crude Extracts of Young Leaves

Dilution	% RSA of Extracts			
	Petroleum Ether	Chloroform	Methanol	Water
20 µg/ml;	2.50	10.075	26.849	25.659
40 µg/ml	6.80	14.845	32.887	30.456
60 µg/ml	10.50	22.04	38.925	36.443
80 µg/ml	15.60	30.859	46.052	44.390
100 µg/ml	20.30	42.06	59.179	55.63
150 µg/ml	28.00	53.048	68.529	64.52
200 µg/ml	35.00	65.997	83.549	80.21
250 µg/ml	42.00	79.157	99.179	95.63
300 µg/ml	50.20	94.377	114.599	111.05

Figure- 8% RSA Graphs of Various Extract of Young Leaves of *Murraya koenigii*

Scavenging Activity

The DPPH radical scavenging activity of both young and mature *Murraya koenigii* leaves increased in a dose-dependent manner. Mature leaf extracts showed significantly higher DPPH scavenging activity at all tested concentrations compared to young leaf extracts.

At a concentration of 100 µg/mL, the mature leaves exhibited a maximum scavenging activity in all four solvents, whereas young leaves showed Y% (insert your value). The IC value for mature leaves were found to be **216.67 µg/mL** , **138.6 µg/mL, 47.08 µg/mL** and **58.01 µg/mL** which was lower than that of young leaves which were **298.78 µg/mL**,**153.274 µg/mL** , **87.64µg/mL** and **101.042 µg/mL** for **Petroleum Ether, Chloroform , Methanol and Water** respectively indicating stronger antioxidant potential in mature leaves. These results suggest that leaves of *Murraya koenigii* possess higher free radical scavenging activity, possibly due to increased accumulation of antioxidant phytochemicals such as phenolics and flavonoids with age.

Phytochemical Analysis

Table-5Phytochemical analysis of old leaves in different solvents:

Solvent	Saponin	Tannins	Steroid	Alkaloids	Flavonoids
Petroleum Ether	-	+	+	-	-
Chloroform	-	-	+	+	+
Methanol	-	+	+	-	-
Distilled Water	-	-	+	-	+

*__Note__: **(+)** : Present , **(-)** Absent

Table-6 Phytochemical analysis of young leaves in different solvents

Solvent	Saponin	Tannins	Steroids	Alkaloids	Flavonoids
Petroleum Ether	-	+	+	-	+
Chloroform	-	-	+	+	-
Methanol	-	+	+	-	-
Distilled Water	-	-	+	-	+

*__Note__: **(+)** : Present , **(-)** Absent

Discussion

The present study compared the antioxidant activity of young and mature leaves of *Murraya koenigii*. It was observed that leaves showed higher antioxidant activity compared to young leaves based on the DPPH assay. The increased antioxidant activity in leaves may be due to the higher concentration of phenolic compounds and flavonoids. As the leaf matures, it is exposed to more environmental stressors, which may trigger increased biosynthesis of protective antioxidant molecules. These results align with a previous study[37], who reported higher antioxidant content in mature leaves of medicinal plants. However, another study[38] found higher antioxidant activity in younger neem leaves, indicating that age-related trends may vary by species. The findings suggest that mature curry leaves may be a more effective source of antioxidants for use in food and pharmaceutical applications. One limitation of the study is that it only used in vitro assays and did not consider seasonal variations. Also, detailed compound-specific analysis was

not performed.

Conclusion

In this study, a comparative analysis of antioxidant content and radical scavenging activity between young and mature leaves of *Murraya koenigii* was conducted. The findings clearly indicate that maturity significantly influences the phytochemical profile of the leaves. Mature leaves were found to contain a higher total antioxidant content compared to young leaves, suggesting a more developed secondary metabolite profile with age. Furthermore, radical scavenging assays demonstrated that mature leaves exhibited superior free radical neutralizing ability, confirming their potential as a more effective natural antioxidant source. These results highlight the importance of leaf maturity in determining the medicinal and nutraceutical value of *Murraya koenigii*. The enhanced antioxidant properties in mature leaves could be leveraged in the development of plant-based health supplements and functional foods. Future studies may further explore the specific compounds responsible for this variation and assess the bioavailability and efficacy of extracts in vivo.

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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Chemical constituents from the leaves of *Sarcococca saligna*

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Abstract- From the Methanolic extract of the leaves of *Sarcococca saligna* 7, 4'-dihydroxy-6,3'-dimethoxy flavanone and 3, 5, 7-trihydroxy- 6, 8- dimethoxy flavanone have been isolated. The structures of the isolated compounds were identified with the help of chemical and spectral studies.

Key words: Sarcococca saligna, Buxaceae, flavanone glycosides.

Introduction

Sarcococca saligna is an evergreen shrub with a slender, arching habit, known for its narrow, willow-like leaves and fragrant, creamy-white flowers in late winter. The flowers are followed by small, purple berries, and the plant is shade-tolerant, making it ideal for shady spots, low hedges, or as a filler plant. The leaves of *Sarcococca saligna* are used as laxative, blood purifier and muscular analgesic[1-2].The species is native to parts of the Indian subcontinent and East Asia, specifically found in areas such as the Himalayas, Pakistan, Nepal, China, and Taiwan. It typically grows in the undergrowth of subtropical and temperate forests at elevations between 1200 and 3500 meters.

The members of Sarcococca plants are used as TCM and traditional folk medicine for the treatment of stomach pain, rheumatism, swollen sore throat and traumatic injury. The ethanolicextract of *S. saligna indicate anti-* bacterial activity against several humanpathogenic bacteria and anti-fungal against Aspergillus species[3-4]. The extract of the plant is also used to increase the antifungal activity of fluconazole against resistant *Aspergillus* species. The present paper deals with isolation and identification of secondary metabolites from the methnolic extract of the leaves of the plant.

Material and Methods

Melting points were recorded on a perfit melting point apparatus. UV spectra on perkin-Elmer, Lambda-25 spectrometer (methanol as solvent), IR spectra recorded on Perkin-Elmer, spectrum RX I FT-IR spectrometer (KBr discs). NMR spectra were obtained on JEOL NMR spectrometer (300 MHz for ^{1}H and 125 MHz for ^{13}C,

DMSO-d_6 as solvent, TMS as internal standard). LCMS spectra were recorded on Finniagan MAT spectrometer (CA, USA, xcalibur ver-2 software). Column chromatography was performed on silica gel (Merck 60-120 mesh, 15×100 cm). Thin layer chromatography was carried out on silica gel (Merck 10-40 µm) precoated plates were visualized by spraying with 7% H_2SO_4 as a universal spray reagent.

Plant Material

Fresh flowers (10kg) of*Sarcococca saligna* were collected from Nandanagar Chamoli (Garhwal) UK., during March 2025 and identified by Taxonomist (Department of Botany H.N.B. Garhwal University Srinagar).

Extraction and Isolation

Air dried powdered leaves were extracted exhaustively with 95% ethanol at 30-50˚C (for 15 hours, 3 times) on a heating mantle. The solvent was distilled on water bath and evaporation of solvent under reduced pressure afforded (250g) crude extract, which was subjected to column chromatography on silica gel column using solvent gradient systems in the order of increasing polarity $CHCl_3$: MeOH (97:3-85:25). The fractions obtained from column were collected (100ml) and combined on the basis of TLC analysis. The $CHCl_3$: MeOH (97:3-85:25) afforded R-1 and R-2. These fractions were concentrated at room temperature.

R-1 -White amorphous powder (0.252 g); mp. 120-123 ˚C; M.F. $C_{17}H_{16}O_6$; IR: 3560, 1650, 1715, 1725 cm^{-1}; ^1H, ^{13}C and 2D NMR data: Table 4.1; LCMS(m/z): 316 $[M]^+$, 286[M-OCH_3]; 270 [M-OCH_3+OH]; 240[M-2×OCH_3+OH]; 224 [M-2×OCH_3+2×OH]; 148 [M-2×OCH_3+2×OH+benzyl].

Table-1^{13}C (125 MHz), ^1H (300 MHz) NMR and HMBC data of R-1 in DMSO d^6:

Position C/H	$\delta_C$ppm	$\delta_H$ppmJ(Hz)	HMBC
2	78.90	5.46(dd,12.0,4.0)	C-3,C-2',C-6'
3	42.36	2.73(dd,16.0,4.0)	-
		3.23(dd,12.0,16.0)	-
4	197.27	-	-
4a	103.09	-	-
5	112.89	7.50(d,9.0)	-
6	146.95	-	-
7	167.89	-	-
8	98.09	6.80(d,2.0)	C-8a
8a	163.25	-	-
1'	135.43	-	-
2'	114.35	6.94 (d,2.0)	C-6',C-3',C-2
3'	163.67	-	-
4'	149.03	-	-
5'	107.05	7.02(d, 7.0)	-
6'	119.29	6.90 (dd,7.0,2.0)	-
OCH_3-3'	56.14	3.38s	C-2',C-3'
OCH_3-6	56.63	3.28s	C-5, C-6
OH-4'	-	9.20s	C-4'
OH-7	-	12.09s	C-7

R-2 - White amorphous powder, m.p.272-274˚C; M.F. $C_{17}H_{16}O_7$; IR:

3410, 1650, 1715 and 1725 cm^{-1}; ^{1}H, ^{13}C and 2D NMR data: Table 2; LCMS (m/z): 332 $[M+H]^{+}$, 302 $[M-OCH_3]$, 286 $[M-OCH_3+OH]$, 256 $[M-2\times CH_3+OH]$, 240 $[M-2\times OCH_3+2\times OH]$, 224 $[M-2xOCH_3+3\times OH]$ and148 $[M-2\times OCH_3+3\times OH+benzyl]$.

Results and Discussion

R-1 - It was crystallized from chloroform-methanol as white amorphous powder; m.p. 120-123°C. It gave green colour with alcoholic ferric chloride indicating the presence of phenolic hydroxyl group. An absorption maximum at 287 nm in the UV spectrum was indicative of flavanone derivative. The UV absorption band at 287 (bathochromic shift 10 nm) indicated substitution at 6 and 7 position in ring A [5-6]. After addition of NaOMe in methanolic solution of R-1, a bathochromic shift of 15 and 10 nm in band I and II was observed of reduced intensity indicated substitution at C-3' and C-4' positions, whereas bathochromic shift of 14 nm after the addition of NaOAc indicated hydroxyl group present at C-7. The IR spectrum exhibited bands at 3560 cm^{-1} characteristics of hydroxyl group, 1650 cm^{-1} for aromatic ring and a band at 1715 and 1725 cm^{-1} was clearly expressive for the stretching in a carbonyl group. The LCMS of compound showed molecular ion peak at m/z 316 $[M^{+}]$, other fragmentation peaks observed at m/z 286$[M-OCH_3]$; 270 $[M-OCH_3+OH]$; 240$[M-2\times OCH_3+OH]$; 224 $[M-2\times OCH_3+2\times OH]$; 148 $[M-2\times OCH_3+2\times OH+benzyl]$ and the two prominent Retro-Diels-Alder fragmentation peaks at m/z 162 and 154 respectively. These peaks were indicative of the nature and number of the substituents in the ring A and B (monohydroxy and monomethoxy). The ^{1}H- NMR spectrum showed one proton signal at δ 12.09 confirming the presence of a hydroxyl group at C-7. Two double doublets at δ 2.73 (dd, J=16.0, 4.0 Hz) and δ 3.23 (dd, J=12.0, 16.0 Hz) a proton as double doublet at δ 5.46 (J=12.0, 4.0 Hz, H-2) as expected for the presence of C-3 and C-2 protons of flavanone were also observed in the ^{1}H-NMR spectrum. The ^{13}C NMR spectrum of the compound showed that the C-2 and C-3 atoms appeared at δ 78.90 and 42.36. Two proton signals for one proton each which appeared at δ 7.02 (d, J=7.0 Hz) and δ 6.90 (dd, J=7.0, 2.0 Hz) were assigned to the H-5' and H-6' while a doublet at δ 6.94 (1H, J=2.0Hz) was also attributed to H-2'. A singlet integrating of one proton at δ 9.20 was assigned to the H-4' proton of ring B. ^{13}C-NMR spectrum further indicated that the carbon of carbonyl function at C-4 appeared at δ197.27. In HMBC spectrum H-3 proton showed the correlations with C-2 and C-4. The H-8 proton showed its multiple cross peaks with C-7, C-8 and C-4a carbon atoms while the hydroxyl function attached to C-7 also showed its correlations with C-5, C-6 and C-8 in HMBC spectrum. All these confirmed by ^{1}H-^{13}C and HMBC spectral data. On the basis of spectral data (IR, UV, ^{1}H, ^{13}C, 2D-NMR and MS) and the data reported in the literature [7], it was characterized as **7,4'-dihydroxy-6,3'-dimethoxyflavanone** (Fig.1).

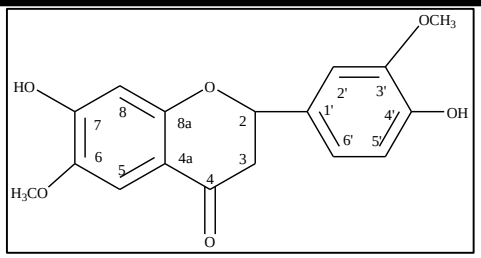

Figure-1

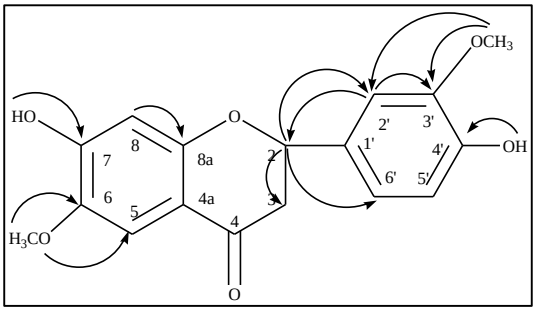

Figure- 1a. ^{1}H-^{13}C HMBC correlation of R-1

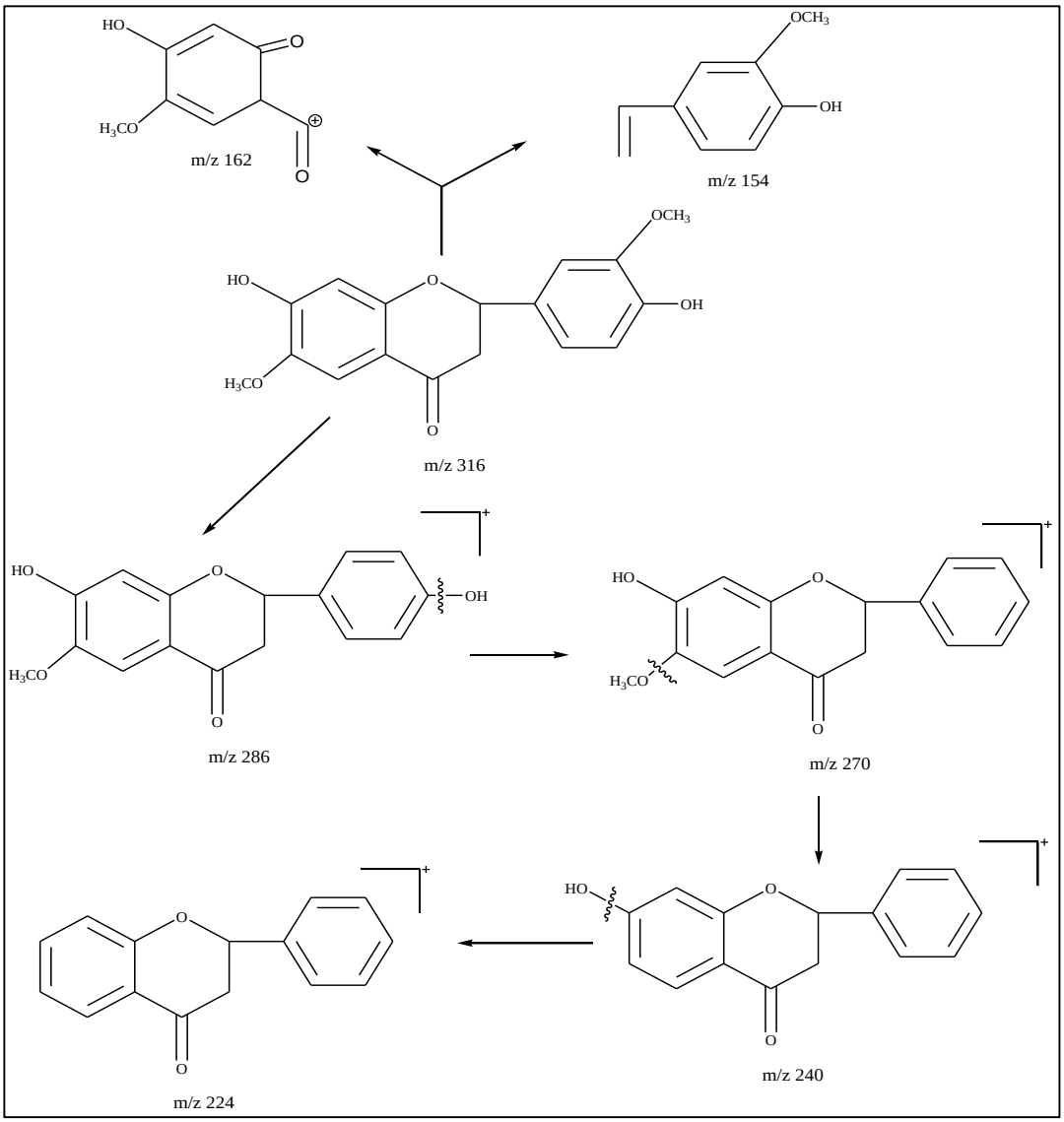

Figure-1b. Proposed mass fragmentation of R-2

Compound R-2 was crystallized as white amorphous powder from $CHCl_3$: MeOH, m.p. 272-274°C. Its molecular formula deduced as $C_{17}H_{16}O_7$ by molecular ion peak at m/z (332.304) in its LCMS. Bathochromic shift of band I by 27 nm in the UV spectrum R-2 in the presence of $AlCl_3$/HCl confirmed the presence of hydroxyl function at C-5 and other at C-3[8]. The C-6 and C-8 position was blocked by methoxyl function was also evidenced from negative response towards gossepetone test, by the parent compound as well as the characteristic mass spectral fragmentation pattern[9]. Batho-chromic shift of 14 nm after the addition of NaOAc indicated free hydroxyl group present at C-7. The IR spectrum exhibited bands at 3410 cm^{-1} characteristics of hydroxyl group, 1650 cm^{-1} for aromatic ring and a band at 1715 and 1725 cm^{-1} was clearly expressive for the stretching in a carbonyl group. The appearance of intense green colour with ferric chloride imparted by the parent compound, locates one of the hydroxyl groups at C-5 position as also revealed from its IR and ^{1}H-NMR spectra. In the LCMS spectrum, the molecular ion peak was observed at m/z 332[M^+] and other fragmentation peaks were observed at 302 [M-OCH_3], 286 [M-OCH_3+OH], 256 [M-2×CH_3+OH], 240 [M-2×OCH_3+2×OH], 224 [M-2×OCH_3+3×OH] and148 [M-2×OCH_3+3×OH+benzyl]. These peaks are indicative of the nature and number of the substituents in the ring A (dihydroxy and dimethoxy) and ring C (monohydroxy). The ^{1}H-NMR spectrum showed one proton signal at δ 12.08 confirming the presence of a hydroxyl group at C-7. Two double doublets at δ 2.73 (dd, J=16.0, 4.0Hz) and δ 5.47 (J=12.0, 4.0 Hz, H-2) as expected for the presence of C-3 and C-2 protons of flavanone were also observed in the ^{1}H-NMR spectrum.

^{1}H NMR spectrum furnished three doublets at δ_H 7.53 (2H, dd,J=8.0, 2.0 Hz, H-2',6'), δ_H 7.41 (2H, d,J=8.0, 2.5Hz, H-3',5') and δ_H 7,39 (1H, d,J=8.0Hz, H-4'). Two singlets each for three protons at δ3.78 (3H) and δ3.65 (3H) specified the presence of two methoxyl groups assigned for H-6 and H-8 which were further authenticated by their ^{13}C-chemical shifts at δ56.11 and δ56.37[3]. A singlet integrating of one proton at δ 9.20 was assigned to the H-5 and other at δ6.44(1H, s) due to free phenolic hydroxyl group of ring A. The downfield singlet at δ167.89 and 197.23 in the ^{13}C NMR spectrum were assigned to C-5 and C-4. All these confirmed by ^{1}H-^{13}C and HMBC spectral data (**Table-2**). On the basis of spectral data (IR UV, ^{1}H, ^{13}C, 2D-NMR and MS) and the data reported from literature. The structure of R-2 was characterized as **3,5,7-tri-hydroxy-6,8-dimethoxyflavanone** (**Figure-2**).

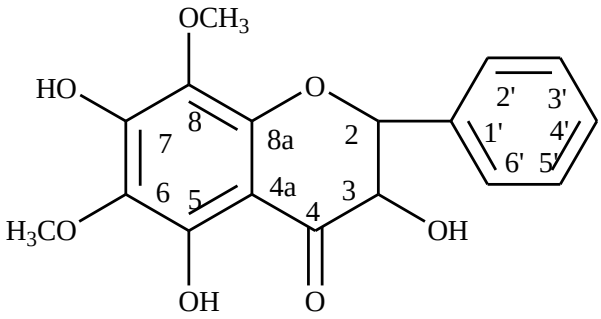

Figure-2

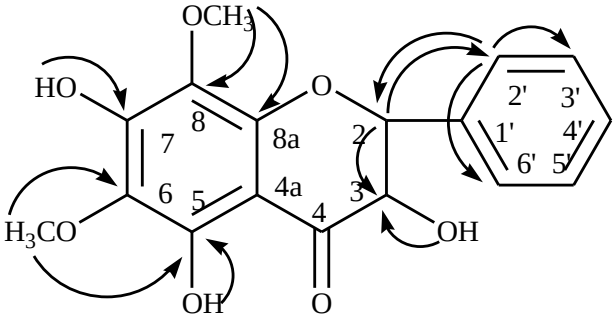

Figure- 2a

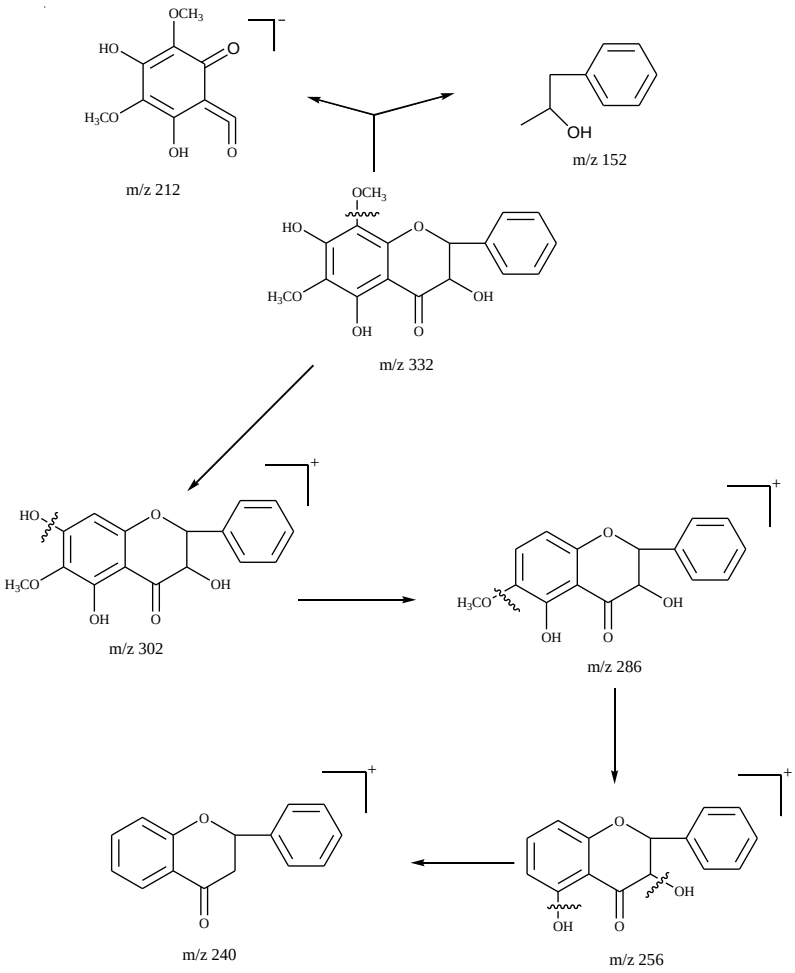

Figure- 2b. Proposed mass fragmentation of R-2

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 Evaluation of Solvent Extraction on the Phytochemical Profile and Antioxidant Potential of *Moringa oleifera* leaf extracts

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Abstract - Antioxidants are molecules that prevent or counteract the harmful effects of reactive oxygen species and free radicals, which are unstable molecules produced during metabolic processes or from external factors such as pollution, UV radiation, and smoking. These reactive molecules induce oxidative stress, resulting in cellular damage and DNA mutations, and play a role in aging, inflammation, and chronic diseases like cancer, diabetes, and cardiovascular disorders. Antioxidants mitigate this damage by donating electrons to free radicals, stabilizing them and preventing further oxidative damage chain reactions. Antioxidants sourced from plants, especially fruits, vegetables, and herbs, are increasingly recognized for their natural effectiveness and possible health advantages. *Moringa oleifera* is known for its numerous pharmacological attributes, with antioxidant activity being prominent. In this study, the antioxidant properties of *Moringa oleifera* were studied and evaluated using DPPH Scavenging Activity. IC5050 value for different solvents i.e., 33.96 µg/mL for methanol, 41.7 µg/mL for ethanol, and 146.9 µg/mL for chloroform, which indicated ethanolic extract showed good scavenging activity. The results were found to be aligned with previous studies on the antioxidant properties of *Moringa oleifera*.

Keywords: Antioxidants, DPPH Scavenging Activity, Free radical, Ascorbic acid

Introduction

Moringa oleifera is a tropical tree indigenous to the lower parts of the Himalaya ountain range. It is now spread throughout tropical regions in Africa, Asia, and Southern-America[1].The tree is one of the 13 known species of the single genus Moringaceae family, classified in the order of Brassicales[2]and has earned the name ‘miracle’ tree due to its versatile properties.

The bark, sap, roots, leaves, seeds, and flowers are used in traditional medicine[3-4]. Research has examined how it might affect blood lipid profiles and insulin secretion[5]. Extracts from leaves contain various polyphenols, which are under basic research to determine their

potential effects in humans[6]. Despite considerable preliminary research to determine if moringa components have bioactive properties, no high-quality evidence has been found to indicate that it has any effect on health or diseases[5].Oxidative stress seems to play a significant role in many human diseases, including cancers[7], and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases[8], as well as inflammation and problems caused by cell and cutaneous ageing[9]. Antioxidants play a crucial role in neutralizing free radicals, reducing oxidative stress, and preventing cellular damage linked to chronic diseases such as cardiovascular disorders, diabetes, and cancer. As a consequence of action of antioxidants against freeradicals, the cellular components are prevented from damage[10].*Moringa oleifera*, has well-documented antioxidant properties due to its rich bioactive compound profile, which includes vitamins, polyphenols, and flavonoids. *Moringa oleifera* leaves are rich in polyphenols and flavonoids, both of which have potent antioxidant effects. These compounds scavenge free radicals, inhibit lipid peroxidation, and enhance the body's natural antioxidant enzymes (such as catalase, superoxide dismutase, and glutathione peroxidase), which collectively protect cells from oxidative damage[11]. *Moringaoleifera* contains high levels of Vitamin C and beta-carotene, which play a crucial role in reducing oxidative stress by scavenging free radicals.

Aim and Objective

Aim- In vitro evaluation of antioxidant potential of *Moringa oleifera* leaves.

Objective- To achieve the above aim the research project have been divided into following objectives:

1. To evaluate the antioxidant activity of *Moringa oleifera* leaves through extraction and assessment of free radical scavenging activity using standard in vitro assays.

2. In vitro **evaluation of** the antioxidant activity of the extracts was done using as DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

3. Evaluating antioxidant activity of *M. oleifera* leaves and explore their potential in preventing or managing diseases like cancer, diabetes, and cardiovascular issues.

Material and Methodology

Collection of Plant Material

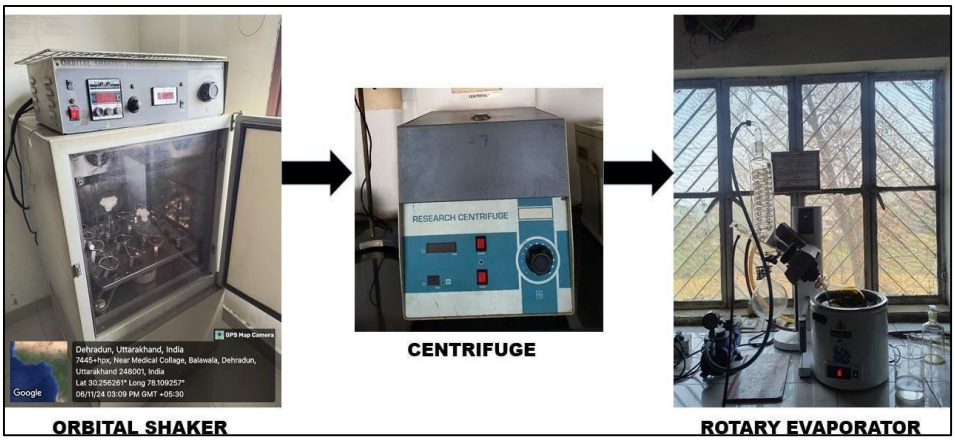
Leaves of *Moringa oleifera*were procured from the locality of Balawala, Dehradun, Uttarakhandin the month of January 2024. A selected plant was identified and authenticated by the Botanical Survey of India, Dehradun and voucher specimen was deposited at the herbarium. The leaves were shade dried for few days until there was no moisture left in them then crushed by hands for further extraction.

Extraction

An orbital shaker was used for extraction preparation. Dry grounded Moringa leaves were mixed with 200 mL of different solvents i.e., ethanol, methanol, chloroform and petroleum ether and placed on the shaker for 24 hrs at 100 rpm. Samples were then centrifuged (MPW®-352R, Med. Instruments) at 5,000 rpm for 15 min. The supernatant of the samples was again filtered with Whattman filter paper, and the filtrates obtained were

carried to a rotary evaporator for evaporation of alcohol under reduced pressure at 50°C, 120 rpm. The extracts obtained were stored at 4°C until further analysis[12].

Figure-1 Instrumentation of extraction process



DPPH Spectrophotometric assay

Series of dilutions of the methanolic extracts ranging from 20-100µg/ml was prepared from stock solution (1mg/mL). A measure of 1 ml of the extract was mixed with 3 ml of 0.3 mg/ml of DPPH radical. The mixture was shaken vigorously and allowed to stand for 30 minutes at room temperature under dark conditions. The absorbance of the mixture was read at 517 nm using UV-Vis spectrophotometer. The absorbance of the resulting solution was converted into a percentage of antioxidant activity (% inhibition) by the use of the following formula:

% Inhibition = (Ao – A1) /Ao× 100

Where Ao = Absorbance of the control solution containing only DPPH solution; A1 = Absorbance in the presence of extract in DPPH solution. Ascorbic acid was used as a reference standard. The IC50 values were determined from extrapolation curves of the antioxidant activity of extracts on DPPH[13].

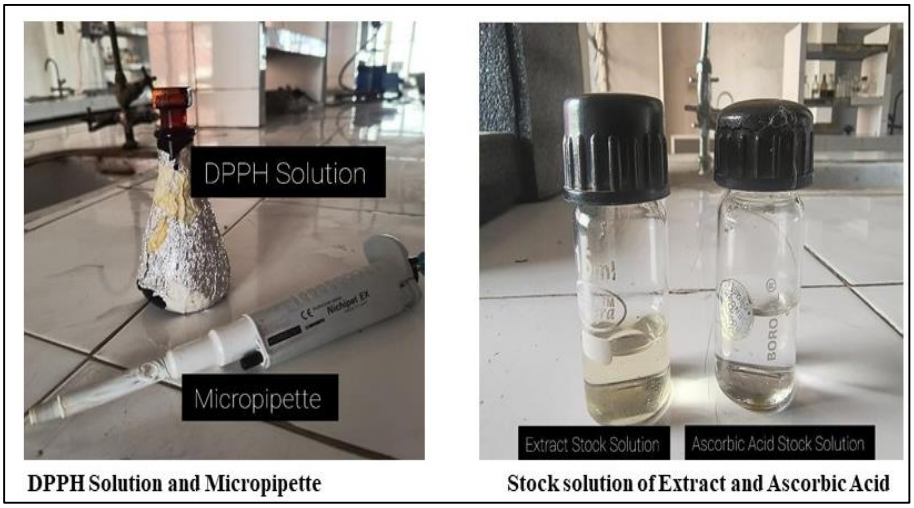


Figure-2 DPPH solution and stock solution

Phytochemical Screening

Qualitative phytochemical analysis of

the crude extracts was performed using standard protocols to detect the presence of tannins, saponnins, flavonoids, steroids, terpenoids, and alkaloids.

Result and Discussion

Extraction Yield

The percentage yield of the extracts increased with solvent polarity: chloroform (5.75%), methanol (9.63%), and ethanol (12.7%).

Table-1 Percentage yield of extracts

Extract	Percentage yield
Chloroform	5.75%
Methanol	9.63%
Ethanol	12.7%

Phytochemicals analysis

The qualitative phytochemical screening (**Table-1**) indicated that methanol and ethanol extracts contained a wider range of phyto-constituents, including tannins, saponins, flavonoids, and alkaloids. The chloroform extract showed a more limited profile.

Determination of antioxidant activity

All extracts demonstrated concen-tration dependent DPPH radical scavenging activity (**Table-2**). The methanolic extract exhibited the highest activity (89.1%) inhibition at 100 µg/ mL, followed by the ethanolic (82.4%) and chloroform (39.2%) extracts. The IC50 values (**Table-3**) confirmed the superior efficacy of the methanolic extract (IC50 = 33.96 µg/ mL), which was close to that of the sta-ndard ascorbic acid (IC50 = 24.2 µg/ mL).

Table-2 Qualitative analysis of different extracts of *M. oleifera* leaves

Constituents	Chloroform extract	Methanol extract	Ethanol extract
Tannins	+	+	–
Saponins	+	+	+
Steroids	–	–	–
Flavonoids	–	+	+
Terpenoids	–	–	+
Alkaloids	+	+	+

Table-3 The absorbance of standard solution for various concentrations

Ascorbic acid (Dilution)	% Inhibition
1µg/mL	16.96
5µg/mL	25.03
10µg/mL	31.25
15µg/mL	39.83
20µg/mL	45.36

Table-4 Absorbance of crude extracts

Dilution	% Inhibition		
	Chloroform	Ethanol	Methanol
20µg/mL	12.4	28.9	35.4
40µg/mL	21.9	48.6	57.8
60µg/mL	29.7	65.3	73.2
80µg/mL	34.5	76.8	84.6
100µg/mL	39.2	82.4	89.1

Table-5 The IC_{50} values of Standard solution and Solvent solution

Samples	IC_{50} value (µg/mL)
Ascorbic acid	24.2
Methanol	33.96
Ethanol	41.7
Chloroform	146.9

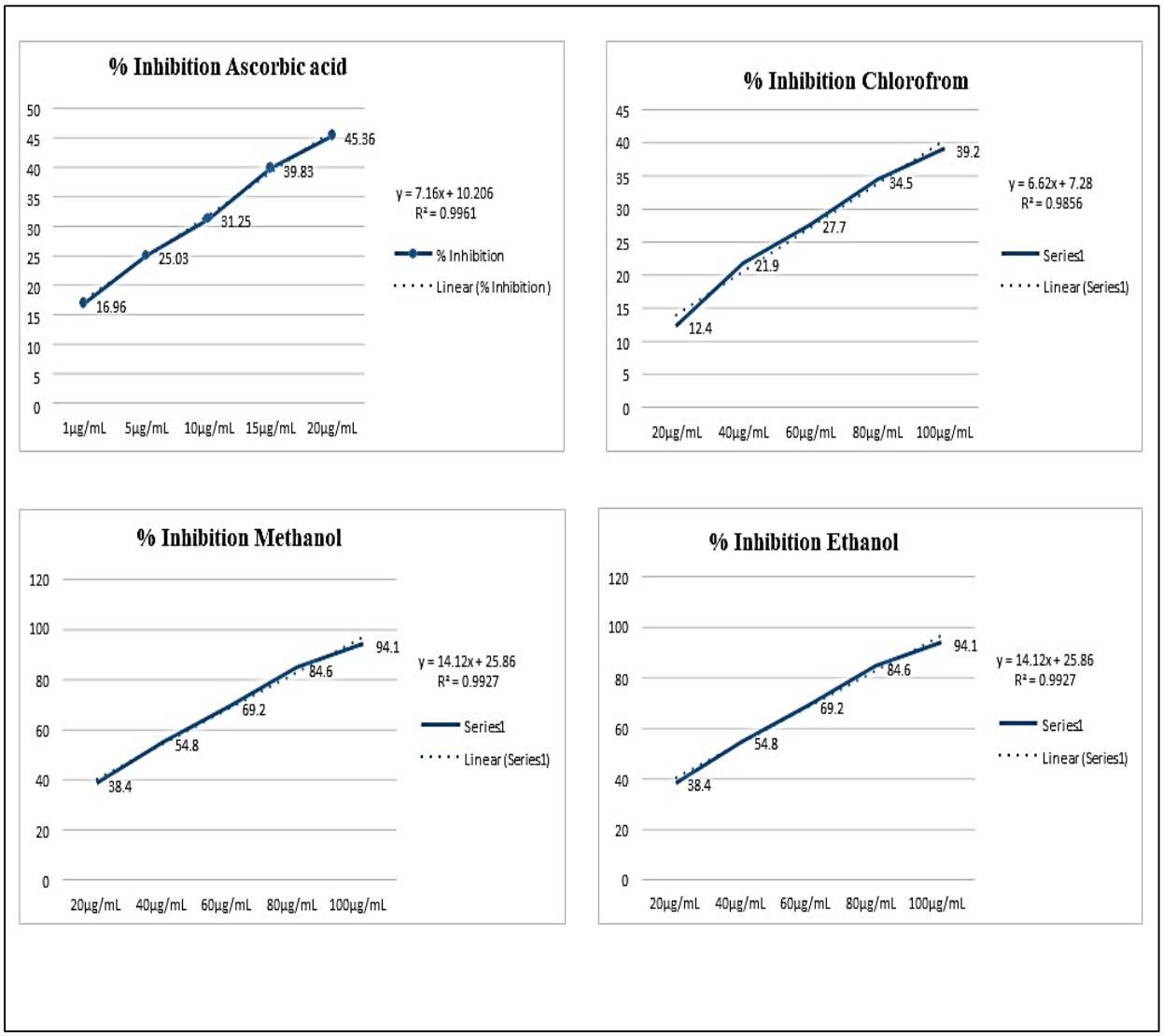


Figure-3 % RSA Graphs of Various Extract of *Moringa oleifera* leaves

According to the results, the solvent extracts of *Moringa oleifera* showed a very good DPPH radical scavenging activity. Ascorbic acid was used as a positive control which showed a maximum percentage of free radical scavenging activity i.e., 85% at aconcentration of 5.0mg/ml. Also, this free radical scavenging activity (%inhibition) increased with increasing concentration of the crude extracts.

Discussion

In the present study, we have investigated the different fractions of *M. oleifera* leaves for in vitro antioxidant activity.The results demonstrate that solvent polaritycritically influences the extraction efficiency of antioxidants from*Moringa oleifera* leaves. Methanol, a moderately polar solvent, out- performed other solvents such as chloroform and methanol in isolating phenolic and flavonoid compounds, which are strongly associated with free radical scavenging activity. This aligns with studies showing that methanol effectively disrupts plant cell membranes and solubilizes glycosylated phenolics, such as quercetin-3-glucoside and chloro-genic acid, which dominate Moringa's antioxidant profile[14]. Ethanol, though slightly less efficient, remains advantageous for food and pharmaceutical applications due to its lower toxicity[15].

Methanol extracted *Moringa olei-fera* leaves demonstrated the highest free radical scavenging activity, achieving 89.1 % inhibition of DPPH radicals at a concentration of 100 µg/mL. This was comparable to the reference standard ascorbic acid (45.36 % inhibition at 20µg/mL). Ethanol extracts followed closely with 82.4% inhibition, non-polar solvents like chloroform exhibited negligible scavenging capacity (<10% inhibition), indicating that polar solvents are more effective in isolating radical neutralizing compounds.

Conclusion

The investigation into the antioxidant potential of *Moringa oleifera* leaf extracts, as evaluated through the DPPH radical scavenging assay, underscores the plant's remarkable capacity to neutralize free radicals, positioning it as a valuable natural source of antioxidants. Methanolic extracts emerged as the most efficacious, exhibiting an IC50 value of 33.96 µg/mL, a performance closely rivaling the reference antioxidant ascorbic acid (IC50 = 24.2 µg/mL). This robust activity can be attributed to the high concentration of polar bioactive compounds, such as glycosylated flavonoids and ascorbic acid, which are efficiently extracted by methanol due to its intermediate polarity and ability to disrupt plant cell matrices. Ethanolic extracts, while slightly less potent (IC50 = 41.7 µg/mL), demonstrated significant radical scavenging activity, making them a safer and industrially viable alternative for food and pharmaceutical applications. The low activity of chloroform extracts

(IC50 = 146.9 µg/mL) highlights the limited solubility of hydrophilic antioxidants in non-polar solvents.These findings emphasize the critical role of solvent selection in optimizing antioxidant recovery, with methanol and ethanol being ideal for research and commercial purposes, respectively.

The study advocates for the integration of *Moringa oleifera* leaf extracts into functional foods, nutra-ceuticals, and natural preservatives, where their radical scavenging properties could mitigate oxidative deterioration and enhance product shelf life. Future research should focus on in vivo validation of these findings, particularly the bio-availability and metabolic fate of Moringa antioxidants, as well as the development of eco-friendly extraction methods to align with sustainable practices.

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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Anti-Microbial Activity of Aerial Parts of *Morina longifolia*

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Abstract-Different extracts of aerial parts of Morina longifolia was screened for their phytochemical constituents and anti-microbial activity. The anti-microbial activity of chloroform (MCH), ethyl acetate (MEA), acetone (MAT) and ethyl alcohol (MAL) extracts of aerial parts of the plant was carried out against four fungal species *Fuserium graminearum*, *Aspergillus flavus, Nigrospora oryzae*, and*Aspergillus niger* and four bacteria species, *Escherichia coli, Bacillus pumilus, Salmonella typhi and Bacillus cereus.* The results of the study showed that the all the extracts showed significant anti-microbial activity against all the microorganism under investigation.

Keywords: Morina Longifolia, Caprifoliaceae, Antimicrobial Activity and Phytochemicals.

Introduction

Antimicrobial resistance poses a major challenge to global health, reducing the effectiveness of conventional antibiotics. Medicinal plants have long been used in traditional medicine for the treatment of infectious diseases, primarily due to their rich phytochemical composition. Recent studies show that plant extracts exhibit strong antibacterial and antifungal activities, offering promising alternatives to synthetic drugs[1]. Extracts obtained from leaves, roots, bark, seeds, or whole plants contain diverse secondary metabolites responsible for antimicrobial effects.

Morina genus belongs to family *Caprifoliaceae* formerly *Dipsacaceae,* is a small genus of small perennial herbs used traditionally in Tibetan, Himalayan and central Asian herbal medicine system. M. species has been reported to possess anti-oxidant, antimicrobial, anti-inflammatory, anti-arthritic and anti-asthmatic activities[2-4]. Morina species have been reported the presence of iridoids, phenolic acids, flavonoids, terpenoids, aromatic glycosides and essential oils, which possess wide range of pharmacological activities[5-8].

Morina longifolia is a small perennial herb, found in temperate and alpine regions of Himalayas from Kashmir to

Bhutan at an altitude of 2400-4200 meters, is commonly known as "Whorlflower". *M. longifolia* produces very beautiful flowers initially of white colour and then turn pink once they are pollinated. They appear in mid-summer on an elongating flower stem. M. longifolia has traditional uses in Tibetan and Indian medicine in curing digestive issues, as an incense, and in treatment of wounds and boils. It is also used for its astringent, emetic and stomachic properties[9-11].

Material and methods

Preparation of extracts

The aerial parts of *M. longifolia* were collected from Dayara Bugyal (3300-3500 m asl) District Uttarkashi, Uttarakhand during August, 2024. Air-dried areal parts of *M. longifolia* were packed in a soxhlet apparatus and were extracted sequentially with chloroform (MCF), Ethyl acetate (MEA), Acetone (MAT), Ethanol (MAL). The organic extracts were dried over vacuum evaporator. Few grams of each extract were subjected to series of purification as per pharmacological profiles. The dried extracts were dissolved in dimethyl sulfoxide (DMSO), ethanol or water prior to analysis depending upon their solubility. The extracts were subjected to further analysis and all the assays were done in triplicates. Qualitative analysis of different extract like solubility test, foam test, alkaloid Meyers test, $FeCl_3$ test, carbohydrate test and ninhydrin test were performed and are shown in **Table-1**.

Table-1 Qualitative analysis of crude extracts of different aerial parts of *M. longifolia*

Extract	Solubility test	Foam Test	$FeCl_3$ Test	Alkaloid Meyers Test	Carbohydrate test	Ninhydrin
MCF	+	++	-	++	++	-
MEA	+	-	+	-	-	-
MAT	++	-	+	-	-	-
MAL	+++	-	-	-	-	-

Antimicrobial Activity

The microorganisms were obtained from IMTECH, Chandigarh. The organisms were stored on agar slant in McCartney bottles and kept in the refrigerator prior to subculture.

Antibacterial Activity

Four bacterial species viz., *Escherichia coli, Bacillus pumilus, Salmonella typhi* and *Bacillus cereus* mutans were taken to evaluate antibacterial potential of the different extracts. All the extracts were dissolve in 30% DMSO and the antimicrobial activity of the extracts were carried out with the concentration of 100mg/ml by agar well diffusion method.

The Muller Hinton Agar media for antibacterial assay was prepared in a sterile Petri dish and the bacterial

culture (10µl) was introduced to the solid surface of agar media with the help of micropipette. Then spread across the surface of solid agar media by means of a sterile spreader and kept at room temperature for 15 min. in agar plate well were prepared by sterile cork borer and 100µl of extracts were poured in each well using micropipette. The Petri dish then incubated in incubator for 24 hrs. at 37±2^{0}C temperature [12,13]. After incubation the degree of sensitivity was determined by measuring the zone of inhibition around the disc **Table-2**.

Table-2 Effect of different extracts of aerial parts of *M. longifolia* on zone of inhibition (mm) of some selected bacterial species tested by well diffusion method.

Bacteria species	Extracts			
	MCF	MEA	MAT	MAL
Escherichia coli	22±1.01	18±1.15	13±1.08	9±0.65
Bacillus pumilus	10±1.14	15±0.52	14±1.13	8±1.12
Salmonella typhi	17±1.18	13±1.14	15±1.00	11±1.14
Bacillus cereus	14±0.65	12±0.82	10±0.52	9±1.21

Anti-fungal Activity

Antifungal activity of the extracts was carried out against four pathogenic fungi namely *Fusarium gramin-earum, Aspergillus flavus, Aspergillus Niger,* and *Nigrospora oryza* by the well diffusion method. The fungal culture (0.1 ml) was introduced to the assay media kept in sterile petri dishes with the help of cotton swap and was spread evenly on the surface of solid agar media by means of a swapper. The wells were punctured on agar growth medium kept in petri dishes using sterile well puncher syringe. 100µg/ml of extracts were poured in each well using micropipette. The Petri dishes then kept in incubator at 25±2ºC for 72 hrs[12]. After incubation plates were observed for the degree of sensitivity of extracts by measuring the zone of inhibition **Table-3**.

Table-3 Effect of different extracts of aerial parts of *M. longifolia* on zone of inhibition (mm) of some selected fungal species tested by well diffusion method.

Bacteria species	Extracts			
	MCF	MEA	MAT	MAL
Fuserium graminearum	15±057	16±1.11	11±1.18	7±0.65
Aspergillus flavus	17±1.53	14±0.58	12±1.00	9±0.78
Nigrospora oryzae	16±1.0	13±0.53	19±1.13	10±0.65
Aspergillus niger	21±1.11	16±1.14	13±1.00	9±1.15

Results and Discussion

Results of qualitative analysis for

phytochemicals of different extracts

of *M. longifolia* are shown in **Table-1**. The chloroform extract was found positive for alkaloid, saponins and carbohydrate whereas the ethyl acetate extract and acetone extracts showed positive test for polyphenolic compounds. The alcoholic extract shows negative test for polyphenolic compounds. The results of antibacterial and antifungal activity against the selected microorganism of different extracts of aerial parts of *M. longifolia* are shown in **Table-2** and **Table-3** respectively. The anti-bacterial activity of different extracts was carried out against four bacterial species, *E. coli, S. Typhi, B. cereus* and *B. pumilus*. The chloroform extract showed significant inhibition zone against all the tested microorganisms and maximum inhibition (22±1.01) was observed against *E. coli*. The ethyl acetate extract showed maximum inhibition (18±1.15) against *E. coli* and minimum inhibition (12±0.82) against *B. cereus,* whereas, the acetone extracts showed maximum inhibition (15±1.00) against *S. typhi* and minimum inhibition (10±0.52) against *B. cereus*. The alcoholic extract showed least inhibition against all the bacterial species under investigation.

The results of antifungal activity of different extracts against four fungal species, *S. flavus, A. niger, F. graminearum* and *N. oryzae* ae presented in Table-3. From the table it is evident that the maximum antifungal activity shown by the chloroform extract. The chloroform extract showed maximum inhibition (21±1.11) against *A. niger* and minimum inhibition against *F. graminearum*. The ethyl acetate extract showed equal zone of inhibition against *A. niger* and *F. graminearum*. The acetone extract showed maximum inhibition against *N. oryzae* (19±1.13). Among all the tested extracts alcoholic extract showed minimum inhibition against all the fungal strain under investigation. *M. logifolia* have been reported to contains a variety of phytochemicals including terpenoids, triterpenoids, sterols, phenolic compounds like para hydroxy benzoic acid caffeic acid and essential oils[2,4,5]. The antimicrobial activity of various extracts is due to the presence of bioactive compounds. The active antimicrobial constituent like terpenoids, poly phenolics and essential oils may exert their toxic effects through the disruption of bacterial and fungal membrane integrity[14-15].

Conclusion

From the above study it can concluded that different extract of aerial parts of M. longifolia possess antimicrobial activity against all bacterial and fungal species. Phytochemical screening showed presence of polyphenolic compounds in these extracts which are responsible for their antimicrobial activity.

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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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Ground Water Quality Assessment & Hydroponics Cultivation

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Abstract- Groundwater serves as a primary drinking water source for the rapidly growing population of Uttarakhand, where both natural factors and anthropogenic activities contribute to the deterioration of water quality. This study evaluates the seasonal variation in drinking water quality in the central region of Dehradun over a seven-month period (January–July 2024), covering winter, summer, and rainy seasons. Five physicochemical parameters—Colour, Turbidity, Total Dissolved Solids (TDS), pH, and Total Hardness—were analyzed following BIS IS: 10500 (2015) standards. The Horton Water Quality Index (WQI) method was applied to interpret overall water quality. Results indicate that pH remained within desirable limits throughout the study, while turbidity exceeded the desirable limit of 1 NTU in all seasons but stayed below the permissible limit of 5 NTU, colour values were within acceptable limits except in July, when monsoon runoff increased the concentration to 7 Hazen. TDS remained below the desirable limit of 500 mg/L, with the highest concentration observed in July. Total hardness was low in January but exceeded desirable limits in April and July, though remaining under the permissible level of 600mg/L. Seasonal WQI values ranged from 26.36 to 33.88 consistently placing the groundwater in the ―Good‖ (Grade B) category. High grade ground water is very promising for cultivation of hydroponics. Groundwater quality varies; deep sources are usually clean, but high salt content might require treatment (like reverse osmosis) before use in hydroponics Overall, despite minor excedances in turbidity, colour, and hardness during the rainy and summer seasons, the groundwater of central Dehradun remains suitable for drinking purposes. Continuous monitoring is recommended to detect early signs of contamination and ensure long-term water safety.

Key words: Ground Water, TDS, WQI, Total hardness

Introduction

While hydroponics reduces freshwater demand, it still needs water, often from bore wells (groundwater) or municipal sources, but can be made sustainable by integrating rainwater harvesting, which provides naturally soft, chlorine-free water, minimizing reliance on groundwater and lowering costs, though groundwater quality (salinity) must be monitored for hydroponic use.

The concept of water quality is not particularly the purity of water, but it attribute to physical, chemical and biological analysis of water that determine its use for different purposes. The most common use of evaluation of the water quality is associated to the health of the human beings and safety of the ecosystem. For the survival every human on this earth need drinking water. Beside their good health may be directly affected by the quality of drinking water. The depletion in the quality and quantity of drinking water and their sources has become a problem in Uttarakhand[2-4].

For the deterioration of drinking water quality, the contamination of drinking water sources is mainly responsible. Degradation of water quality of drinking water resources is caused by both geogenic as well as anthropogenic activities including hydrological activities, climate change, precipitation, agricultural runoff and sewage discharge[5, 6]. Other factors responsible for contamination of water sources are open defecation, dumping of household wastes, road side construction and development of infrastructure. Besides, rapid growth of tourism industry, urbanization, industrialization and rapid growth of population of state also other important factors, which degrade drinking water sources. Moreover, awareness of local people, insufficient enforcement of laws and lack of continuous monitoring of water quality of water sources are other leading causes due to which drinking water quality is greatly being influenced. These water sources are also getting contaminated due to increased deforestation, weathering of rocks, erosion, growing construction activities and other exogenic factors along with a very high number of tourists as floating population. Also turbidity is generally found high in water sources owing to high velocity of water from upstream to downstream, as a result of which dust, soil, rock particles get suspended into fast flowing water. Moreover, disposal of human and animal faeces containing bacterial, viral and protozoan, pathogens as well ashelminthes parasites in hill areas may result into serious water borne diseases such as typhoid, cholera, dysentery, polio myelitisetc due to slop factors of mountains and hills. Therefore, this is a big challenge for the drinking water supply and maintenance agencies to provide good quality of drinking water for the public use. The water-retaining capacity of these underground water storage depends on geomorphology, hydrology, rock type, porosity and permeability of rocks and soil. These natural sub-surface waters are mainly a very rich source of minerals, calcium, magnesium, potassium, sulfur and iron[7-9]. However, due to the increase in urbanization, Industrialization and tourism in Uttarakhand these water resources have been contaminated with both chemical and biological contaminants. Almost all the springs and water sources are contaminated with microbial contamination. The density of microbes increases at the springs or a natural water source when it is located near a village or in an area with extensive wildlife and animal populations.

Water Quality of Dehradun

Monitoring of water quality of drinking water sources is therefore necessary to

collect the information about existing status of water quality and source of contamination for the implementation of strategies by water management agencies to overcome the source of pollution and provide good quality water for the public use in future. Process of such water quality monitoring includes the continuous sampling, analyses and characterization of drinking water sources on regular time intervals.

Water Quality Index (WQI) is the most effective tool to analyse the water quality of the particular area and to produce information regarding water quality in the simplest form to the general community. The water quality data of Dehradun district has been established by the Central Ground Water Board (CGWB, 2013) in the study during 2007-2008 by observing 149 hand pumps, 9 dug wells, 23 springs and 4 exploratory wells. Water quality characteristics of these sources have been assessed by adopting pH, EC, carbonate, bicarbonate, chloride, total hardness, calcium and magnesium parameters. Most of the analyzed sources express the fresh water quality for domestic and irrigation purposes. Only some sources have high hardness, calcium or magnesium concentration than the desirable limits. Hand pump water at Maldevta location show high hardness, calcium and magnesium concentration as 485 mg/l, 104 mg/l and 55 mg/l, respectively. Spring water quality at Barlowganj has 400 mg/l total hardness and 63mg/l magnesium concentration[10-12] . Water quality of two Sahastradhara springs has also revealed high hardness, calcium and magnesium as 445 mg/l, 100 mg/l and 47 mg/l for first and 435 mg/l, 96 mg/l and 47 mg/l for second spring water sample. The ground water scenario of Uttarakhand expresses the deteriorated water quality of district due to high nitrate concentration (CGWB, 2010). According to survey of drinking water quality of India it was predicted that the drinking water resources of various districts in our country were contaminated due to the predominant concentrations of different ions or bacteria. For this study, water qualityof28 cities was assessed under CLEAN-India programme and output of the study showed the alarming condition of water. This study has also predicted the high degree of hardness in water sources of Dehradun district[13-15].

Hydroponics is basically a contemporary method of horticulture or basically growing plants using farm produce, and the thing is without using soil. The method of hydroponics is nearly easy but it takes time to understand the method. In the method, crops are planted and grown on water. These crops are also found to be very efficient and filled with benefiting nutrients.

According to the scientist‘s plants in hydroponics method have direct connection to the roots in form of aqueous solvent instead of the soil. Hydroponics produce between three and ten times more food than conventional agriculture in the same space. The plants also grow in half the time. There is no need for herbicides or pesticides. While hydroponics reduces freshwater demand, it still needs water, often from bore wells (groundwater) or municipal sources, but can be made

sustainable by integrating rainwater harvesting, which provides naturally soft, chlorine-free water, minimizing reliance on groundwater and lowering costs, though groundwater quality (salinity) must be monitored for hydroponic use[16,17].

Hydroponics and Groundwater

Source Dependency: Hydroponic systems need a consistent water supply, often sourced from groundwater via bore wells, especially in large setups.

Water Quality- Ground water quality varies; deep sources are usually clean, but high salt content might require treatment (like reverse osmosis) before use in hydroponics.

Sustainability- Using groundwater heavily can lower water tables, creating environmental concerns, which rainwater harvesting helps mitigate[18].

Figure-1 Hydroponics Cultivation

Study Area description- Dehradun district is situated in the north-western part of Uttarakhand state, extending from N Latitude 29°56′39.33″ to 30°58′41.70″ N and from E Longitude 77°34′29.04″ to 78°18′41.21″ E (GWBDDU, 2011). For this study, central region of Dehradun have been selected for sampling and monitoring of water quality for a period of 7 months i.e. from January to July of 2024 covering 3 seasons i.e. winter, summer and rainy. The selection criteria of drinking water source was based on information provided by Uttarakhand Jal Sansthan, Dehradun, which provided a piped drinking water supply to the study area. 3-3 samples of drinking water have been collected in the months of January, April and July which covers winter, summer and rainy seasons respectively.

Adopted Methods- The Bureau of Indian Standard, 2015 has recommended Indian water quality standards for water quality parameters, to examine the water quality of water sources for the suitability of drinking purpose by describing their desirable and permissible limits. According to IS: 10500 (2015), desirable limits are the acceptable limits above which water is not suitable for drinking, but still may be used for drinking purpose up to permissible limits in the absence of alternate drinking water sources[19]. The protocols and adopted methods for

analysis of 5 drinking water quality parameters including both physico-chemical adopted as per guidelines of Bureau of Indian Standard {IS:10500(2015)} are given under **Table-1** for

(a) on site analysed 2 parameters namely turbidity and pH and
(b) laboratory analyzed parameters i.e. Colour, Total Hardness, TDS.

Table-1 Methodology Adopted and Instrumentation Technique used for Analysis

S. No.	Water Quality Parameter	Type of Parameter	Adopted Methodology	Instrumental Technique Used	Protocol used
1	Colour	Physical	Spectrophotometric single wavelength method	UV-Vis Spectrophotometer	APHA24thEd. 2120 C
2	Turbidity	Physical	Nephelometric method	Nephelometer	BIS 3025Pt-10 (2015)
3	Total Dissolved solid	Physical	Gravimetric Method	TDS meter APHA	24thEd. 2120-C
4	pH	Physical	Electrometric method	pHmeter	BIS 3025Pt-11
5	Total Hardness	Chemical	EDTA Titration method	Volumetric titration	BIS 3025Pt-21 (2015)

Procedure used for Analysis of Water Quality Parameters Analysis of Colour

Water colour is an important visual indicator fits quality. It can be influenced by natural factors such as dissolved organic matter or human activities like industrial discharges or pollution. For the appearance of colour in drinking water, various factors are responsible such as turbidity, corrosion, algal bloom and suspended solid particles etc.

Unusual or significant changes in water colour can suggest the presence of contaminants or pollutants. The Hazen color scale (APHA 24th Ed. 2120-C) was utilized to measure the color of analyzed drinking water samples using a UV-Visible spectrophotometer (Hach, USA; Model: DR-5000). The spectrophotometer was set to operate within a wavelength range of 450 – 465 nm, as recommended by APHA guidelines (2012). Specifically, the instrument was typically configured at a wavelength of 456 nm to establish standard curve. Prior to analysis, the spectrophotometer was

warmed up for 15 minutes, following the manufacturer's instructions[20].

Analysis of Turbidity

The cloudiness of water, measured as turbidity, is a crucial parameter for assessing water quality. In the case of drinking water samples, turbidity was quantitatively measured using the Nephelometric method in Nephelo-metric Turbidity Units (NTU). This measurement was conducted using a Nephelometer (Model: Turbi Check; Make: Aqualytic, Germany) directly at the water source site.

The Nephelometric method (IS 3025 Pt-10-2006) quantifies turbidity by measuring the intensity of scattered light by the water sample. This is compared to the intensity of light scattered by a standard reference solution (Formazin) under identical experimental conditions. Higher intensity of scattered light indicates higher turbidity in the sample.

Before starting the analysis, the turbidity meter was allowed to warm up for approximately 15 minutes to stabilize. Calibration of the instrument was then performed using standard reference suspensions with turbidity levels of 1 NTU, 10 NTU, 100 NTU, and 1000 NTU.

Analysis of Total dissolved Solids

The Total Dissolved Solids (TDS) in drinking water samples were analyzed using the electrometric method (APHA 24th Ed. 2540-C) with a TDS meter (Make: Hach, USA; Model: Sension-5) in a laboratory setting. The TDS meter was calibrated using a standard sodium chloride solution with a conductivity of 1000 µS/cm. Calibration ensures that the instrument provides accurate readings base don known standards. After calibration, the electrode of the TDS meter was rinsed thoroughly with de-mineralized water to remove any residues and wiped dry with tissue paper. To verify the accuracy of the TDS meter, it was cross-checked using the earlier standard solution. This step ensures that the instrument maintains its accuracy over time and between measurements.

Analysis of pH

The acidity or alkalinity of a drinking water sample, expressed as pH or negative logarithm of hydrogen ion concentration, is a critical characteristic that varies with naturally occurring minerals and biological activities. Typically, the pH of natural drinking water sources ranges from 5.0 to 9.0, reflecting important ecological characteristics of aquatic environments. The pH measurement (BIS 3025 Pt-11-2002) utilizes the electromotive force (EMF) of a cell containing two electrodes: a glass electrode (indicator electrode) to measure hydrogen ions in the sample, and a calomel electrode (reference electrode). These electrodes are in contact through a liquid junction, and the EMF of the cell is measured using a pH meter.

Analysis of Total Hardness

Total hardness of water indicates the

presence of calcium and magnesium salts. To determine total hardness using the EDTA method (as per BIS 3025 Pt-212015), volumetric titration method was employed. Eriochrome Black T served as the complexometric indicator in the titration process. Initially blue in color, Eriochrome Black T turns red upon binding with calcium and magnesium ions to form complexes.

Determination of Water Quality Index

Water quality of analyzed drinking water sources is often summarized using a single number known as the Water Quality Index (WQI) [21]. The primary aim of WQI is to simplify complex water quality data and present it in a comprehensible form. In the current study, the hort on water quality index WQI method was employed to assess the water quality of drinking water sources in Dehradun. This method provides a score between 0to 100, which reflects the quality of drinking water in term so find exnumber. The higher the index number, poor the quality of drinking water.

Table-2 Water Quality Index Vs Water Quality Grade

S. No.	WQI Values	Water Quality Grade	Water Quality Rating
1	0-25	A	Excellent
2	26-50	B	Good
3	51-75	C	Average
4	76-100	D	Poor
5	100 and above	E	Unsuitable to drink

Table-3 Indian Standard for selecting Drinking Water parameters

S. No.	Nature of Parameter	Water Quality parameters	Accepted Limit	Permissible Limit
1	Physical	Colour	5hazen	15 hazen
2	Physical	Turbidity	1NTU	5NTU
3	Physical	Total DissolvedSolids	500 mg/l	2000 mg/l
4	Physical	pH	6.5-8.5	6.5-8.5
5	Chemical	Total Hardness	200 mg/l	600 mg/l

Result and Discussion

Water Quality Analysis of Drinking water

Water quality data of all 6 water quality parameters are described in **Table-4**. The overall description of water quality data is described under:-

Colour- The analytical data of colour is compiled in **Table-4**. The concentration of colour in water sample of January and April were under its desired limit i.e. 5 hazen while the sample of July month showed the maximum value for colour which was over the desired limit of 5 hazen but under the permitted limit i.e. 15 Hazen.

Turbidity- The variation in Turbidity is compiled in table. The turbidity for all months were well over its desired limit i.e. 1 NTU but were under the permitted limit of 5 NTU. The sample collected in the month of July showed the maximum turbidity value respectively.

High turbidity of water attributed the presence of suspended solid matter as clay, silt and organic solids etc. due to various geographical activities such as precipitation, weathering of rocks, soil erosion and growth of some phytoplankton and other microorganisms [22, 23].

Total Dissolved Solids (TDS)-The variation in TDS value is described in Table 4. The result of analysis of data of TDS was found to well below desired limit i.e. 500 mg/l. It was also noticed that the sample collected in month of July had the maximum TDS value.

The Characteristics of TDS may be attributed to the influence of various natural activities, like leaching of minerals and percolation of water from rock minerals and soil, and Anthropogenic activities like waste dump water run-offs, agricultural field run-offs and industrial water dumping[24].

pH-The pH of all analyzed water samples from January to July were found to be within the desired limit range of 6.5-8.5 as per BIS drinking water standards.

Total Hardness- The variation of total hardness in drinking water samples for period of 6 months are depicted in table. Result of drinking water quality showed that the total hardness for the month of January was under the desired limit of 200 mg/l while for the months of April and July, the total hardness values were above desired limit but lower than the permissible limit i.e. 600 mg/l as per BIS (2015) drinking water standards.

The characteristic values of Total hardness indicates of interaction (percolation) of water with minerals in soil and rocks and weathering impact as well as anthropogenic sources like industrial, agricultural, and untreated water run-off[25, 26].

Table-4 Water Quality Data of central region of Dehradun during January to July 2024

S. No.	Water Quality Parameters	Months of Sample Collection (2024)			As per IS10500-2015	
		January	April	July	Acceptable Limit	Permissible Limit
1	Colour(Hazen)	2	4	7	5	15
2	Turbidity (NTU)	1.51	1.87	2.83	1	5

3	Total Dissolved Solids(mg/l)	300	345	372	500	2000
4	pH at25˚C	7.90	7.67	7.41	6.5-8.5	No Relaxation
5	TotalHardness as $CaCO_3$(mg/l)	125	246	298	200	600

Table-5 Calculated Water Quality Index with Grade and Rating

Month	WQI	WQI Grade	WQI Rating
January	26.36	B	Good
April	27.89	B	Good
July	33.88	B	Good

Conclusion

The overall results of the research work carried for the time period of 7 months from January to July, 2024 have been tabulated.

Analysis of 5 Physico-chemical Water Quality Parameters under 2 categories such as

(i) Physical Parameters
(ii) Chemical Parameters for Drinking Water of central region of Dehradun as per IS:10500 (2012) have been covered.

From the result above, the conclusion can be made that the water quality analysis of central region of Dehradun, conducted over the months of January, April, and July 2024, reveals the following key points regarding the water quality parameters:

1. **pH Value**: The pH levels of the water samples were consistently within the desirable range of 6.5-8.5 as per BIS standards. This indicates that the water is neither too acidic nor too alkaline, which is ideal for drinking purposes.

2. **Turbidity:** The turbidity values were above the desirable limit of1 NTU but remained below the permissible limit of 3 NTU for all sampled months. This suggests that while the water is somewhat turbid, it does not exceed acceptable levels, likely due to natural processes affecting particle suspension.

3. **Colour:** The colour of the water samples was well within the desirable limit of 5 Hazen in January and April months however, raised to 7 in the month of July.

   This indicates that the water is clear and free from significant color contamination in January and April. In July due to heavy muddy rains water on ground the value might have got affected, which are the signs of some contamination.

4. **Total Dissolved Solids(TDS)**: The TDS levels were above the desirable limit of500 mg/L in all months but remained under the permissible limit of2000 mg/L. While the levels were higher than ideal, they were still within an acceptable range for drinking water.

5. **Total Hardness**: Total hardness was under the desirable limit of 200 mg / L in January but exceeded this limit in April and July, although it remained below the permissible limit of 600 mg/L. This suggests that the water may have some

mineral content but is not excessively hard. The Water Quality Index (WQI) calculated for February, May, and July month indicated a rating of "Excellent" for all months. This reflects that, despite some parameters exceeding the desirable limits, the overall water quality remains within acceptable standards for drinking purposes.

In summary, the drinking water of central region of Dehradun, is generally of high quality with only minor issues that do not significantly compromise its safety or suitability for consumption. It can be useful for the hydroponics cultivation for sustainable agriculture and adopting this technique can increases food production.

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Phytochemical analysis and effects of Haridra *(Curcuma longa)* on *diabetes mellitus* (Madhumeha)

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Abstract- Diabetes mellitus (Madhumeha) is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both. Traditional medicinal systems such as Ayurveda describe Haridra (*Curcuma longa* Linn.) as a drug with *Prameha-hara* (anti-diabetic) properties. Haridra is one of an important dravya described in Ayurveda. It is used clinically externally as well as internally for the treatment of variety of ailments. Haridra is most popular among Indian women to improve the texture and lustre of the skin. It is used in religious functions of Hindus since centuries. Drugs containing Tikta, Katu Rasa like Haridra, is specially indicated in the management of Prameha/Madhumeha. Acharya Vagbhata has praised it, as a drug of choice in the treatment of diabetes mellitus. Recently Turmeric has received a lot of attention from all section of scientific communities in the field of health care system and pharma-ceutical industry, regarding its anti-diabetic, antioxidant, hepato-protective, anti-inflammatory, anti-carcinogenic and antimicrobial properties. The present research article focuses exclusively on preliminary phytochemical screening of *Curcuma longa* rhizome extracts, their qualitative testing, observed results, and discussion of how these phytochemicals contribute to anti-diabetic activity. The study highlights the relevance of phytochemical screening as a foundational step in identifying bioactive constituents responsible for anti-diabetic effects.

Introduction

Diabetes mellitus is a rapidly increasing global health problem associated with severe complications such as neuropathy, nephropathy, retinopathy, and cardiovascular disorders. In Ayurveda, Madhumeha is classified under *Prameha* and is associated with derangement of *Kapha* and *Vata* doshas along with impaired *Agni* and *Medo dhatu*

metabolism. Herbal drugs play a crucial role in the management of Madhumeha due to their multi-targeted action and fewer side effects.

Haridra (*Curcuma longa* Linn.), belonging to the family Zingiberaceae, is widely used as a spice and medicinal plant (Sharma, 2005; Srinivasan, 2007). Classical Ayurvedic texts describe Haridra as *Tikta* and *Katu rasa, Ruksha guna,* and *Ushna virya,* making it beneficial in metabolic disorders. Modern scientific studies attribute its pharmacological activities to the presence of diverse phytochemicals. Therefore, phytochemical screening of *Curcuma longa* extracts is essential to understand the basis of its anti-diabetic potential (Harborne, 1998; Kokate, 2010).

The Sanskrit word Haridra literally means the drug which improves the complexion of the skin. In India, it is popularly known as Haldi (turmeric). In Atharvaveda (Shaunakiya recession 1-22), it was mentioned that the food of rice pap mixed with turmeric should be given to a patient of Hariman (Yellowness/Jaundice). In Atharvaveda (Pipalada recession P.I. 16.1) four plants were mentioned, turmeric is one of them, named as Oshadhi. In Ayurvedic texts, its various synonyms describing its virtues like Kanchani -renders golden tinge to the skin, Nisha- beautiful like a starry night, Harita- yellow, Varavarni- imparts color to the skin etc. The great sage Charaka has categorized it under Lekhaneeya Varnya Vishaghna Kushṭhaghna. Sushruta has classified it under Stanya Shodhaka Mahakashaya. Later on, many Ayurvedic texts have categorized it as Kandughna, Kamala nashaka, Shonita sthapana and Sirovirechana.

In the classical texts of Ayurveda (C.S. Ci.-6/26, 27, 38, 39, S.S. Ci.-6/20, 1118, 9, Su. 13/11, A.S. Ci. -14/12, 18, 25, A.H.D. -40/48), Haridra is clearly mentioned for the treatment of Prameha/Madhumeha alone or in combination with other preparations. Drugs containing Tikta, Katu Rasa like Haridra *is* specially prescribed in the management of Prameha/ Madhu-meha. Acharya Vagbhata has described it as the drug of choice in the treatment of diabetes mellitus. A large number of synonyms were given in the lexicons of Indian Pharmacopoeia. These synonyms were based either on the pharmacological action of the drug or on its morphology.

Botanical description of Haridra

Common Name : Indian Saffron or Turmeric.

Botanical Name : Curcuma longa

Synonym : Curma domes.,

Family : Zingiberaceae

Flowering and Fruiting Time : from October to December

Propagation :Tubers of previous crop are planted 6-8 cm deep at distance of 30 to 40 cm in April August.

Useful parts : Rhizome and Some-time flowers.

Doses : Powder, 1-3 gm., Juice 10-20 ml

Phytochemical Analysis of Curcuma longa

The phytochemical screening of the C. longa rhizome extract showed the presence of several bioactive compounds including alkaloids, tannins, phenolic compounds, terpenoids, saponins, flavonoids, cardiac glycosides, and fixed oils / fatty acids. This remarkable phyto-chemical diversity contributes to the multitude of pharmacological activi-ities and therapeutic potential associated with turmeric. Alkaloids are well known for their anti-microbial, analgesic, and antioxidant properties, play a crucial role in the traditional use of turmeric for treating various infectious diseases and inflammatory conditions. The presence of tannins and phenolic compounds, which exhibit potent antioxidant, anti-inflammatory, and antimutagenic effects, may contri-bute to the anticancer properties attributed to turmeric.

The **terpenoid** class, encompassing the curcuminoids (curcumin, de-methoxycurcumin, and bisdemeth-oxycurcumin), has garnered significant attention for its anticancer, anti-inflammatory, and neuro-protective activities. These bioactive compounds may be responsible for the observed therapeutic effects of turmeric in various chronic diseases, including cancer, inflammatory disorders, and neurodegenerative conditions. **Saponins**, known for their ability to enhance immune function and exhibit hypocholesterolemic effects, may contribute to the traditional use of turmeric in promoting overall health and well-being. **Flavonoids**, another class of polyphenolic compounds present in the extract, possess remarkable antioxidant, anti-inflammatory, and anticancer properties, further enhancing the therapeutic potential of turmeric.

The diverse phytochemical composition of C. longa rhizomes provides a strong scientific rationale for its widespread traditional use in various therapeutic applications and highlights the potential for further exploration and development of novel therapeutic agents derived from this plant source. The DPPH free radical scavenging assay is a widely accepted and reliable method for evaluating the antioxidant capacity of various compounds. In various studies, the C. longa rhizome extract exhibited a concentration-dependent increase in radical scavenging activity, reaching a maximum of around 81% at the highest concentration tested (3 mL). This potent antioxidant activity can be attributed to the unique chemical structure of curcumin, the principal bioactive compound present in the extract.

Curcumin’s remarkable antioxidant potential is attributed to its ability to donate hydrogen atoms and electrons efficiently, thereby neutralizing free radicals and reactive oxygen species (ROS). The presence of methoxy, phenoxy, and carbon-carbon double bonds in the curcumin molecule facilitates this process, enabling the formation of relatively stable

phenoxy radicals and preventing further oxidative damage to cellular components. Additionally, curcumin has been reported to chelate metal ions, which can catalyze the formation of free radicals through the Fenton reaction. By sequestering these metal ions, curcumin prevents the generation of harmful free radicals, further contributing to its overall antioxidant activity. Moreover, curcumin exhibits indirect antioxidant mechanisms by modulating the activity of transcription factors like nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 regulates the expression of various antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). By activating the Nrf2 signaling pathway, curcumin can enhance the levels of these enzymes, thereby strengthening the body's endogenous antioxidant defense system against oxidative stress.

The phytochemical constituents present in the methanolic extract were more in number than that of ethanolic extract which may due to more extracting capacity of some specific phytochemicals like phenol, phlobatannin etc. In various studies, phytochemical constituents alkaloids, steroids, tannins, saponins, flavonoids, phenol, carbohydrates, cardiac glycosides, phytosterol, protein, anthocyanin, coumarin, emodins, diterpenes, amino acids, phlobatannin, leucoanthocyanin, anthroquinone, chalcones, terpenoids were analysed. In the methanolic extract saponin, protein and amino acids, coumarin and phlobatannin were absent. In the ehanolic extract phenol, phlobatannin, protein, amino acids, coumarin and diterpenes were absent.

Ayurvedic Pharmacodynamics of Haridra

Rasa : Tikta, Katu-bitter and pungent.
Guna : Ruksha, Laghu
Veerya : Ushna
Vipaka : Katu
Prabhava : anti-diabetic, anti-dermatitis, digestant and haemo-poeitic.
Dosha Karma: Kapha Vatahara and Pitta shamaka.

Haridra in Ayurvedic Classics

In **Charaka Samhita** it is mentioned in Lekhneeya, Kushthaghna and Vishaghna Mahakashayas. It is indicated in for the treatment of Prameha, Kushtha, Pandu, Shotha, Krimi etc. It is enumerated in Tiktaskandha and Shirovirehcana Dravyas. In Cikitsasthana it is used for Samshodhana before taking Rasayana and as an ingredient of Brahma Rasayana and other Rasayana yogas in Prameha, Kushṭha, Mahakushtha etc. It is frequently prescribed in Vishadosha Chikitsa, internally as well as externally. It is also used for Vamana and Virechana in combination with other drugs and as an ingredient of Anuvasna- Vasti.

In **Sushruta Samhita** it is mentioned in Haridradi gana, Mustadi gana, Lakshadi gana and Vidaryadi gana. In

vata sanshamana Varga and Kapha shamana Varga and in Tikta Varga. It promotes Àrogya, Bala, Medha and Ayu of infants. It is indicated in Prameha and also in Asthapana vasti. It is also used as Rasayana and also in the management of Sthavara and Jangama visha etc.

In **Ashtanga Sangraha** it is described as the best drug among Prameha hara Dravyas, (Haridra Pramehaharanam A.S.Su. 13/3). It is mentioned in Tikta skandha and is described as Katu, Tikta, Meha nashaka etc. It is mentioned in Lekhaneeya and Kushthaghana gaṇas, in Haridradi and Mustadigana. It is an ingredient of Atisthaulyahara Yogas, Vishanashaka Yogas. It is also used in Vaman Kalpa and in disease like Jvara, Raktapitta, Prameha, Pandu, and Kamala etc. It is an ingredient of Brahma Rasayana and other Rasayana yogas.

In **Ashtanga Hridaya** it is mentioned in Tiktavarga, Kaphanashaka varga, Haridradi gana and Mustadigana. It is used in Vishashamaka yoga, Atisthaulyahara yoga and Dhooma-Dravyas. It is prescribed in Prameha and externally used in Vidradhi. It is regarded as the best drug in treating Prameha along with Amalaka, ("Meheshu Dhatrinishe "(A.H. U. 40/48).

Table-1 Therapeutic Indications of Haridra in Ayurvedic text

Clinical conditions		Modern correlate	Textual References
1.	Prameha	Urinary Disorder and Diabetes	C.S. Ci.-6/26, S.S. Ci.-6/17-20, K.N., B.P.N., R.N., D.N.
2.	Tvagvikara	Skin Diseases	K.N., C.S. Ci. 12/39.
3.	Arsharoga	Haemorrhoids	C.S. Ci.-14/52, B.P. 5/57
4.	Bhagandra	Fistula in ano	B.P.-50/31, C.D.- 46/26
5.	Pandu	Anemia	K.N., B.D.N., C.D., C.S. Ci. 6/53
6.	Visha	Allergy	K.N., D.N., G.N., 6/3/11, C.S. Su. 4
7.	Vrana	Ulcers	K.N., R.N., B.P.N., D.N.
8.	Ashmari	Urinary calculus	Vangasena Aśmarī-48, B.P.-37/48
9.	Kamala	Jaundice	C.S. Ci.-16/53, A.V.S. 1.22, A.H. Ci. 16/44
10.	Shleepada	Filariasis	C.D.-42/12, B.P.-45/14, V.M.-42/14
11.	Vatavyadhi	Neuropathies	V.D. -12-2/15
12.	Kasa	Cough	S.B.M.- 4/393.
13.	Mashurika	Chickenpox	C.D.- 45/9
14.	Vyanga	Chloasma	R.R.S.- 24/45
15.	Visarpa	Anthrax/Erysipelas	C.D.- 55/139, V.M.- 57/97
16.	Stanapeeda	Pain in the breast	B.P.- 70/74
17.	Medoroga	Obesity	B.P.- 39/72
18.	Chippa and Kunakha	Infection of nails	B.P.- 61/75, V.M.- 27/18
19.	Netrabhishyanda	Conjunctivitis	C.D.- 59/58
20.	Arbuda Apachi	Tumors	K.N., C.D.- 41/60

21.	Shwasa Kasa Hikka	Dyspnoea, cough, Hiccough	S.B.M.- 4/375, 376
22.	Nadi vrana	Sinus	B.R.- 50/3
23.	Atisara	Diarrhoea	A.H.Su.-15/35
24.	Peenasa	Rhinitis	D.N.-Guduchyadi Varga -54, 55,56
25.	Vatarakta	Gout	B.P. Ci. - 29-79.
26.	Pama	Scabies	C.D.- 50/47

Pharmacological Studies on Haridra

Extensive pharmacological investigations on *Haridra* (Curcuma longa) and its active constituents—primarily **curcumin**, **turmerin**, and **tetrahydrocurcuminoids (THC)**—demonstrate a wide spectrum of biological activities validated through **in vitro**, **in vivo**, and some **clinical studies**.

Antioxidant Activity

- Curcumin, turmerin, and tetrahydrocurcuminoids exhibit **strong antioxidant properties**, effectively inhibiting lipid peroxidation and protecting DNA and cellular membranes.
- Turmerin showed up to **80% protection** against oxidative injury without cytotoxicity.
- THC demonstrated stronger antioxidant effects than curcumin, enhancing endogenous antioxidant enzymes (SOD, catalase, GPx, glutathione).

Anti-inflammatory and Anti-spasmodic Effects

- Curcumin and its analogues significantly reduced inflame-
- mation by inhibiting edema in experimental models.
- Certain curcuminoids (demethoxycurcumin, bisdemethoxycurcumin) showed **stronger anti-inflammatory activity** than curcumin.
- Curcumin exhibited **spasmolytic**, depressant, and transient hypotensive effects.

Anti-diabetic and Metabolic Effects

- Curcumin and THC significantly reduced **blood glucose**, **glycosylated hemoglobin**, and improved **insulin levels** in diabetic animal models.
- Restoration of carbohydrate metabolic enzymes and normalization of gluconeogenesis were observed.
- Activation of **PPAR-γ** by curcuminoids and sesquiterpenoids contributes to hypoglycemic action.
- Protective effects against **diabetic complications** such as nephropathy, cataract, oxidative stress, and impaired wound healing were demonstrated.

Antimicrobial, Antiviral, and

Anticancer Properties

- Exhibited **antibacterial activity**, though weaker than standard antibiotics.
- Curcumin showed **cytotoxic effects** against lymphoma cells and growth inhibition in cell cultures.
- Demonstrated **antiviral activity**, including trials in AIDS patients.
- Neuroprotective and anti-amyloid effects (e.g., Calebin-A) suggest potential in neurodegenerative disorders.

Material and Methods

Plant Material and Preparation of Extracts

Fresh rhizomes of *Curcuma longa* were collected, authenticated, washed, shade-dried, and coarsely powdered. The powdered material was subjected to extraction using different solvents such as aqueous, ethanol, and methanol to obtain crude extracts. The extracts were concentrated and stored in airtight containers for phytochemical analysis.

Relationship of Phytochemical Screening of Haridra (*Curcuma longa*) with Anti-Diabetic Activity

Phytochemical screening of Haridra (*Curcuma longa* Linn.) reveals the presence of several bioactive secondary metabolites such as flavonoids, phenolic compounds, alkaloids, terpenoids, saponins, glycosides, and steroids (Harborne, 1998; Kokate, 2010). These phytochemicals are reported to play a significant role in the management of diabetes mellitus through multiple biochemical and physiological mechanisms.

Flavonoids and phenolic compounds exhibit strong antioxidant activity, which is particularly important in diabetes mellitus, where oxidative stress leads to pancreatic β-cell dysfunction and insulin resistance (Srinivasan, 2007; Kumar et al., 2017). By scavenging free radicals, these compounds protect pancreatic β-cells and improve insulin sensitivity, thereby helping in the regulation of blood glucose levels.

Alkaloids identified during phytochemical screening are known to enhance insulin secretion from pancreatic β-cells and inhibit carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, leading to reduced intestinal glucose absorption and better post-prandial glycemic control (Kumar et al., 2017).

Terpenoids and steroids present in turmeric contribute to anti-diabetic activity by improving lipid metabolism, reducing insulin resistance, and enhancing glucose uptake in peripheral tissues (Srinivasan, 2007). These effects are crucial in type 2 diabetes mellitus, where dyslipidemia and insulin resistance are common pathological features. Saponins play a vital role in delaying glucose absorption from the gastrointestinal tract and improving glucose utilization, which helps in lowering hyperglycemia (Pandey and Tripathi, 2014). Glycosides detected

in Haridra extracts have also been reported to exhibit hypoglycemic activity by modulating hepatic glucose production and improving insulin action (Kumar et al., 2017).

Thus, phytochemical screening is an important tool in anti-diabetic research as it helps identify the presence of bioactive compounds responsible for glucose-lowering, antioxidant, and insulin-sensitizing effects. The synergistic action of these phytochemicals provides scientific validation for the traditional Ayurvedic use of Haridra in the management of Madhumeha (Sharma, 2005).

Experimental Procedure for Phytochemical Screening

Each extract (aqueous, ethanolic, and methanolic) was subjected to individual qualitative chemical tests using standard protocols described in pharmacognosy literature (Harborne, 1998; Kokate, 2010). About 1–2 mL of each extract was taken in clean test tubes and treated with specific reagents for the detection of different phytochemical groups.

For alkaloids, the extract was acidified with dilute hydrochloric acid and treated separately with Mayer's and Wagner's reagents; the formation of a cream or reddish-brown precipitate indicated a positive result, as described by Harborne (1998). Flavonoids were identified by the appearance of a pink or red coloration upon addition of magnesium turnings followed by concentrated hydrochloric acid (Shinoda test), according to standard procedures outlined by Kokate (2010).

Phenolic compounds and tannins were detected by the development of a blue-green or dark coloration upon addition of ferric chloride solution, following the method suggested by Harborne (1998). Saponins were identified by persistent froth formation after vigorous shaking of the aqueous extract, as reported by Pandey and Tripathi (2014). Glycosides were tested using Keller–Killiani reaction, where a brown ring at the interface indicated the presence of cardiac glycosides (Kokate, 2010).

Terpenoids were confirmed by the formation of a reddish-brown coloration in the Salkowski test, while steroids were identified by a green or bluish coloration in the Liebermann–Burchard test, following standard phytochemical methods (Harborne, 1998; Pandey and Tripathi, 2014). Carbohydrates were detected by the appearance of a violet ring at the junction of two liquids in Molisch's test (Kokate, 2010).

All experiments were carried out in triplicate to ensure reproducibility, and observations were recorded carefully based on visible color changes or precipitate formation.

Results

The preliminary phytochemical screening of *Curcuma longa* rhizome extracts revealed the presence of several bioactive constituents. The results are summarized in **Table-2**.

Table-2 Extraction of phytochemical screening of *Curcuma longa* rhizome

Phytochemical Constituents	Aqueous Extract	Ethanolic Extract	Methanolic Extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols & Tannins	+	+	+
Saponins	+	−	−
Glycosides	−	+	+
Terpenoids	+	+	+
Steroids	−	+	+
Carbohydrates	+	+	+

(+ = Present, − = Absent)

Discussion

Phytochemical screening serves as a crucial preliminary step in identifying bioactive compounds responsible for therapeutic effects (Harborne, 1998; Pandey and Tripathi, 2014). The presence of flavonoids, phenolic compounds, alkaloids, terpenoids, and glycosides in *Curcuma longa* extracts strongly supports its traditional use in diabetes mellitus.

Flavonoids and phenolic compounds are well-known for their antioxidant properties, which play a significant role in reducing oxidative stress-induced pancreatic β-cell damage in diabetes (Kumar et al., 2017; Srinivasan, 2007). These compounds also improve insulin sensitivity and glucose uptake in peripheral tissues. Alkaloids are reported to enhance insulin secretion and modulate carbohydrate metabolism enzymes (Kumar et al., 2017).

Terpenoids and steroids contribute to anti-diabetic activity by improving lipid metabolism, reducing insulin resistance, and protecting pancreatic tissue (Srinivasan, 2007). Saponins are known to inhibit intestinal glucose absorption and delay carbohydrate digestion, thereby controlling postprandial hyperglycemia (Kumar et al., 2017).

The synergistic action of these phytochemicals explains the multi-targeted anti-diabetic potential of *Curcuma longa* in Madhumeha.

Conclusion

The present study confirms that *Curcuma longa* rhizome contains diverse phytochemical constituents with proven relevance to diabetes management (Srinivasan, 2007; Sharma, 2005). Preliminary phytochemical screening reveals the presence of flavonoids, phenols, alkaloids, terpenoids, glycosides, and saponins, all of which contribute to anti-diabetic activity through antioxidant, insulin-sensitizing, and glucose-lowering mechanisms. Phyto-chemical screening

thus provides a scientific basis for the traditional use of Haridra in Madhumeha and forms a foundation for further anti-diabetic research using herbal extracts.

Haridra is an Ayurvedic classical drug having various pharmacological actions and it is used in the treatment of variety of physical and mental disorders since antiquity. In recent year Haridra has received a lot of attention from all the scientific communities in the field of health care system and pharmaceutical industry and to do extensive research, regarding its anti-diabetic, antioxidant, hepato-protective, anti-inflammatory, anti-carcinogenic and antimicrobial properties.

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 analysis of *Inula Cappa*

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Abstract- *Inula cappa* is a medicinal shrub widely distributed across the Himalayan region and Southeast Asia. Used extensively in traditional systems of medicine, the plant is known for its pharmacological potential, particularly its antioxidant, and anti-inflammatory activities. This study provides a comprehensive evaluation of *Inula cappa*, focusing on its phytochemical constituents of the leaves and stems. The results validate the traditional use of *Inula cappa* and suggest its potential application in modern herbal medicine. The analysis of *Inula cappa* resulted in the identification of chemical constituents. These compounds were identified based on their respective retention indices.

Key words: Phytochemical, antioxidant and anti-inflammatory.

Introduction

Inula cappa (syn. *Duhaldea cappa*) is a medicinal plant belonging to the Asteraceae family and is naturally distributed in the high-altitude regions of the Himalayas. It grows commonly along forest edges, open grasslands, and mountain slopes between 1,500 and 3,000 meters. The plant is easily identified by its aromatic character, hairy leaves, and bright yellow, daisy-like flowers[1]. Although not widely known outside local communities, it holds a steady place in traditional herbal practices.

For generations, people living in the Himalayan regions of India, Nepal, Bhutan, and China have used *Inula cappa* as a home remedy for common ailments. Its leaves and stems are typically prepared as teas, decoctions, or pastes to treat cough, sore throat, chest congestion, diarrhoea, and minor skin infections. The plant is also applied externally to soothe wounds and reduce inflammation. In many areas, it is collected seasonally, dried, and stored for year-round use[2].

Studies suggest that extracts of the plant, especially methanolic and chloroform extracts, may support respiratory and digestive health, aligning with traditional claims.

Phytochemical investigations have revealed that *Inula cappa* contains several important secondary metabolites, including flavonoids, phenolic compounds, and terpenoids. The presence of such compounds

indicates that *Inula cappa* may possess significant therapeutic value and could serve as a source of new bioactive molecules[3].

With the increasing global interest in herbal medicines, proper scientific evaluation of plants like *Inula cappa* has become essential. Herbal drug evaluation ensures correct identification, assesses the safety of plant materials, and measures the consistency of active compounds[4]. This not only validates traditional knowledge but also supports the development of reliable, evidence-based herbal formulations and potential new therapeutic agents.

Figure- 1 *Inula cappa* **plant**

Morphology of *Inula cappa*

Inula cappa, a distinctive herb native to the Himalayan region, may seem modest in appearance, yet its structure reflects an elegant and well-adapted design shaped by its natural environment. This section provides a clear and simplified overview of the plant's morphology, describing the characteristics of its leaves, stems, flowers, and roots. Understanding these structural features not only helps in proper identification of the species but also highlights the biological adaptations that enable *Inula cappa* to thrive in high-altitude ecosystems.

Leaves- The leaves of *Inula cappa* are a vital part of its identity. They not only help the plant survive and thrive, but are also the parts most commonly used in traditional medicine[5]. They are arranged alternately on the stem, which means each leaf grows on its own at different levels rather than directly opposite another. The edges (margins) of the leaves are usually entire or slightly toothed, and the tip is pointed. The base is typically rounded or narrowed, attaching to a short leaf stalk (petiole) or sometimes directly to the stem (sessile).

Flowers- The part of *Inula cappa* that truly makes it stand out—its flowers[6]. These bright, yellow blossoms are not only beautiful but also carry much of the plant's medicinal potential. They are arranged in a capitulum (a dense flower head), which is typical of the Asteraceae family. Each head consists of many small florets packed together to look like one large flower. They are

usually bright yellow to golden-yellow, giving the plant a radiant and eye-catching look during its blooming season, which is typically from June to September.

Each flower head consists of two types of florets:

Ray florets: These are the long, strap-like petals around the edge that make the flower look daisy-like[7].

Disc florets: These are the small, tubular flowers in the centre. They may have a mild scent and are known to attract bees, butterflies, and other pollinators, making *Inula cappa* important not just for medicine, but for the environment as well.

Figure- 2 Leaves of *Inula cappa*

Figure- 3 Flowers of *Inula cappa*

Fruits and Seeds- After the blooming season, the flowers of *Inula cappa* mature into small fruits, which contain the seeds—nature's way of ensuring the plant's slopes where the wind is strong[8]. This helps it colonize new spaces naturally.

Roots- The root system of *Inula cappa* plays a major role in anchoring the plant and helping it absorb nutrients. They develops a taproot system, meaning it has one main central root that grows deep into the soil, with several smaller side roots branching from it. The roots are usually thick, fibrous, and slightly woody, especially in older plants.

They penetrate well into the ground, which helps the plant survive in dry, rocky soils.
In traditional medicine, not just the leaves and flowers, but also the roots are sometimes used for their therapeutic properties. They are believed to help with inflammatory issues, coughs, and digestive problems.

They are traditionally harvested for medicinal use and are known to contain:

i. Sesquiterpene lactones
ii. Terpenoids
iii. Alkaloids

Decoctions made from the roots are used to treat **liver ailments, rheumatism, sore throats**, and various forms of **inflame-mation**. The root system also plays a crucial role in anchoring the plant in its mountainous terrain and storing bioactive compounds during adverse seasonal conditions.

Pharmacological Activity of *Inula cappa*

Inula cappa has long been valued in traditional medicinal systems for its effectiveness against various health

conditions. Emerging scientific studies now support many of these traditional uses by identifying several important bioactive compounds, including flavonoids, phenolic acids, terpenoids, and sesquiterpene lactones, which are responsible for its medicinal properties[9]. This research provides an overview of the pharmacological activities of *Inula cappa.*

Phytochemical Constituents of *Inula cappa*

Inula cappa is known for its diverse array of secondary metabolites, which play a key role in its therapeutic properties. Phytochemical analyses indicate that the plant's leaves, stems, and roots contain substantial amounts of flavonoids, phenolic acids, sesquiterpene lactones, essential oils, alkaloids, tannins, and various other biologically active compounds. The specific composition and concentration of these constituents can differ based on the plant part examined and the extraction solvent used[10].

Flavonoids

Flavonoids represent one of the most abundant groups of phytochemicals present in Inula cappa. These polyphenolic compounds are well recognised for their strong antioxidant, anti-inflammatory, and liver-protective activities. Studies have shown that the methanolic extracts of the plant's leaves and stems contain particularly high levels of flavonoids. Several key flavonoids, including quercetin, kaempferol, luteolin, and apigenin, have been identified in Inula cappa[11]. These bioactive molecules play a crucial role in neutralising free radicals, preventing lipid peroxidation, and regulating pro-inflammatory cytokines, thereby contributing to the overall therapeutic potential of the plant.

Phenolic Compounds

Phenolic compounds present in *Inula cappa* are well known for their strong antioxidant, antimicrobial, and anti-aging effects. These bioactive molecules play a key role in defending the body against oxidative stress by neutralising harmful free radicals and limiting cellular damage caused by environmental pollutants, aging processes, and chronic inflammation[12]. Because phenolics dissolve more readily in polar solvents like methanol and ethanol, these solvents are commonly used to obtain phenolic-rich extracts from the plant. Among the major phenolic acids identified in *Inula cappa* are chlorogenic acid, caffeic acid, and gallic acid. These compounds not only demonstrate powerful antioxidant activity but also show therapeutic potential in reducing the risk of cancer, cardiovascular diseases, and neurodegenerative disorders. Their protective effects against oxidative injury further justify the plant's traditional use in various medicinal preparations.

Tannins

Tannins found in *Inula cappa* are naturally occurring polyphenolic compounds recognised for their astringent, antimicrobial, and wound-healing properties. These compounds are predominantly present in the leaf extracts and contribute significantly to the plant's therapeutic value. Owing to their ability to precipitate proteins, tannins create a protective layer over mucosal tissues, which helps reduce irritation and inflammation. This mechanism makes them particularly useful in managing gastrointestinal disorders such as diarrhoea by decreasing fluid loss and supporting

the repair of intestinal lining. Their antimicrobial effects also aid in preventing infections in wounds and skin lesions. These beneficial properties correspond well with the traditional use of *Inula cappa* leaves in treating diarrhoea, minor wounds, and various skin infections.

Alkaloids

Although present in relatively low concentrations, alkaloids in *Inula cappa* are thought to contribute to the plant's analgesic and antibacterial properties. Alkaloids are nitrogen-containing compounds widely distributed in medicinal plants and are known for their broad range of pharmacological effects. In the case of Inula cappa, the specific alkaloid types and their exact quantities are still being explored through ongoing research. Early findings indicate that these compounds may help relieve pain by influencing neural pathways involved in pain perception[13]. Their antibacterial activity is believed to result from their ability to disrupt the integrity of microbial cell membranes, thereby inhibiting bacterial growth.

Material and Methods

Plant collection and preparation for extraction

Proper collection and preparation of plant material is essential in herbal research to ensure that the resulting extracts are pure, effective and suitable for laboratory analysis. Fresh leaves and stems of *Inula cappa* were gathered from a high-altitude region of Uttarakhand during the non-flowering season. After confirming the plant's identity, the required parts were collected using clean, sterilized tools such as gloves and scissors to maintain hygiene and prevent contamination. Only mature, healthy, and undamaged samples were selected, while any discoloured, infected, or wilted parts were excluded. To avoid environmental contaminants, plants growing near roadsides, industrial sites, or polluted areas were not selected for sampling.

Collection was carried out early in the morning, shortly after the dew had dried, to preserve the plant's active constituents, particularly those sensitive to heat or moisture. The collected material was immediately cleaned to remove soil, insects, and other impurities. Initially, the samples were rinsed under running tap water to remove visible debris, followed by a final wash with distilled water to ensure purity. Care was taken to avoid excessive handling or bruising, as damage to tissues may affect the stability of phytochemicals.

Drying the plant material is a crucial step, as it prevents microbial growth and preserves the therapeutic compounds. Shade drying was chosen because it is gentle and helps retain the natural colour, aroma, and bioactive components of Inula cappa. The cleaned leaves and stems were spread in a thin, even layer on clean papers or mesh trays and kept in a cool, dry, and well-ventilated area, away from direct sunlight. Exposure to sunlight was avoided to prevent degradation of sensitive phytochemicals. The shade-drying process lasted for approximately 10–15 days, allowing gradual and uniform removal of moisture.

Once completely dried, the plant material was ground into a fine powder using a mechanical grinder. Powdering increases the surface area of the material, thereby improving extraction efficiency and enabling accurate weighing for experimental use. The powdered material was sieved to obtain a uniform particle

size and then stored in airtight containers, preferably glass or food-grade plastic. These containers were kept in a cool, dry, and dark place to prevent moisture absorption, oxidation, or loss of phytochemical activity.

Before extraction, the powdered material was inspected for quality. This included checking for signs of fungal contamination, residual moisture, or abnormal odours. Only samples that were thoroughly dried, clean, and well preserved were used for extraction to ensure the reliability and accuracy of subsequent experimental results.

Figure- 4 Dried leaves of *Inula cappa*

Figure- 5 Dried stems of *Inula cappa*

Extraction Procedure

Approximately 100 grams of powdered leaves and stems were subjected to **successive solvent extraction** using the following solvents in increasing polarity:

- **Hexane** (non-polar)
- **Chloroform** (moderately polar)
- **Methanol** (polar)

Preliminary Phytochemical Screening

Standard qualitative tests were performed to detect the presence of major phyto- chemical groups in all three extracts (hexane, chloroform, and methanol). The following tests were employed:

Chemical tests performed on plant extracts using solvents like methanol, ethanol, chloroform, or water—to detect the presence of important bioactive compounds.

Test for Alkaloids

Reagents Used: Mayer's, Dragendorff's, and Wagner's reagents.
Procedure: A few drops of reagent are added to the extract.
Positive Result: Formation of a **white or reddish-brown precipitate** indicates the presence of alkaloids.

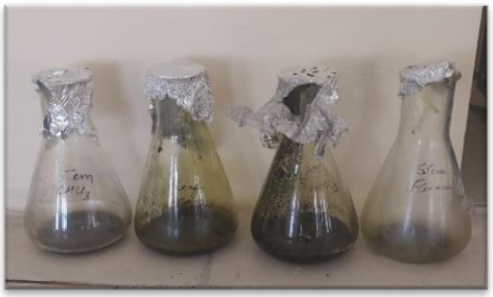


Figure-6 Various Extractions

Test for Flavonoids

Reagents Used: Magnesium ribbon and concentrated HCl (Shinoda test).
Procedure: Add magnesium and HCl to the extract.
Positive Result: A **pink, orange, or red coloration** indicates the presence of flavonoids.

Test for Tannins

Reagent Used: Ferric chloride solution.
Procedure: Add ferric chloride to the extract.
Positive Result: A **blue-black or greenish-black colour** confirms the presence of tannins.

Test for Phenolic Compounds

Reagent Used: Ferric chloride.
Positive Result: Formation of **deep blue or green colour** indicates phenols.

Test for Saponins

Foam Test: Shake the extract with water.
Positive Result: Persistent froth or foam indicates saponins.

Test for Terpenoids

Salkowski Test: Mix extract with chloroform and add concentrated sulphuric acid.
Positive Result: A **reddish-brown interface** shows terpenoids.

Test for Steroids

Liebermann-Burchard Test: Mix extract with acetic anhydride and sulfuric acid.
Positive Result: A **green or blue coloration** confirms steroids.

Test for Glycosides

Keller-Killiani Test:
Procedure: Mix the extract with glacial acetic acid containing a trace of ferric chloride. Carefully add concentrated sulfuric acid along the side of the test tube to form two layers.
Positive Result: A **blue or bluish-green ring** appears at the **interface**, indicating the presence of **cardiac glycosides** (deoxy sugar).

Table- 1 Test for phytochemical group

Phytochemical Group	Test Used	Indicator
Alkaloids	Mayer's, Wagner's, Dragendorff's	Cream/orange precipitate
Flavonoids	Shinoda Test	Pink/red coloration
Phenolic Compounds	Ferric Chloride Test	Deep blue/green colour
Tannins	Gelatin Test	White precipitate
Terpenoids	Salkowski's Test	Reddish-brown interface
Steroids	Liebermann–Burchard Test	Green/blue ring
Saponins	Foam Test	Persistent foam
Glycosides	Legal's Test	Pink to red colour
Proteins	Biuret Test	Violet colour

Results and Discussion

Phytochemical Screening Results

The preliminary phytochemical screening of *Inula cappa* extracts confirmed the presence of a wide array of secondary metabolites. The methanolic extract demonstrated the richest phytochemical profile, showing positive tests for **flavonoids, phenolics, tannins, alkaloids saponins, steroids, terpenoids, and glycosides**.

Chloroform extract also showed moderate results, while the hexane extract exhibited limited constituents, primarily **terpenoids and essential oils**.

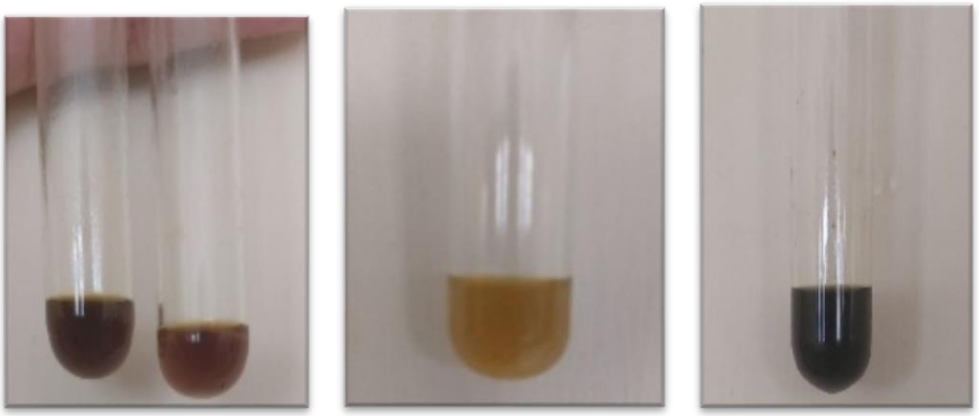
These findings suggest that polar solvents like methanol are more effective in extracting diverse phytoconstituents from *Inula cappa*, consistent with the solubility of phenolics and flavonoids in polar media.

Table – 2 Phytochemical properties from various extract of *Inula cappa*

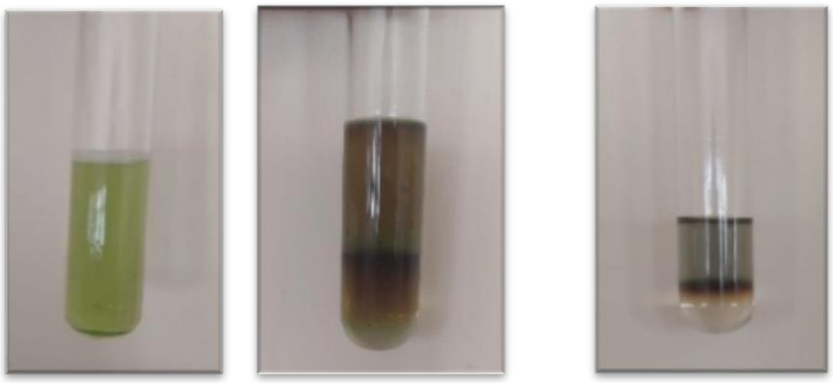
Phytochemical Group	Hexane Extract	Chloroform Extract	Methanol Extract
Alkaloids	–	+	+
Flavonoids	–	+	+++
Tannins	–	–	++
Phenolic Compounds	–	+	+++
Saponins	–	–	+
Terpenoids	++	+	+
Steroids	++	++	+
Glycosides	–	+	++
Essential Oils	++	+	–

Notes:

- (+++) = Strong presence
- (++) = Moderate presence
- (+) = Mild presence
- (–) = Absent or negligible



a) Alkaloids b) flavonoids c) tannins/phenolic compounds



d) Saponins e) glycosides f) terpenoids/ steroids

Figure- 7(a-f) Phytochemical Tests of various phytochemical constituent

Traditional uses of *Inula cappa*

Traditional uses of *Inula cappa*—for fever, joint pain, sore throat, and liver conditions—align with its **validated anti-inflammatory, antioxidant, and hepatoprotective activities**. The presence of specific phytochemicals (e.g., luteolin, quercetin, chlorogenic acid) provides a mechanistic basis for its ethnomedicinal value[15]. The findings also suggest its potential in developing herbal formulations, Neutraceuticals, or lead compounds for future drug discovery.

Conclusion

The results of preliminary phytochemical screening revealed that *Inula cappa* contains a broad spectrum of secondary metabolites, including flavonoids, phenolic compounds, tannins, alkaloids, saponins, steroids, glycosides**,** and terpenoids**.** These compounds are known for their wide-ranging biological activities, including antioxidant, antimicrobial, antiinflammatory, and hepatoprotective effects. Notably, the methanolic extract showed the highest presence of these phyto-chemicals, indicating the effectiveness of polar solvents in extracting bioactive constituents.

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 antimicrobial screening of *Dracaena trifasciata* leaf extracts

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Abstract- *Dracaena trifasciata* (syn. *Sansevieria trifasciata*) species belongs to the genus *Dracaena*, commonly known as "snake plant". It is widely used as a common indoor plant, cultivated worldwide. The plant is recognized for pollutant-absorbing ability, it also produces large amount of oxygen at night and considered as air purifier plant for indoor environments. It can eliminate toxins, benzene, formaldehyde, trichoethylene and toluene from the surrounding. *Dracaena trifasciata* can reduce CO2 concentration by 10% to 20%. It's also been used as traditional remedies across Asia and Africa for cough, flu, respiratory tract inflammation, diarrhoea, wound healing, and snakebites etc. The plant contains bioactive compounds found in the roots and leaves include alkaloids, tannins, terpenoids, saponins, phenols, sterols- beta-sitosterol, stigmasterol, polyphenols, carbohydrates, and steroid-abamagenin (a glycoside that can break toxins), cardenolides (cardiac active steroids). It also possesses antibacterial, anti-fungal, antioxidant, anti-diabetic and anticancer activity.

The aim of this study to provide insights into the phytochemical constituents of methanol and hexane plant extracts prepared using solvent extraction method, along with the antimicrobial activity of *D. trifasciata* against *Staphylococcus aureus* (*ATCC 6538)* and *E.coli* (ATCC 8739) using agar well diffusion method. The study will also be helpful in exploring its use in traditional medicine and prospects for further advancement to promote the broader application.

Key words: Anti-toxic, antibacterial, anticancer, antioxidant, Sansevieria, snake plant

Introduction

Dracaena trifasciata, commonly known as the cylindrical snake plant or mother-in-law's tongue, (Said et al., 2015; Yumna et al., 2018) is categorized under the Genus *Dracaena*. *D.trifasciata* is a member of the family Asparagaceae, characterised by sword-shaped, dark green leaves with attractive patterns. It is a xerophytic perennial succulent plant, adapted to dry tropical and subtropical regions and high-salt soils and high-temperature climates (Takawira and Nordal, 2003; Lu and Morden, 2014; Gaylor, Juntunen et al., 2018). It is native to tropical West

Africa, specifically Nigeria, southern Asia, Madagascar and the Congo region. It is one of the poorly explored species, despite its relevance in folkloric medicine, very limited is known about its phytochemicals and biological activity. The *D.trifasciata* is recognized for its capability to absorb pollutants both indoors and outdoors, functioning as an air purifier, effectively absorbing harmful gases including formaldehyde, xylene, and total volatile organic compounds (Ullah et al. 2021; Guo et al. 2023; Sutrisno et al. 2023; Weerasinghe et al. 2023).

Dracaena trifasciata is valued for its leaf fibre's, ornamental value and ethnopharmacological background (Khalumba, Mbugua et al., 2005). The plant has high economic value, primarily due to the abundant fiber content, being used as a natural textile raw material (Adeniyi et al. 2020; Papaj 2022; Raj et al. 2023). The elastic, white, and strong fibers derived from D. trifasciata are used in the production of ropes, clothing, fishing lines, bowstrings, fine weaves, and binding cords due to their high strength (Sathishkumar 2016; Widyasanti et al. 2020). *D. trifasciata* has been used in traditional medicine in Asia and Africa in the form of juice or decoction from its leaves for the treatment of gonorrhea, earaches, toothaches, respiratory tract inflammation, flu, diarrhoea, coughs, haemorrhoids, influenza, inflammatory ailments and snakebites (Morton, 1981). The leaf latex of this plant is applied externally to treat bruises, sprains, wounds, abscesses, scabs, itchiness, and ear diseases, alongside being used as a natural antibiotic, hair tonic, and pain reliever (Sunilson et al. 2009; Andhare et al. 2012; Berame et al. 2017; Aseptianova 2019; Hijrah et al. 2019; Nahdi and Kurniawan 2019; Sun et al. 2019; Hartanti and Budipramana 2020; Thu et al. 2020; Pathy et al. 2021). In Africa, the latex from this plant can be used as a snake and insect repellent (Umoh et al. 2020; Sharma et al. 2023). Antibacterial agents are also derived from D. trifasciata. In tropical countries, D. trifasciata is used for treating inflammatory diseases and is sold in markets as a crude oil for treating snake bites, earaches, swelling, boils, and fever (Aliero et al. 2008; Anbu et al. 2009).

The plant has glycosides, phenolics, tannins, saponins, flavonoids and steroids, dicarboxylic acids, coumarins, and fatty acids (Yumna et al., 2018; Umoh et al., 2020). The abundance of such potent bioactive phytoconstituents makes it a biologically active plant species. *D.trifasciata* possess antibacterial activity, which has been ascribed to the presence of a number of bioactive compounds like quinolone, 3,4-dimethoxybenzoic acid, palmitaldehyde, 1,2-benzenedicarboxylic acid, and delta-undecalactone (Yumna et al., 2018). Fatty acids and their esters are the predominant phytochemicals in the leaves and rhizomes; there has been concrete evidence of their antimicrobial and anticancer potential (Jo´´zwiak et al., 2020).

Several fatty acids have shown anticancer activity by inducing apoptosis and autophagy and by inhibiting DNA topoisomerase (Jiang et al., 2017; Bharath et al., 2021).

Considering the apparent lack of available literature on the phytochemical constituents of different parts of S. cylindrica and their biological activity, the current study was intended to explore the phytochemicals in *D.trifasciata* and to evaluate its antimicrobial activity.

Material and Methods

Plant collection: Plant sample was collected from himalaya wellness company.

Part used: Leaves

Extract preparation: 50g of the ground powder was added into 250ml of solvent each solvent in wide mouth conical flask followed by 6-7 hours of shaking. Methanol and hexane extract was prepared, extracts were filtered using Whatman filter paper No 1. The filtrates were then concentrated by using water bath 60°-80°c and stored in screw-capped tubes in refrigerated prior to use.

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out for extracts using the standard procedures. For each procedure, details of these have been furnished below-

1. **Test for alkaloids-** The presence of alkaloids in the plant extract was tested 2 ml of the plant extract was treated with 1ml of each reagent separately.
   Mayer's reagent - Dull white precipitates indicated the presence of alkaloids.
   Dragendroff's reagent-Formation of orange or orange red precipitates indicated the presence of alkaloids.

2. **Test for flavonoids**
   Alkaline reagent test- to the test solution few drops of sodium hydroxide solution was added, Intense yellow colour was formed which turned to colourless on addition of few drops of dilute acid it indicated presence of flavonoids.

3. **Test for saponins-** This was carried out by taking 2ml solution of test sample in the test tube was then shaken vigorously. A persistent froth that lasted for at least 15mins indicated the presence of saponins.

4. **Test for tannins-** Ferric Chloride Test- The extracts were treated with 3 drops of 5% ferric chloride solution. A green-black or blue-black colour indicated the presence of tannins.

5. **Test for steroids-** Salkowski's tests- Treat the extract few drops of concentrated sulphuric acid poured by sides of the test tube red colour layer indicates presence of steroids and sterol compound in the extract.

6. **Test for triterpenoids-** Salkowski's tests -Extract was treated with few drops of concentrated sulphuric acid formation of yellow coloured lower

layer indicated presence of triterpenoids.

Determination of anti-microbial activity

Well-Diffusion method:

Pre-inoculated nutrient agar media was poured in Petri dishes, the plates were allowed to cooled and settled inside the laminar air flow. After the plates get solidified 8mm well was made using a well cutter. And 200µl sample was loaded in the well. The inoculated plates were incubated in incubator at 30-35°c for 12-24 hrs.

Strains used- *Staphylococcus aureus* (*ATCC 6538*), *E.coli* (ATCC 8739).

Results and Discussions

Table-01 Phytochemical analysis

Test	Methanol	Hexane
Alkaloids	+	-
Flavonoids	+	-
Saponins	+	-
tannins	+	-
Steroids	+	-
Triterpenoids	-	+

Phytochemicals like alkaloids, flavanoids, saponins, steroids, tanins were found present in the methanolic extract, whereas hexane have only triterpenoids present. But overall plant showed positive result for the above tests. From the **(Table-1)** results plant found rich in phytochemical content, which make the *D.trifasciata* anti-microbially active against microbes.

Table-02 Antimicrobial activity

SOLVENT	***S.aureus***	***E.coli***
Methanol	21	16
Hexane	11	NA
Positive control	32	29

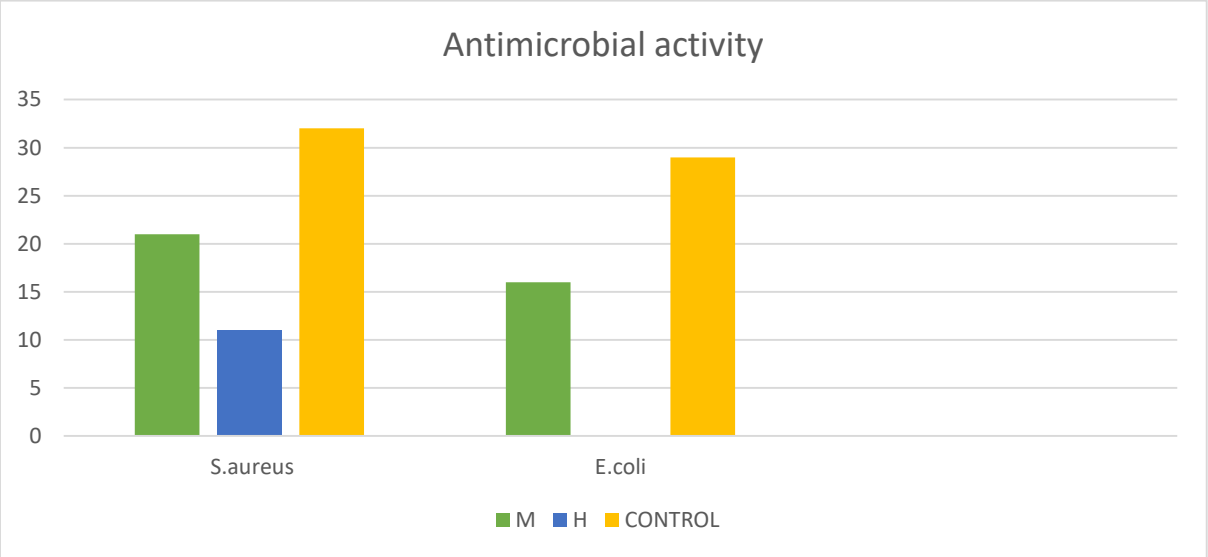

Figure-1

The assessment of the antibacterial activity of different extracts in this study was based on measurement of diameter of Zones of inhibition formed around the wells as depicted in **Tables-2** and **Figure-1**. Methanolic extract of *D.trifasciata* leaves was found more active with relatively larger zones of inhibition against tested pathogens. Hexane extract had smaller zones in comparison with methanol long with little activity against *Staphylococcus aureus* and no activity against *E.coli*.

But all extracts has shown a significant and modest antibacterial activity against *S.aureus*, *E.coli* except hexane extract against *E.coli* which exhibited no activity.

Conclusion

It can be concluded from our study and analysis results, that this plant is rich in phytochemicals like tannin, flavonoid, alkaloid, steroids, saponins etc, which makes the plant active against the microorganisms. Hence the plant was found active against *S.aureus* and *E.coli* .Comprehensive literature studies showed that the attributes of D. trifasciata can be applied as an, having a promising source of natural compounds for novel drug development. Further investigations are needed in this direction to assess the long-term and short-term toxicity associated with the use of this plant material, thereby making it a potential source for the development of modern drugs from natural sources.

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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HPTLC Fingerprinting and Antimicrobial Analysis of Trikatu: A Comprehensive In-Vitro Study

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Abstract- Piper nigrum and Piper longum both belonging to family Piperaceae and Zingiber officinale belonging to Zingiberaceae family, are well known in traditional medicine. Trikatu is a traditional herbal formulation containing three herbs mixed together in equal quantities. Trikatu churma plays an important role in balancing respiratory and digestive health. The major aim of present study was to study the antimicrobial activity and chromatographic evaluation of Trikatu to scientifically validate its traditional uses.

The chromatographic evaluation of Trikatu using High-Performance Thin-Layer Chromatography (HPTLC) to identify key phytochemical markers. Distinct bands corresponding to major bioactive compounds were observed, enabling comparative profiling and confirmation of formulation integrity. The developed HPTLC fingerprint provides a reliable tool for routine quality assessment, authentication, and standardization of Trikatu.

Extract of Trikatu i.e. P. nigrum, P. longum and Z. officinale were tested for their antimicrobial activity by agar well diffusion method. The Methanol, Hexane and Aqueous extract of the

Trikatu was studied against Staphylococcus aureus and Escherichia coli as test microorganisms. The results reveal that the Methanol has shown significant activity against Staphylococcus aureus and Escherichia coli as compared to Hexane and Aqueous extract indicating the plant can restrain these organisms.

This project also explores the antiviral relevance of Trikatu by reviewing ancient textual references and correlating them with modern antiviral pharmacology. References such as the Vedas, Puranas, and Ayurvedic Samhitas. Through a comparative analysis of scriptural descriptions and current scientific literature, this study proposes that the synergistic phytoconstituents of Trikatu may exhibit antiviral actions through immunomodulation, bio

enhancement, and respiratory health support.

Key words: Piper nigrum, Piper longum, Zingiber officinale, Antimicrobial, Chromatographic evaluation, Well diffusion, Staphylococcus aureus, Escherichia coli, Synergistic phytoconstituents, Antiviral actions, Immunomodulation, Bio enhancement.

Introduction

The word Trikatu is derived from a Sanskrit word where "Tri" means three and "Katu" means bitter. Trikatu is a traditional herbal formulation containing three herbs mixed together in equal quantities. These three herbs are Marich (Piper nigrum), Pippali (Piper longum), and Sunthi (Zingiber officinale). Trikatu churma plays an important role in balancing respiratory and digestive health. In stomach, it increases production of digestive juices thereby stimulating digestion. It has a powerful effect on mucous membrane of the gastrointestinal tract and regulates the intestinal functions to facilitate absorption. Trikatu acts primarily by its effect on stomach, liver, and pancreas. In liver, it acts as Cholagogue and increases production of bile salts by stimulating gallbladder functioning.

Dosage: Indigestion & Bloating ¼ to ½ teaspoon of Trikatu powder with warm water before meals. Weight Loss ½ teaspoon Trikatu with honey in the morning. Cough & Cold Mix ¼ teaspoon Trikatu with honey and consume twice daily. Joint Pain & Inflammation ¼ teaspoon Trikatu powder with warm turmeric milk before bedtime.

Precaution: Trikatu may worsen gastritis and cause a burning sensation if taken in higher doses due to its hot potency. It is not recommended for pregnant and breastfeeding women without a doctor's consultation.

Piper nigrum

P. nigrum belonging to the family Piperaceae is commonly known as "Maricha", is a climber plant. Maricha is formed by drying the green unripe fruit of the pepper plant. For many days it is Sun-dried or machine-dried, during which pepper gains black wrinkled skin. Maricha is an herb which can aggravate pitta. Black Pepper is exceedingly a very popular and important ingredient that is used in cooking worldwide. It is usually used in flavouring food but beside this the herb provides medicinal benefits. It has ability to improve the function of the digestive system, which reduces flatulence and bloating along with other medicinal properties.

Piper longum

Long pepper or Pippali is a fragrant, thin vine with creeping, segmented stems. Its fleshy fruits are set into elongated spikes. Spike turns red in colour once ripened. Long pepper has its reference from the ancient textbooks of Ayurveda. Ayurveda has explained various uses of long pepper for dietary purposes as well as for various health purposes. It is basically used for a healthy respiratory system. Long pepper is

also beneficial for healthy digestion and healthy metabolism as well. Pippali is a useful herb for cold and mucus conditions of the lungs as it boosts vasodilatation and increases circulation to the lung. It acts as a bronchodilator, decongestant and expectorant. Pippali is a strong rejuvenative for the lungs. This herb is recommended as it will prevent colds.

Zingiber officinale

Zingiber officinale is a flowering plant, which is commonly known as ginger. It belongs to the Zingiberaceae family. The Indian name of this plant is dry ginger or Saunth/ Sunthi or Adrak. It is very commonly used spice. Gingerols is its natural oil that provides a unique flavour and aroma to this spice. Ginger is famous in medicine system because of its medicinal properties. It has very powerful anti-inflammatory and anti-oxidant properties.

Chemical Composition

Piper nigrum and Piper longum belonging to the family Piperaceae has a distinct sharp flavour attributed to the presence of the phytochemical, piperine. Piperine, the active compound in Black pepper and long pepper offers several health benefits, including enhancing nutrient absorption, reducing inflammation and acting as an antioxidant. Piperine displays numerous pharmacological effects such as antioxidant, antimicrobial, and immunomodulatory effects in various in vitro and in vivo experimental trials.

Gingerols and Shogaols are the major pungent compounds present in the rhizomes of ginger i.e. Zingiber officinale and are renowned for their contribution to human health and nutrition. They have anti-inflammatory and digestive stimulant properties. Both gingerols and shogaols exhibit a host of biological activities, ranging from anticancer, anti-oxidant, antimicrobial, anti-inflammatory and anti- allergic to various central nervous system activities.

In Ayurvedic practice, Trikatu is primarily used to stimulate "Agni" i.e. the digestive fire and eliminate "Ama" i.e. undigested toxins.

Present work was carried out with the objective of Chromatographic evaluation and investigate antimicrobial activity of different solvent extracts of Trikatu against S. aureus and E. coli.

Material and Methods

Samples and Chemicals- Herbal sample of Trikatu, Solvents: Methanol, Ethanol, Chloroform, Distilled water

Phases- **a. Stationary phase:** Pre-coated silica gel 60 F254 plates (10×10 cm)
b. Mobile phase: Chloroform: Methanol (90:10)

Instruments- a. HPTLC applicator (Linomat)Twin-trough developing chamber
b. UV cabinet (254/366 nm)
c. CAMAG TLC Visualiser 3
d. Computer (VisionCATS Software)
e. Analytical balance

f. Water bath

Accessories- a. Microliter syringe
b. Whatman filter paper
c. Glassware: Volumetric flasks, Conical flasks, round bottom flasks, Measuring cylinder.

Chromatographic evaluation of Trikatu

High-Performance thin layer chromatography serves as an extension of thin-layer chromatography, offering robustness, simplicity, speed, and efficiency in the qualitative analysis of compounds.

The three herbs of Trikatu were collected and Grinded into fine powder. Each herb powder was mixed in equal proportion. The sample extract was prepared by accurately weighing 20 g of Trikatu and dissolving it in 150 mL Methanol, Ethanol and Aqueous solvent respectively. The solution was mixed well. After mixing, the solution was filtered through Whatman filter paper to obtain a clear sample extract suitable for chromatographic analysis. To concentrate the sample, it was put in the water bath.

For chromatographic separation, pre-coated silica gel 60 F254 plates were selected as the stationary phase. The sample extract was then applied on the plates using a CAMAG Linomat applicator. Application was done in the form of measured bands of 12 mm in width, ensuring uniform distance from the edges of the plate and uniform spacing between them.

A suitable mobile phase was selected. The mobile phase components i.e. Chloroform and Methanol (90:10) were mixed and poured into a twin-trough glass developing chamber. To ensure a stable and uniform saturation, the chamber was lined with saturation pads. Then the prepared Silica gel plate was carefully placed inside the chamber, ensuring that the sample application line remained above the mobile phase level. Development was allowed to occur until the solvent reached a marked distance of 8.5 cm. The plate was then removed and dried.

Once dried, the developed plate was observed under TLC visualizer. UV light was used at 254 nm and 366 nm to visualize the bands. The Vision CATS software generated TLC bands. These data were used for qualitative analysis.

Observation

The fingerprint profile generated from the HPTLC analysis provided information on the chemical composition, purity, and consistency of the herbal sample. The clear bands indicate good quality of raw material and absence of adulteration.

Antimicrobial activity of Trikatu

The agar well diffusion method is used to evaluate the antimicrobial activity of a Trikatu on test microorganisms i.e. S. aureus (gram positive) and E. coli (gram negative) bacteria. Two separate Conical flasks of 250 mL were taken. Agar medium was prepared measuring 6g Nutrient

agar medium (Mueller Hinton agar) and dissolved it 200 mL Purified water. The medium was then sterilized in an autoclave at 121°C for 30 minutes. After cooling the medium, grown bacteria of S. aureus and E. coli from separate test tube was inoculated using an inoculating needle. Bacteria inoculated was then suspended into the Nutrient agar medium and labelled.

The nutrient agar medium was then poured in 8 petri dishes (4 for S. aureus and 4 for E. coli) and allowed to solidify. Wells of 6–8 mm diameter were made aseptically in the agar using a sterile cork borer. Each well was filled with Trikatu extract of Methanol, Hexane and Aqueous solvent. 2 petri dishes were filled with positive control i.e. Methanol. After loading the wells, the plates were left undisturbed diffusion of the extract into the agar.

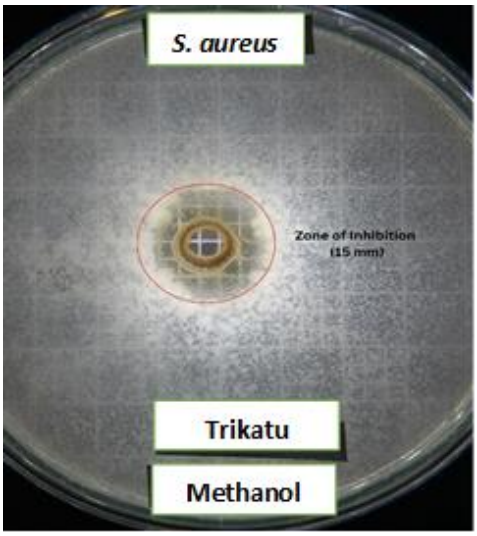
The prepared plates were incubated at 37°C for 18–24 hours. After incubation, the plates were examined for the presence of clear zones around the wells, indicating inhibition of bacterial growth. The diameter of each zone of inhibition was measured in millimetres using a transparent ruler. Larger inhibition zones indicated stronger anti-microbial activity. The results were recorded and compared across samples and controls, and concentrations to assess the potency of Trikatu against both bacterial strains.

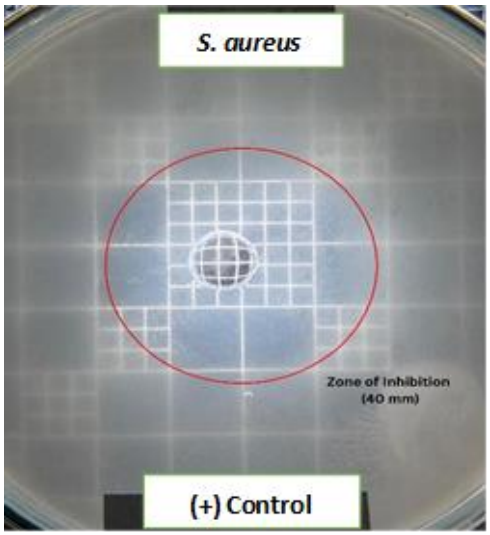
Table-1 *Staphylococcus aureus* (Gram positive bacteria)

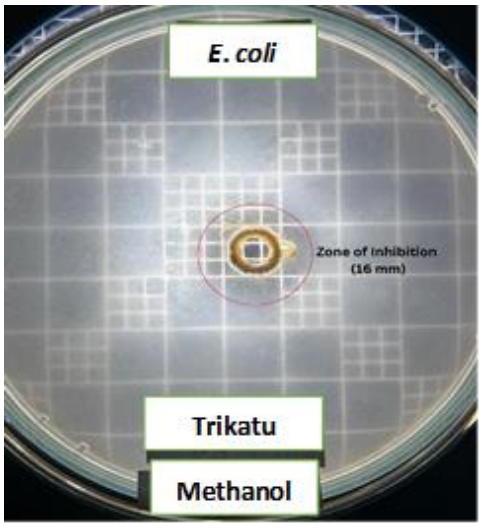
S. no.	Extract	Bacteria	Zone of Inhibition
1	Trikatu-Methanol extract	*S. aureus*	15 mm
2	Trikatu-Hexane Extract	*S. aureus*	Not detected
3	Trikatu-Aqueous Extract	*S. aureus*	Not detected
4	Positive control	*S. aureus*	40 mm

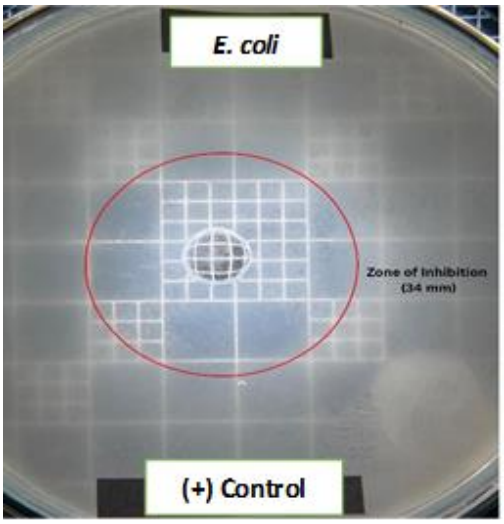
Table-2 *Escherichia coli* (Gram negative bacteria)

S. no.	Extract	Bacteria	Zone of Inhibition
1	Trikatu-Methanol extract	*E. coli*	16 mm
2	Trikatu-Hexane Extract	*E. coli*	Not detected
3	Trikatu-Aqueous Extract	*E. coli*	Not detected
4	Positive control	*E. coli*	34 mm









Result and Discussion

Chromatographic evaluation of Trikatu showed the presence of all its key bioactive markers, validating the authenticity of the formulation. The clear confirms good-quality raw materials and absence of major adulteration. This also indicates the presence of Piperine and Gingerols. Piperine is responsible for enhancing bioavailability and contributing to the pungency of the formulation, while gingerols play a key role in digestive activity. Their confirmed presence supports the pharmacological relevance and

expected therapeutic effects of Trikatu.

Effectiveness of different extracts is determined by the size of the Growth inhibition zone (Diameter measured in mm) around the well prepared. In table 1 Trikatu-Methanol extract showed larger Inhibition zone against Staphylococcus aureus as compared to Trikatu Hexane and Aqueous extract. It showed the highest inhibition zone of 15 mm and positive control of 40 mm. In table 2 Trikatu- Methanol extract showed larger inhibition zone against Escherichia coli as compared to Trikatu Hexane and Aqueous extract. It showed the highest inhibition zone of 16 mm and positive control of 34 mm. These findings suggest that

methanol is a more efficient solvent for extracting bioactive constituents responsible for antimicrobial activity in Trikatu.

Overall, the results indicate a correlation between chromatographic composition and antimicrobial performance. The confirmed presence of piperine and gingerols aligns with the observed antibacterial activity, as both compounds have documented inhibitory effects against pathogens. Thus, the study not only validates the authenticity of Trikatu but also highlights its potential as a natural antimicrobial agent, especially when extracted using methanol.

Conclusion

The study confirmed the authenticity and quality of Trikatu through HPTLC analysis, which showed the presence of key bioactive markers such as piperine and gingerols. These compounds support the traditional digestive and therapeutic properties. The antimicrobial evaluation demonstrated that the methanolic extract of Trikatu exhibited the strongest inhibitory activity against both Staphylococcus aureus and Escherichia coli, indicating that methanol is the most effective solvent for extracting antibacterial constituents. Overall, our study validates the purity of Trikatu and highlight its moderate antimicrobial potential, supporting its traditional medicinal use.

Acknowledgement

We acknowledge with thanks for the facilities provided to carry on the study.

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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Antimicrobial efficacy of Indian Medicinal Plants as alternative source of antimicrobial compounds against Drug Resistant Pathogenic Microorganisms

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Abstract- New sources of antimicrobial compounds need to be identified and improved methods should be developed to combat rising multidrug resistance problem in pathogenic bacteria. Plant extract and phytochemicals demonstrating antimicrobial action needs to be exploited for their antimicrobial efficacy to exploit it in modern phytomedicine and combinational therapy. In the present study alcoholic extracts of *Rosmarinus officinalis* was screened for their antimicrobial efficacy against drug resistant bacteria and pathogenic yeast. The extract of *Rosmarinus officinalis* showed promising action against one or more drug resistant bacteria as well as against *Candida albicans*.

Key words: Antimicrobial activity, MDR bacteria, *Rosmarinus officinalis*, pathogenic yeast.

Introduction

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 group of multidrug resistant bacterial pathogens. Bacteria have evolved numerous defenses against anti-microbial agents and drug resistant pathogens are on the rise and such bacteria have become a global helath problem. Nearly twenty years ago over 90% *Staph aureus* strains were reported β-lactamase positive. Strains of β-lacatam resistant *Staphylo-coccus 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 mechanism of bacterial-resistance to β-lactamase antibiotics.

Numerous compound 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 Entero-bacteriaceae 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).

A large portion of the world population especially in the developing countries depends on the traditional-system of medicine for a variety of diseases. 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).

According to an estimate about 119 secondary plant metabolites are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angio-sperms have been investigated chemically and that 74% of pharmacologically active plant 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 semisynthetic 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. The antibacterial and antifungal activities of Indian medicinal plants are widely known against a variety of pathogenic and opportunistic micro-organisms. However, targeted screening with improve 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 need 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 *Rosmarinus officinalis* on the basis of their traditional uses, ethano-pharmocoloical data and local availability. The present screening programme has been planned to identify most effective plants with broad spectrum activity against drug resistant microbial pathogens *in vitro.*

Material and methods

Plants material

The authentic plant material was obtained from the Himalaya Wellness Company, Dehradun an. The identification of the samples was further confirmed by the plant taxonomist in the Department of Pharmacognosy.

Drug resistant and sensitive bacterial strains used in the screening programme

The Standard strains were obtained from Microbiologics U.S.A. While Multidrug resistant bacteria include the strains of *Staphylococci* including methi-cillin resistant *Staphylococcus aureus* (MRSA), strains of *E. coli.* Were isolated in our laboratory.

Source of Antibiotics

All the antibiotic discs were purchased from Hi-Media Lab Pvt Ltd, Mumbai, India.

Bacterial cultures

Bacterial isolates were obtained from different sources 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 production 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 extract was prepared as described earlier (Ahmad and Mehmood,1998) 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.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Mehmood,1998) 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 & Ciplox) 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.

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 elsewhere (Ahmad and Beg 2001).

Result 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, ciprofloxacin, oflaxacin etc.) in many pathogenic bacteria. In the present study, clinical isolates and ATCC cultures of *Staph. aureus E. coli,* and *Candida albicans* were used. These microbial strains (clinical

isolates) 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). In the present study, *Rosmarinus officinalis* 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 *Rosmarinus officinalis* against Gram positive bacteria (isolates of *S. aureus*) and Gram- negative bacteria (*E.coli*,) and a yeast (*C. albicans*) is presented in Table 1. Activity of ethanolic crude extracts against Gram positive bacteria showed broad spectrum activity (Table 1). On the other hand broad spectrum activity against Gram negative MDR bacteria was also exhibited by this plant as evidenced with fair size of zone of inhibition (Table1). Similarly, anticandidal activity of this plant extracts demonstrated that they exhibit good anticandidal activity (Table 1).

Thus our antimicrobial screening results also justify the traditional uses of this plants in ailments and localized skin infections caused by *Staph.aureus*, *E.coli*, and *Candida albicans*.

This preliminary investigation of this plants open an avenue to evaluate the possible additive, synergistic or antagonistic interaction of this plant extracts in different combinations to obtain enhanced activity of herbal preparations. Although, it will also require an additional data on *in vivo* studies.

Multiple antibiotic therapy is now considered an effective way to control infectious diseases caused by drug resistant bacteria. Phyto-compounds 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 will be tried to see the possible synergistic effect between plant extracts *Rosmarinus officinalis* and antibiotics.

Table-1

S. No.	**Test Microorganisms**	**Alcoholic extract of *Rosmarinus officinalis***	**Antibiotics as positive control**	
			Ciplox	*Chloramphenicol*

		Diameter of zone of inhibition(mm)		
1.	*Staph aureus(ATCC)*	25	38	26
2.	*Staph aureus(Clinical isolate)*	22	25	R
3.	*E.coli(ATCC)*	24	40	10
4.	*E.coli(Clinical isolate)*	22	32	R
5.	*Candida albicans ATCC*	24	40	16

Conclusion

This preliminary investigation indicated that *Rosmarinus officinalis* showing broad spectrum antimicrobial activity against standard cultures and clinical isolates comparable with chloramphenicol antibiotic and synergy could be further tested to determine the efficacy *in vivo* against MDR bacteria. Active fractions of this plant 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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HPTLC Standardization and Antioxidant activity of Leaves of Rosemary (*Rosmarinus officinalis L.)*

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Abstract- *Rosmarinus officinalis L. a fragrant perennial shrub with needle-like leaves, belongs to the mint family (Lamiaceae)* and is popularly known as rosemary. The plant is widely used for its medicinal properties, particularly its antioxidant activity, which is mainly attributed to its phenolic constituents. Proper standardization of herbal drug is essential to ensure their identity, quality and consistency. The present study focuses on the qualitative standardization and antioxidant activity of rosemary leaves. HPTLC was employed for fingerprint profiling without the use of any reference marker compound. Different mobile phase system of varying polarity were optimized to achieve effective separation of phytoconstituents and to develop a characteristic chromatographic fingerprint. Methanolic extract of rosemary leaves was subjected to HPTLC analysis using silica gel plates and suitable mobile phase. The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging assay. The study demonstrates the HPTLC fingerprint profiling, combined with antioxidant evaluation, provides a simple, reliable, and economical approach for the quality control and a standardization of *Rosmarins officianalis.*

Keywords: *Rosmarins officianalis, HPTLC fingerprint, Antioxidant activity, DPPH assay.*

Introduction

Rosmarinus officinalis (rosemary) is an aromatic medicinal plant widely used for its therapeutic properties. Rosemary leaves are commonly consumed as a fragrant, oxidant-rich herbal tea and are known for their benefits in enhancing memory, aiding digestion, reducing stress and alleviating inflammation. Traditionally, rosemary has been used in herbal medicine for the treatment of digestive disorders, infections and inflammatory conditions. The leaves are rich in bioactive phytochemicals, including phenolic acids, flavonoids, and diterpenes,[(1)] which are recognized for their strong antioxidant properties.

Due to the increasing demand for natural antioxidants, there has been growing interest in identifying plant based compounds that can limit or replace synthetic antioxidants. Numerous scientific studies have investigated the chemical

composition and biological activities of rosemary for this purpose. These studies highlight the plant's long standing medicinal use and its richness in secondary metabolites and phytochemicals. The therapeutic relevance of rosemary is largely attributed to its high content of bioactive compounds.

Among the various phytoconstituents present in rosemary leaves, the most significant antioxidant polyphenolic compounds include carnosic acid, rosmarinic acid[(2)]. These compounds play a major role in the antioxidant potential of rosemary extracts. Plant phenolics, the most abundant secondary metabolites, are the main source of dietary antioxidants and have many health benefits[(3)]. Rosemary and other members from the Lamiaceae family are a rich source of natural antioxidants. Rosemary were shown to have similar patterns of phenolic compounds and their antioxidant activity was attributed mainly to theier carnosic acid, carnosol and rosmarinic acid components[(4)]. This research paper standardizes the methanolic extract of rosemary with the in vitro antioxidant activity of the standardized rosemary extract.

Material and Methods

Collection of Plant Material- Fresh leaves of *Rosmarinus officinalis L.* (Rosemary) were collected from the herbal garden and the plant material was authenticated by using a standard herbarium voucher specimen.

Extraction of plant Material- Wash the rosemary leaves with distilled water to remove any dust or contaminants. Dry them by a shaded area or using an oven at low heat (around 40–45°C) to prevent degradation of the active compounds.Place the powdered rosemary leaves into a clean beaker.

Maceration Process- Once dried, grind the rosemary leaves into a coarse powder using a mortar and pestle or a grinder. This increases the surface area and helps to release more active compounds into the solvent. Pour the chosen solvent over the powdered leaves to ensure that the plant material is fully submerged. Shake the beaker gently for the first few minutes to ensure that the plant material is well mixed with the solvent. You can also stir the mixture with a glass stirring rod to improve extraction. Let the mixture sit at room temperature for 48–72 hours. During this time, the solvent will slowly extract the active compounds from the rosemary leaves. Shake or stir the mixture once or twice a day to facilitate the extraction process.

TLC/HPTLC fingerprinting- TLC finger printing profile was done for methanol extract to find out the nature of compounds present. When standardizing rosemary (*Rosmarinus officinalis L.*) extracts through techniques like High-Performance Thin-Layer Chromatography (HPTLC), choosing the appropriate mobile phase is crucial for separating and identifying various bioactive compounds. The mobile phase, which consists of a mixture of solvents, plays a significant role in determining the retention factors (Rf

values) of the compounds, as well as the resolution of spots or peaks on the chromatogram.

Here are some commonly used mobile phases for the standardization of rosemary extracts,for different types of compounds (phenolic acids, flavonoids, essential oils, etc.) present in rosemary[5]:

a) Toluene: ethyl acetate: Formic acid= 5:4:1(v/v/v)- **separate non-polar to moderately polar compounds**.
b) Ethyl acetate: Acetic acid: Formic acid: water= 100:11:11:26 (v/v/v/v)- **polar compound separation**,
c) Chloroform: Acetone: Formic acid= 76: 16:08 (v/v/v)- **non-polar to moderately polar compounds** such as **essential oils**, **terpenes**, and some **phenolic acids** from rosemary.

Determination of Antioxidant Activity of Rosemary Leaf Extract

DPPH radical scavenging activity

Using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich, Germany), Al Tarawneh (2022) methodology[6] was utilized to evaluate the DPPH scavenging actions of Rosemary methanol extraction 0.1 mM DPPH methanol solution was combined with 0.1 ml of different concentrations of RE. After giving the mixture a good shake and letting it at room temperature for thirty minutes, then at 517 nm, by utilizing the HITACHI U-5100 UV-VIS spectrophotometer, to get the absorbance against a blank. 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.

Result and Discussion

a) The Rf values and color of the resolved bands were noted after development of the silica plate up to the height of 8.5cm. The bands correspond to different constituents in rosemary. Fig-1 shows the plates on the mobile phase Toluene: ethyl acetate: Formic acid= 5:4:1. This solvent system **separates non-polar to moderately polar compounds and** is useful for resolving less polar phenolic compounds or essential oils, giving moderate Rf values that allow good spot separation.

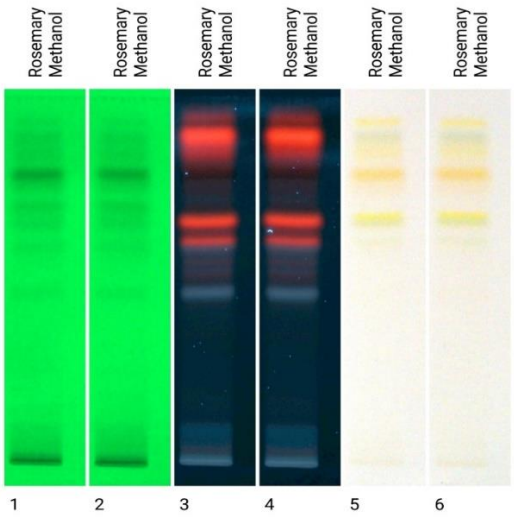

Figure-1 Separation of constituents of rosemary with mobile phase Toluene: ethyl acetate: Formic acid= 5:4:1

b) Using the mobile phase for polar compound separation is Ethyl acetate: Acetic acid: Formic acid: water= 100:11:11:26. This mobile phase is used for separation of polar compound. The water content makes the mobile phase strongly polar, ideal for separating polar analytes like flavonoids and phenolic acids, yielding lower Rf values for polar substances.

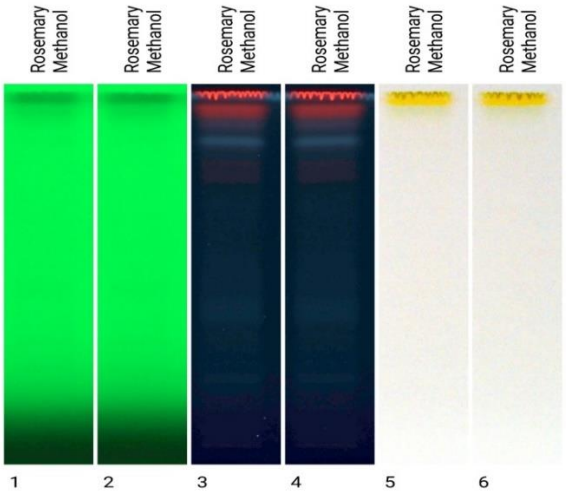

Figure-2 Separation of constituents of rosemary with mobile phase Ethyl acetate: Acetic acid: Formic acid: water= 100:11:11:26

c) Using another mobile phase to separate nonpolar to moderately polar compound, Chloroform: Acetone: Formic acid= (76: 16:08) is employed. This solvent system effectively resolves non-polar to moderately polar compounds, including essential oils and terpenes, thereby providing superior separation of medium polarity components in the rosemary extract.

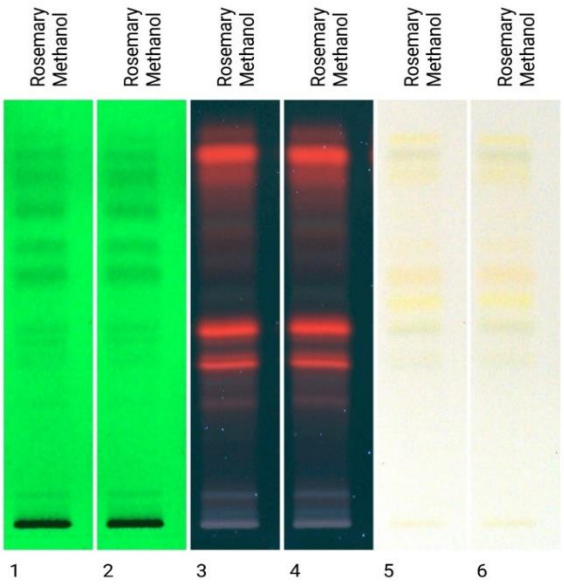

Figure-3 Separation of constituents of rosemary with mobile phase Chloroform: Acetone: Formic acid= 76:16:08

Table-1 Antioxidant activity of Methanolic extract of Rosemary leaves.

Concentration (µg/ml)	Absorbance	Plant extract (%) inhibition
Blank	0.159	-
20	0.109	31.4%
30	0.106	33.3%
40	0.086	45.9%
50	0.081	49.0%
60	0.048	69.8%
70	0.035	77.9%
80	0.010	93.7%
IC_{50}	-	51.6 µg/mL

The radical DPPH is frequently utilized to assess antioxidant activity quickly. Of the natural antioxidants, rosemary extract has been widely recognized as one of the spices/seasoning that exhibits high antioxidant activity in numerous food applications. According to Moreno et al.[8], both antioxidant and antimicrobial activities of rosemary extract are linked to its polyphenol composition. The capacity of the phytochemicals to give hydrogen, that scavenges the DPPH radical, provides the basis for DPPH scavenging action. When a DPPH solution is combined with an element that may supply an electron or a hydrogen atom to DPPH, neutralizing its free radical properties, the resulting reduced form of DPPH (non-radical) loses its violet hue. As the fraction of free radical inhibition rises, so does the radical scavenging activity [7]. The stable radical DPPH's absorbance decreased and its hue changed from purple to yellow at varying doses.

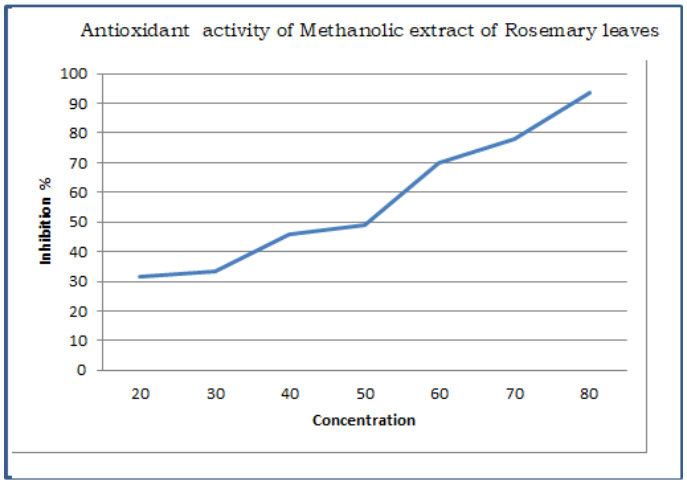

Figure-4 Antioxidant activity of Methanolic Extract of rosemary leaves

According to the research findings, the extraction of methanol exhibited the greatest DPPH scavenging activity (93.7% for rosemary extract at 80µg/ml), while the lowest scavenging value was (31.4 % for rosemary at 20µg/ml). The methanolic extract of rosemary leaves demonstrated a concentration –dependent antioxidant activity, with an IC_{50} of 51.6µg/ml. this indicates that the extract exhibits moderate free radical scavenging potency, effectively inhibiting 50% of oxidative stress at this concentration.

Conclusion

The HPTLC standardization of rosemary extract, conducted using three mobile phases, generated reproducible fingerprint profile. The selected mobile phase system ensured effective separation and quantification of bioactive compounds in the extract and can be used for quality control outline of rosemary. Rosemary also exhibited potent antioxidant activity according to the DPPH assay. The result showed notable antioxidant activity (IC_{50}= 51.6ug/ml). Thus rosemary extract emerges as a validated, moderate-strength natural radical scavenger, rich in polyphenol and capable of protecting against oxidative stress.

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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In -Vitro Antioxidant and Nitric Oxide Scavenging Activity of *Shorea Robusta* Plant Leaves

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Abstract - This study illustrates the discussion of the activity of *Shorea Robusta* plant (known for its medicinal properties) leaves extract prepared via methanolic extraction **to assess** in-vitro antioxidant & nitric oxide scavenging activity**.** The two widely accepted methods that are- 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay along with Nitric Oxide scavenging assay compared with Ascorbic Acid **as** a standard was taken to assess the antioxidant potential. The extract evaluated at five concentrations (20, 40, 60, 80, and 100 µg/mL). In both assays, extract showed increase in concentration-dependent in radical scavenging activity**,** indicating its effectiveness in neutralizing free radicals. The DPPH assay showed a maximum inhibition of 81.76% at 100 µg/mL, in the other hand Ascorbic Acid demonstrated 95.89% inhibition at the same concentration. Similarly, in the NO scavenging assay, the extract exhibited significant inhibition, although slightly lower than the standard. The results highlight that the leaves extract possesses notable antioxidant and NO scavenging properties, which can lead to the presence of flavonoid, phenolic compounds. Although extract exhibited relatively lower activity compared to the pure standard, it still showed substantial free radical neutralizing potential, supporting its traditional medicinal use. This study that *Shorea Robusta* leaves can be a promising source of antioxidants for therapeutic applications, particularly in preventing oxidative stress-related disorders. Future research are warranted to work on the isolation of active compounds as well as investigate their mechanism of action in biological systems.

Key words: *Shorea Robusta*, Antioxidant activity, DPPH assay and Nitric oxide scavenging

Introduction

The generation of reactive oxygen species and the intracellular ability to eliminate ROS are dispro-portionate, resulting in oxidative stress. It causes significant damage to all biomolecules, including lipids, proteins, DNA, and RNA[1]**.** This

damage can lead to the development of cancer, oxygen toxicity, aging, lipofuscinosis, and liver injury[2,3]. They may act as antioxidants against a variety of free radical-related illnesses[4]. Plants' anti-oxidant activity is caused by phytochemicals that interact. Antioxidant are chemicals that inhibits oxidation or oxidative damage cause by free radical. As a result, it has the potential to neutralize reactive oxygen species and free radical. The existence of these phytochemicals in plant products has also led to recent research demonstrating that they can interact with other species in the environment to obstruct the growing of bacteria or fungi. Because these chemicals inhibit infections and have little toxicity to host cells, they are expected to provide the groundwork for the creation of novel antimicrobial drugs[5].

Plants have served as a significant source of medicinal properties for millennia. Plants are utilized for therapeutic purposes in many places and serve as the origin of numerous effective pharmaceuticals. Primarily focused on traditional medicines, particularly plants, which have a historical significance in popular folk medicine[6,7]. In Ayurveda, *Shorea robusta* is considered a significant medicinal plant. S. robusta Gaertn. f. is a member of the Dipterocarpaceae family and has historically been used to cure a variety of conditions, including wounds, ulcers, leprosy, cough, gonorrhea, earaches, headaches, and many more. Traditionally, the plant's many parts have been utilized for a variety of purposes[8,9]. The leaves have applications in Ayurveda to treat anthelmintics, inflammation, antinociception, hyperlipidemia, antioxidants, and alexiteric conditions. Wounds, leprosy, earache, ulcers, itching, gonorrhea, cough and headache are all treated with the leaves. To halt bleeding and encourage wound healing, crushed stem bark administered[10,11]. The resin is used in the Unani medical system to treat menorrhagia, spleen enlargement, and eye irritations. The resin has analgesic, antiulcer, and anti-bacterial properties. Its resin combined with sugar or honey is used for sluggish digestion, gonorrhea, and dysentery. The chopped bark releases oleoresin, which has detergent and astringent qualities[12,13]. Drops made from its bark decoction are used to treat ear issues. Additionally, diarrhea is treated using its fruits[14-17]. The primary purpose of this study was to highlight the traditional and contemporary medicinal pharma-cological profile of the S. robusta plant, which is a member of the Dipterocarpaceae family and might be a source for future research.

Plant Profile [18-19]

Botanical name: Shorea. Robusta
Family: Dipterocarpaceae
Vernacular names: Guggilam, Ashvakarna, Chiraparna, Sal, Sala, Sal tree, Common Sal, Indian Da mmer, Dhuna, Damar, Jall, Sal, Salwa, and Shal
Commonly used Parts: Resin, leaves, bark, and fruit

Chemical constituents: Flavonoids, saponins, steroids, tannins, phenols, triterpenoids.

The plant's resin is fumigated in ill people's rooms and used with sugar or honey to cure bleeding piles and diarrhea. It is thought of as a detergent and astringent. It is also used to treat gonorrhea and bad digestion. Its bark extraction is used as medicine for hearing problems, while its fruits are utilized for alleviating diarrhea. Indian Shorea robusta dammar resin. It comes out of the tree's bark fissure as a gum resin. The European Pharmacopeia states that this drug is advantageous. It comes in two varieties, white and red, is soluble in alcohol, and is used for fumigation similar to frankincense. It is combined with sulfur and applied as an ointment to wounds, etc. It is also used with wax to make plasters for wounds. It is unpleasant, strong, and bitter. It is prescribed by traditional doctors for venereal ailments such as gout and gonorrhea. It works well for bronchitis, leucorrhea, piles, and cough when combined with boiling milk. All of the system's morbid fluid can be absorbed by it. Additionally, a growing number of Hindu homes, temples, and medical facilities employ the resin[20,21].

Figure-1 Leaves part of *Shorea Robusta* plant

Material and Method

Chemicals- For the in-vitro antioxidant and nitric oxide scavenging assays of *Shorea robusta* leaves, the following chemicals and reagents were used. For DPPH radical scavenging assay- 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Methanol, Ascorbic acid remained bought by Shaila enterprise. Analytical grade reagents were utilized in all other cases. For the nitric oxide assay, **sodium nitroprusside** used in order to generate nitric oxide in solution, and **Griess reagent** was used to detect nitrite formation at **546 nm**. Extract and reagents was prepared in **methanol or phosphate buffer (pH 7.4)** as appropriate. All experiments were performed using standard laboratory glassware, pipettes, and cuvettes, and measurements were carried out in **triplicate** for accuracy. **Distilled water** was used for all dilutions.

Collection and authenticfication of Plant material- *Shorea Robusta* plant leaves has been taken from the local area of Dehradun, Uttarakhand and were air dried in the shade.

Authentication of the Plant- Authentication of *Shorea Robusta* was done by Botanical Survey of India, Dehradun, Uttarakhand.

Preparation of plant extract

Shorea robusta plant leaves were gathered, chopped into tiny pieces, shade-dried at room temperature, and then ground into a fine powder using a grinder combination. Shorea robusta powder was macerated for three days at room temperature using 70% methanol. The supernatant was moved onto a china dish after three days. By holding the china dish over a hot water bath at 45°C, the supernatant was entirely eliminated. After the alcohol was completely removed, a semi-solid extract was produced. For later usage, the resulting residue was stored in the refrigerator[22].

In-vitro Antioxidant activities

DPPH assay methods were used to determine the antioxidant activity of the plant extraction. Entirely, assay was carried out in triplicate or typical value were considering and The plant extract's capacity to scavenge nitric oxide radicals produced from sodium nitroprusside in phosphate buffer and Griess reagents was used to assess its antioxidant activity.

DPPH Radical Scavenging Assay

The capacity of Shorea Robusta leaves methanol extract to neutralize free radicals is measured. The solutions (0.004% w/v) were made using 95% ethanol. Stock solutions (10 milligrams per 100 milliliters) were made by combining the methanolic extracts of the plants with 95% ethanol and water, respectively. Two milliliters, four milliliters, six milliliters, eight milliliters, and ten milliliters of this solution in stock were added to five test tubes. & The final volume of each test tube was increased to 10 ml by successive dilutions using comparable solvents, with concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 8060 µg/ml, and 10060 µg/ml for each extract, respectively. Each of these test tubes was filled with freshly made solutions (0.004% w/v), and after ten minutes, the absorbance was measured at 517 nm using a spectrophotometer. In order to create stock solutions with comparable quantities, ascorbic acid was either dissolved in distilled water or used as a reference standard medication. The same quantities were present in a control sample that was prepared. The following formulas were used to calculate the percentage of free radical scavenging. [328-330].

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

(A absorbance of control)

Nitric Oxide Scavenging Assay

At dosages of 20, 40, 60, 80, and 100 µg/ml, the nitric oxide scavenging ability of its leaf extract was evaluated using ascorbic acid as a reference. Sodium nitroprusside (5 mM) was mixed with varying amounts of the extract or standard in phosphate buffer (pH 7.4) and left to rest at room temperature for 150 minutes. After incubation, an equal quantity of Griess reagent was added to each mixture to interact with the nitrite that nitric oxide had created. Using a UV-visible spectrophotometer, the absorbance of the resulting pink solution at 546 nm was measured. The percentage suppression of nitric oxide radicals was determined by comparing the test samples' absorption coefficient with the extract-free control solution.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample})}{(\text{Absorbance of control})} \times 100$$

Formula for sample absorbance for % inhibition-

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

Results

Table-1 Preliminary phytochemical investigation of *Shorea Robusta*

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

Table I: + represents presence; ++ represents present in more concentrations; - represents absence. Thephytochemical testing of methanolic extraction of leave of *Shorea Robusta* shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

Phytochemical screening- *Shorea Robusta* leaves extraction on screening shows plants is riches in various actives ingredient (2^{nd}ry plant metabolite). The results of

phytochemicals screening exposed strongest to moderates occurrence of alkaloid, flavonoid, saponins and carbohydrates (Table-1).

In-Vitro Antioxidant Activity

DPPH radical scavenging activity

Shorea Robusta leaves extract at two different concentrations investigate in this present study was examine along with specific antioxidants (Ascorbic Acid) at their similar concentration. *Shorea Robusta* leaves extract (methanolic extracts) presented significant effects in concentration dependents manner. The extracts exhibit comparatively lower DPPH free radical scavenging potentials when compared to the conventional antioxidant applications in the trials.

Table-2 *Shorea Robusta* leaves extract antioxidant activity by DPPH method.

S.NO.	Methanolic Leaves Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	DPPH % Percentage Inhibition	Concentration (µg/mL)	Percentage Inhibition
01	20	22.12	20	35.23
02	40	38.89	40	55.16
03	60	52.45	60	70.45
04	80	68.23	80	85.56
05	100	81.76	100	95.89

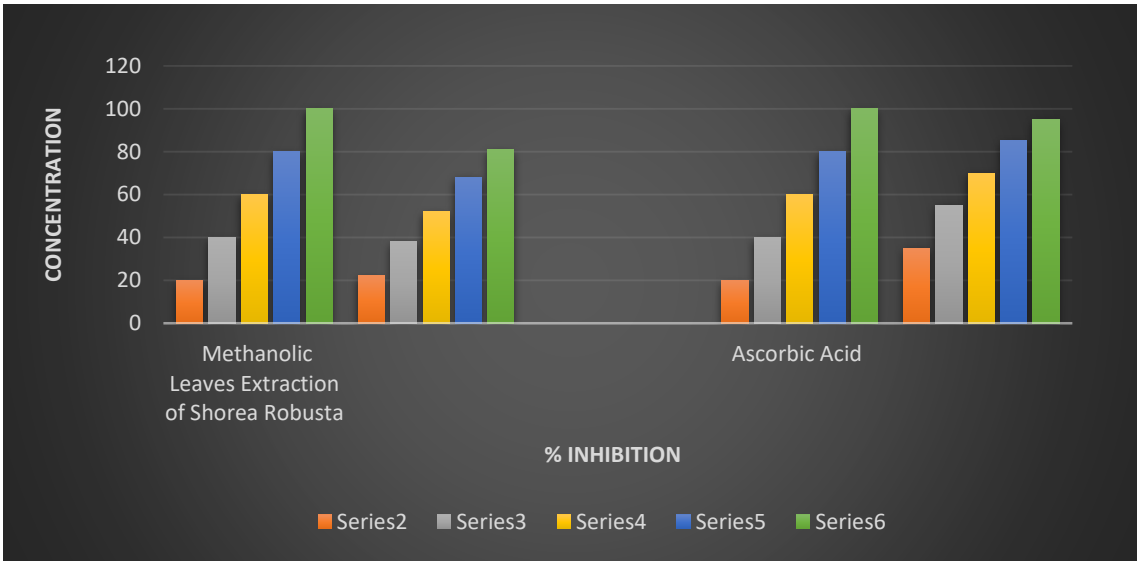

Figure-1 Graphical representation I- shows the % inhibition of DPPH radicals by extraction of *Shorea Robusta*

Table - 3 Antioxidant activities of methanolic extraction of leaves part of *Shorea Robusta* by DPPH method.

S. NO.	Methanolic Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	Absorbance at 517nm	Concentration (µg/mL)	Absorbance at 517 nm
01	20	0.624	20	0.520
02	40	0.496	40	0.360
03	60	0.384	60	0.240
04	80	0.256	80	0.120
05	100	0.152	100	0.040

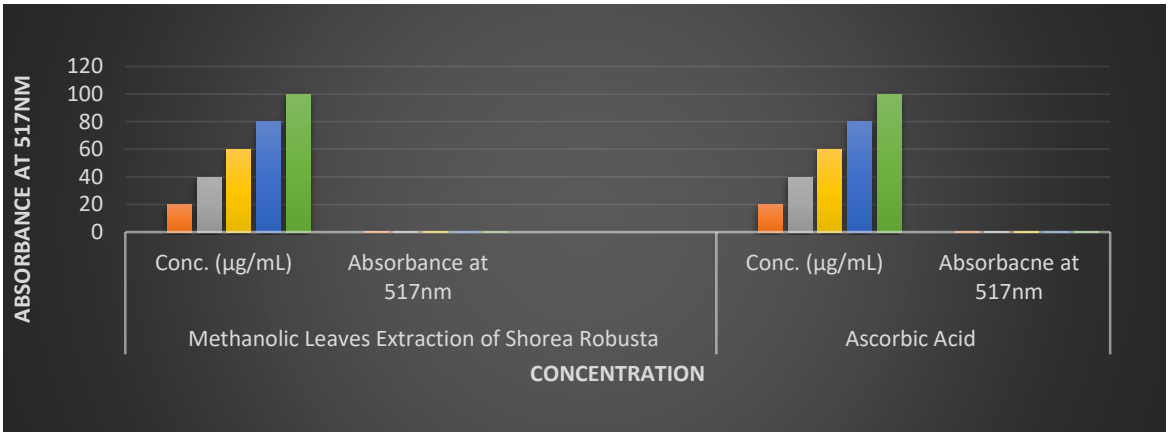

Figure-2 Graphical representation II showing the absorbance of DPPH radicals by extracts of *Shorea Robusta*

Scavenging Activity of Nitric Oxide

The nitric oxide radical scavenging strength of Shorea robusta leaf extract at various doses explored in the current study was compared with Ascorbic Acid at the same concentrations. Shorea robusta leaf extract (methanolic extract) demonstrated considerable nitric oxide radical scavenging activity in a concentration-dependent manner. When compared to the conventional antioxidant employed in the trials, the extract demonstrated much lower nitric oxide radical scavenging capability.

Table -4 Antioxidant activities of methanolic extraction of leaves part of *Shorea Robusta* by Nitric Oxide (NO) Scavenging method.

S.NO.	Methanolic Leaves Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	Nitric Oxide % Inhibition	Concentration (µg/mL)	Percentage Inhibition
01	20	18.12	20	30.34
02	40	34.76	40	50.76
03	60	49.87	60	65.98
04	80	63.32	80	78.14
05	100	77.0	100	88.76

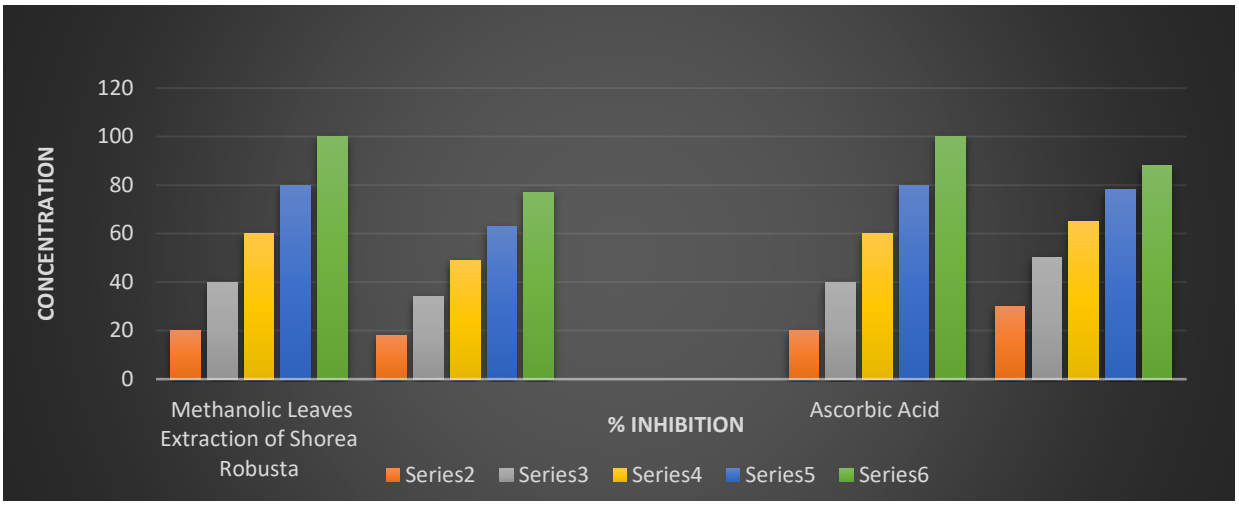

Figure-3 Graphical representation III shows the % inhibition of Nitric oxide radicals by extraction of *Shorea Robusta*

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

S.NO.	Methanolic Leaves Extraction of *Shorea Robusta*		Ascorbic Acid	
	Concentration (µg/mL)	Nitric Oxide Absorbance at (546nm)	Concentration (µg/mL)	Absorbance at (546nm)
01	20	0.656	20	0.560
02	40	0.528	40	0.400
03	60	0.408	60	0.280
04	80	0.296	80	0.176
05	100	0.184	100	0.096

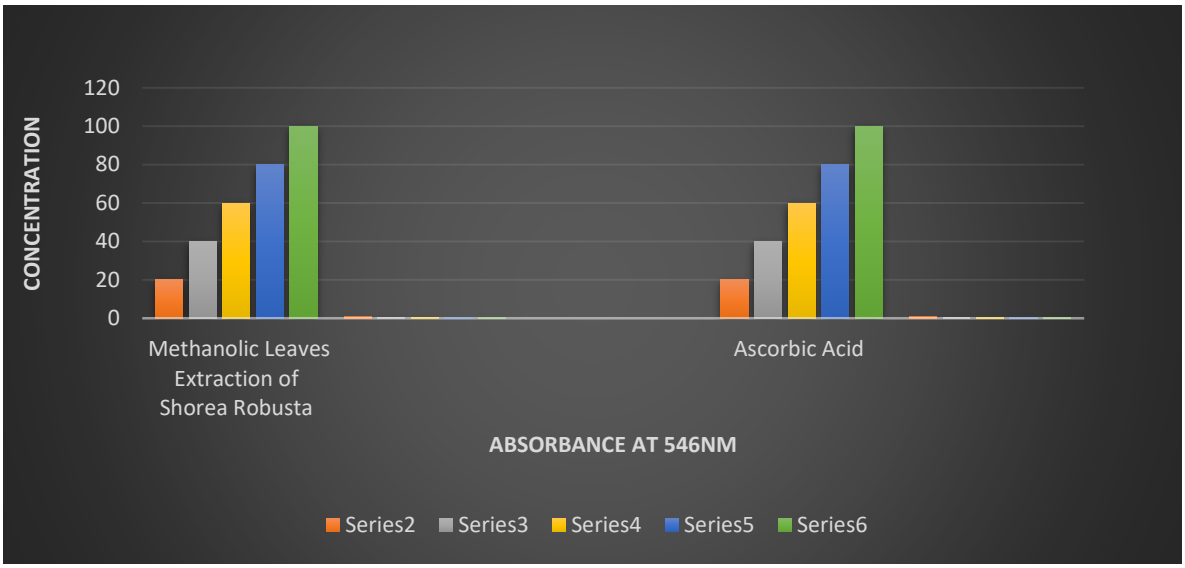

Figure-4 Graphical representation IV shows the % inhibition of Nitric oxide radicals by extraction of *Shorea Robusta*

Discussion

The current study used both DPPH and nitric oxide scavenging tests to assess the in vitro antioxidant activity of Shorea robusta methanolic leaf extract. The findings showed that the extract significantly scavenged radicals in both tests in a concentration-dependent manner. This indicates that higher concentrations were showing good results in neutralizing free radicals when compared to standard. Ascorbic Acid, the *Shorea robusta* extract showed relatively lower % inhibition at all tested concentrations. This is consistent with the observation that natural plant extracts often contain a mixture of bioactive compounds, which may act synergistically but are generally less potent than pure standard antioxidants in vitro. Nevertheless, the extract still showed **substantial scavenging activity**, confirming its potential as a natural source of antioxidant compounds. The DPPH assay, which measures extract's capacity to give hydrogen to stabilize DPPH radicals, showed a maximum inhibition of approximately 81.76% at 100 μg/mL, while Ascorbic Acid showed 95.89% inhibition at the same concentration. Similarly, in the Nitric Oxide scavenging assay, the extract demonstrated effective inhibition of NO radicals, which are implicated in oxidative stress and inflammation. The activity of the extract in the NO assay was slightly lower than in the DPPH assay, suggesting that the extract may have **more pronounced hydrogen-donating antioxidant activity** than NO scavenging potential. These results align with previous studies on plant antioxidants, where methanolic extracts of leaves often contain **phenolic and flavonoid compounds**. The concentration-dependent increase in activity indicates that *Shorea robusta* leaves are a promising candidate for **natural antioxidant development**. Overall, the comparative analysis with Ascorbic Acid confirms that while the extract is less potent than the standard, it still exhibits significant antioxidant and nitric oxide scavenging potential, which may justify its traditional use in herbal remedies and supports further investigation for therapeutic applications.

Conclusion

The current investigation also showed that *Shorea Robusta* leaves portions includes a range of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may having enormous potential uses as a remedy for a variety of long-term conditions. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence. More research is required to produce innovative antioxidant medications.

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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Authentication of herbs by physiological & morphological studies through HPTLC, ICP-MS, UV Spectroscopy, Genetic Engineering & PCR

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Abstract- The increasing global market for herbal medicinal products (HMPs) require stringent quality control measures to combat adulteration, misidentification, and contamination. Traditional reliance on organoleptic and morphological studies alone is inadequate for complex, processed, or powdered materials as it contains more than 50 species. This article focus on research on *Eclipta alba* (Bhringaraj) by combined application of Physiological & Morphological studies, High-Performance Thin-Layer Chromatography (HPTLC), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), UV Spectroscopy (UV), and Genetic Engineering (DNA Barcoding/PCR) as an integrated, multi-disciplinary strategy for the definitive authentication and quality assessment of herbal drugs. This synergistic approach provides a three-tiered confirmation—botanical origin, chemical profile, and safety (heavy metal) profile—essential for the standardization and regulation of herbal medicine.

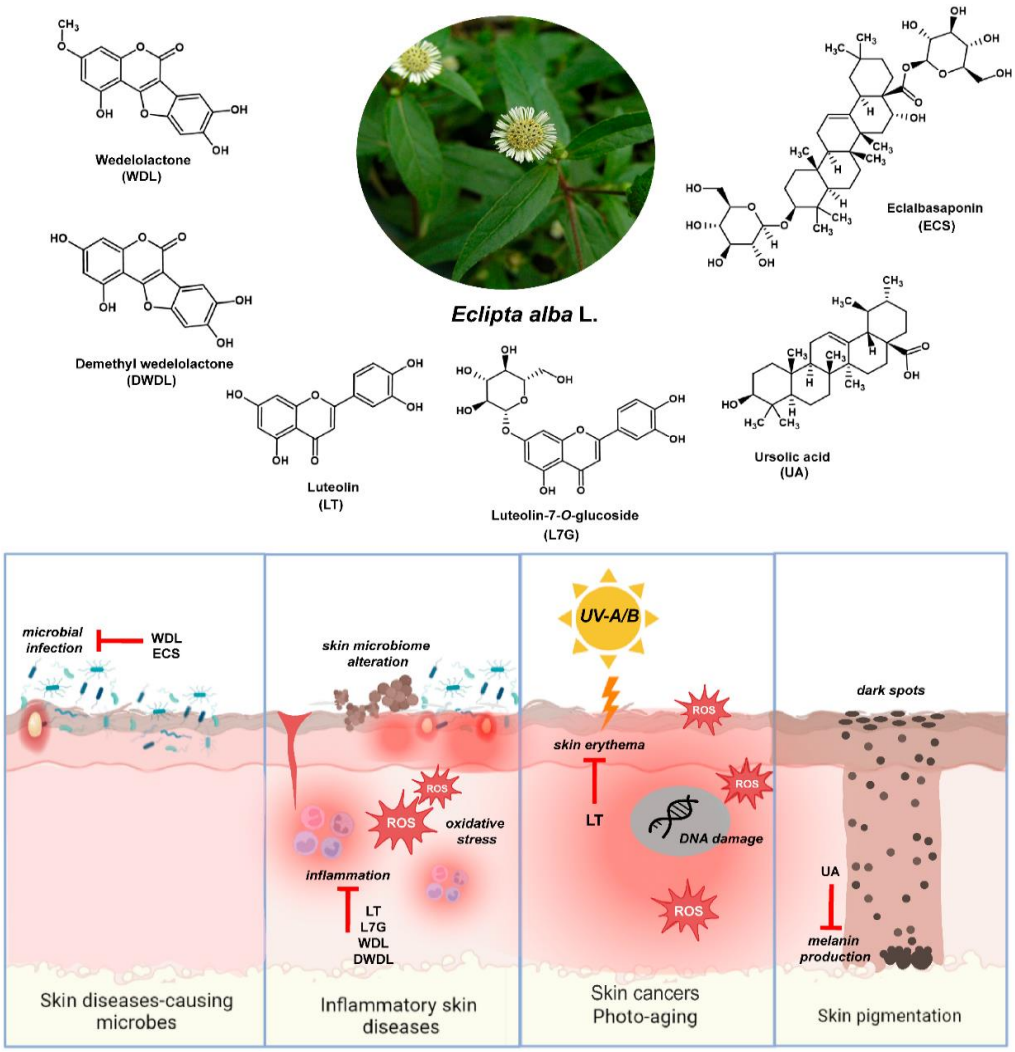
Introduction

For making an herb to globalize it is required to stringent quality control measures to combat adulteration, misidentification, and contamination. Authentication is the fundamental first step in the quality control of herbal materials, ensuring the correct plant species and part are used for therapeutic purposes. The World Health Organization (WHO) and international pharmacopoeias emphasize the need for robust methods to guarantee the safety, efficacy, and consistency of herbal products. Adulteration, where a genuine herb is intentionally substituted with an inferior or toxic alternative, poses a major threat to public health and erodes consumer trust. A comprehensive authentic-cation protocol must integrate classical and modern analytical techniques to provide an irrefutable identity profile. Bhringaraj is a small branched perennial herb. It is commonly found in India, China, Taiwan, Philippines, Japan and Indonesia. This herb has traditionally been used in ayurvedic medicine for being a liver tonic and having beneficial effects on diabetes, eye health, and hair growth.

Bhringaraj (*Eclipta alba*) oil is a well-

known for hair tonic to keep hair dark and stop hair loss. Sometime it is also termed as 'king of the hair'. *E. alba* decocted in coconut oil, is referred as 'cooling' oil. It is used externally for 'hot' and inflammatory like headaches, sinusitis and ear infections. The herb is helpful in heat problems. The whole plant of *Eclipta alba* works as a best medicine for hair growth. The presence of β-sitosterol in *Eclipta alba* help to rebuild hair in androgenic alopecia. β-sitosterol is usually used for heart disease, modulating the immune system, prevention of cancer, as well as for rheumatoid arthritis, tuberculosis, hair loss and benign prostatic hyperplasia. Other activities recorded include trypanocidal and mosquito larvicidal. Standardization, a tool in the quality control process thus, simply provides a quality assurance program for manufacturing of any herbal formulation. For quality control of herbal materials or their extracts one needs to proceed by selecting one of the different phytoconstituents of the product, preferably the one showing maximum desired bioactivity and subsequent method of quantification of that specific constituent is required to be developed.

HPTLC as well as HPLC are modern adaptations of chromatography with better and advanced separation efficiency and detection limits. HPTLC can be utilized to identify as well as quantify
the phytoconstituents present in a medicinal plant. These identification is required for regulatory perspective to ensure the efficacy, quality as well safety of the herbal drugs present in a plant. Presence of trace elements can be seen in medicinal plants. Low quantities of heavy metals like lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) are toxic for human health when exposed for a long time. According to World Health organization the maximum perm-issible levels in food and drug materials of heavy metals as given for cadmium (Cd) and lead (Pb) which are 0.3 and 10 mg/kg respectively[1-11].



Material and Method

Equipments and reagents

All chemical used for HPTLC and UV Spectroscopy as well as physiochemical method are Analytical Grade and acid and standard used for ICP-MS analysis use ICP Grade.

HPTLC from CAMAG, ICP-MS from Perkin Elmer with model 1100, UV Spectroscopy from Shimadzu with model UV 1800.

Physiological and Morpho-logical Studies

Physiological (organoleptic) and morphological (macroscopic and microscopic) evaluations remain the conventional starting point, especially for crude, whole, or fragmented herbal drugs.

- **Macroscopic Examination (Morphology):** Involves assessing external features such as size, shape, color, texture, fracture, and specific features like hairs or venation patterns.
- **Microscopic Examination (Physiological/Anatomy):** Involves the study of cellular and tissue elements, which are often unique to a species. Diagnostic features include the shape of trichomes, stomatal index, arrangement of vascular bundles, and the presence

of crystals (e.g., calcium oxalate) or starch grains.

High-Performance Thin-Layer Chromtography (Hptlc)

HPTLC is a powerful, economical, and high-through put chromatographic tech-nique widely used for herbal quality control and authentication.

- **Principle:** HPTLC separates the complex mixture of compounds in an herbal extract based on their differential partitioning between a stationary phase (e.g., silica gel plate) and a mobile phase (solvent system).
- **Sample Preparation:** weighed about 2 g of powder add 25 ml methanol reflux on water bath for 15 minutes, cool and filter. Standard of authentic E. alba is also prepared in the same manner. Apply 10 micro liter each of the standard and test solution on the TLC plate as a band of 10 mm. Develop the plate about 8 cm from the line of application.
- **Solvent system:** Toluene: acetone: formic acid (11: 6: 1).

Loss on Drying

Dry the evaporating dish for 30 min under the same conditions to be employed in the determination. Place about 5 to 10 g of powder/drug accurately weighed in a tared evaporating dish. Place the loaded bottle in the hot air oven. Dry the test specimen at 105 °C for 3 hours and weigh. Continue the drying and weighing at half an hour interval until difference between two successive weighing corresponds to, not more than 0.25 per cent.

Total Ash Value

Incinerate about 2 to 3 g, accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 600 °C until free from carbon, cool in a desiccator for 30 min and weigh without delay. If carbon free ash cannot be obtained in this way, exhaust the charred mass with hot *water*, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 600 °C. Calculate the percentage of ash with reference to the air-dried drug.

Alcohol Soluble Extractive

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of *alcohol* of specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105 °C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Water Soluble Extractive

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during 6 hours and allowing to stand for 18 hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed

shallow dish and dry at 105 °C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

UV- Visible Spectroscopy (UV)

UV-Visible spectroscopy is a rapid, non-destructive technique used to characterize and quantify compounds containing chromo-spheres. In this research we use UV for determination of Total tannin and total flavonoids.

Estimation of Total Tannin Content

The tannins were determined by A.M. Diaz,

Analytical method. Absorbance for test and standard solutions were measured against the blank at 720 nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Sample preparation

Weight accurately about 0.1 g finely powered sample in 100 ml of purified water extract it with the help of condenser at 100°C using water bath for 1 hr, cool and decant the dissolved extract in to 500 ml volumetric flask. Washed the residue with purified water and make the volume up mark with same solvent. Filter the extract through whatman filter paper no.1. Discard first 50 ml of filtrate use next filtrate for analysis.

Standard preparation

Weight accurately about 100 mg of standard tannic acid in 100 ml standard flask and make up to volume with water (standard stock solution). Pipette out 1 ml from the above solution and makeup to 100 ml with water (standard solution).

Reagent preparation: 1. Prepare 1 % potassium ferri cyanide in water.
2. Prepare 1 % ferric chloride in water

Procedure: Take 1 ml of standard solution in 10 ml volumetric flask. Add 1 ml potassium ferri cyanide and 1 ml of ferric chloride. Mix well and make the volume up to 10 ml with purified water. Exactly 30 min after addition of the reagent read the optical density at 720 nm against reagent blank. Reagent blank is prepared by a diluting 1 ml potassium ferri cyanide and 1 ml of ferric chloride to 10ml with purified water.

Test solution: Take 0.2 ml of test solution and follow the same procedure as that as standard and measure the test absorbance (T) against reagent blank.

Test blank: Take 0.2 ml of test solution and make up to 10 ml with purified water and measure the absorbance (TB) against water.

Note-All the optical density readings should be taken exactly 30 min after additions of the reagents.

Determination of Flavonoids

The flavonoid content was determined by aluminum chloride method using Quercetin as standard. Extracts and Quercetin were

prepared in (10 mg/ mL). 0.1 mL of extract was mixed with 0.9 mL of distilled water in test tubes, followed by addition of 75 µL of 5% sodium nitrite solution. After 6 minutes, 150 µL of 10%aluminium chloride solution was added and the mixture was allowed to stand for further 5minutes after which 0.5 mL of 1M Sodium hydroxide was added to the reaction mixture. Then add 2.5 ml of distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. A calibration curve was generate during various concentrations of Quercetin (20-100µg). Blank consist of all the reagents, except for the extract or Quercetin is substituted with 0.1ml of Results were expressed as the Quercetin equivalence (QE) of the sample was expressed in mg/g of the extract.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is an ultra-sensitive technique for the determination of trace and ultra-trace elements, critical for safety and geographical authentication.

Sample Preparation: sample is prepared with the help of Anton Paar microwave digester model no 3001. Weigh accurately 0.250 g sample in vessel and add 8 ml of ICP grade nitric acid swirl the content and seal the vessel after completion of acid reaction. Now put sealed vessel into the assembly and put the lid of assembly after that put it into the turnable plate of digester and run the program as prepared care should be taken that the sample after digestion should be clear and no residue should be left. The prepared sample will go for ICP-MS Analysis.

Prepare the method and sample file in the ICP-MS instrument by using Syngistix software and run the sample after getting daily performance check passed. Result of heavy metal will present in PPM.

Molecular Authentication: Genetic Engineering and Pcr-Based Tech-niques

DNA-based methods provide the most definitive species identification, unaffected by the plant's age, physiological state, or processing steps.

DNA Barcoding and Polymerase Chain Reaction (PCR)

DNA barcoding, a technique derived from genetic engineering principles, uses short, standardized gene regions to identify species.

Process: 1. DNA Isolation: Extracting DNA from the herbal material.

2. **PCR Amplification:** Using the Polymerase Chain Reaction (PCR) with universal primers (e.g., *ITS2*, *matK*, or *rbcL*) to amplify the target barcode region[10].
3. **Sequencing and Analysis:** Sequencing the amplified fragment and comparing the resulting sequence against validated reference databases (e.g., GenBank or BOLD).

4. **Authentication Value:** PCR-based DNA barcoding provides a **molecular fingerprint** that can reliably distinguish between closely related species (substitutes) and detect low-level adulteration in powdered or multi-component formulations. Species-specific PCR assays are also developed for rapid screening of known adulterants [12-23].

Result and Discussion

Macroscopic Study

The plant is herbaceous; the leaves are opposite and dark green. The stem and leaves bear characteristic white hairs. The inflorescence is found at the axis of the leaves.

Microscopic Study

A transverse section of the root shows the following characters:

1. An outer cork layer which consists of reddish brown cells (Fig.1a).
2. The cortical zone inner to the epidermis consists of Parenchymatous cells, which are thin walled, and loosely arranged (Fig.4b). They are 38-50-75µ X 30-45-50µ. They contain starch grains upto 2.5µ in diameter. The cortical zone shows the presence of resin canals with brownish black contents in them.
3. The stele shows an outermost endodermis, which is not very distinct. Inner to the endodermis, pericyclic fibres are seen (Fig.1c), arranged in patches as a ring. These fibres are 18-50µ in diameter. The phloem lies inner to these fibres (Fig.1d).
4. The centre of the stele is filled with the xylem elements (Fig.1e). The xylem vessels are arranged in close radial rows surrounded by the xylem fibres. 1-2 seriate medullary rays are also seen. The vessels have a diameter ranging from 15-50µ.

A transverse section of the stem shows the following characters:

1. An epidermis consisting of rectangular cells which are 20-25µ X 10-13µ (Fig.3a). Some of the cells bear multicellular glands (Fig.5a).
2. The hypodermis lies inner to the epidermis and consists of collenchyma (Fig. 2a, 3b) which are thick walled cells having a diameter of 15-30µ.
3. The cortex is made up of loosely arranged, thin walled parenchymatous cells filled with starch grains measuring 45-75µ in diameter (Fig.2b, 3c). The inner cortex shows a few resin canals (Fig.4e).
4. The stele consists of the outermost pericyclic fibres (Fig3d, 4b). The cambium is in-between the phloem and is found outer to the xylem (Fig.4c), which occurs towards the pith (Fig.4d). The secondary xylem just beneath the cambium is formed as a continuous ring unlike the primary xylem, which is formed in bundles.
5. The central pith is large and consists of loosely arranged parenchyma (Fig.2d).

A transverse section of the lamina of the leaf shows the following characteristics:

1. An outermost epidermis which has characteristic hairs and multicellular glands (Fig.7a, 8).
2. The palisade parenchyma that

consists of elongated cells (Fig.6a) and the spongy parenchyma which has circular cells (Fig.6b). Both these constitute the mesophyll tissue.

3. The lower epidermis bears characteristic hairs.

Diagnostic features:

1. All parts of the plant except the roots bear characteristic white hairs.
2. The leaves are opposite and bear the inflorescence in the axis.
3. The root and the stem show resin canals in the cortical region.
4. The stem and leaves bear multicellular glands.

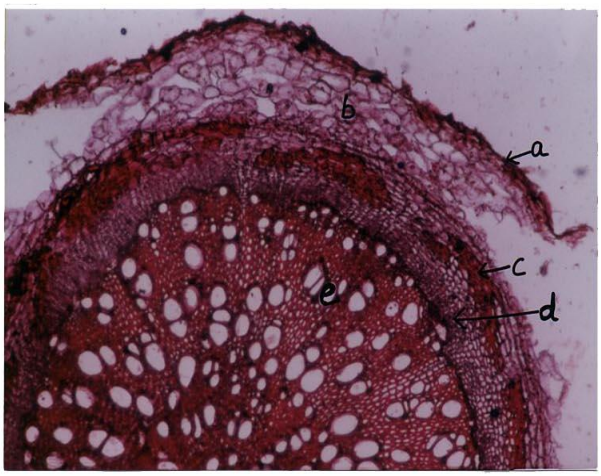


Figure- 1 TS of root (4 x)
a. Cork
b. Cortex
c. Pericyclic fibres
d. Phloem
e. Xylem

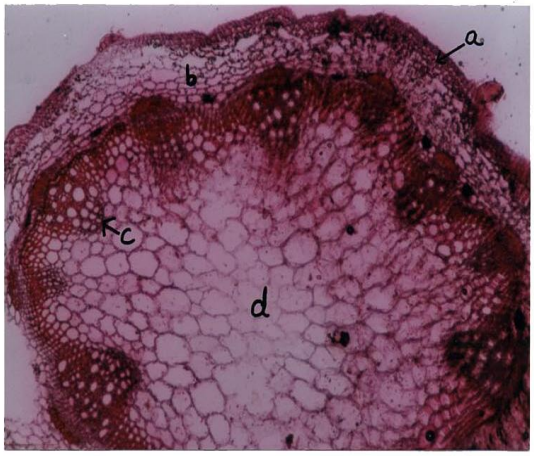


Figure-2 TS of stem (4 x)
a. Hypodermis
b.Cortex
c. Vascular bundles
d. Pith

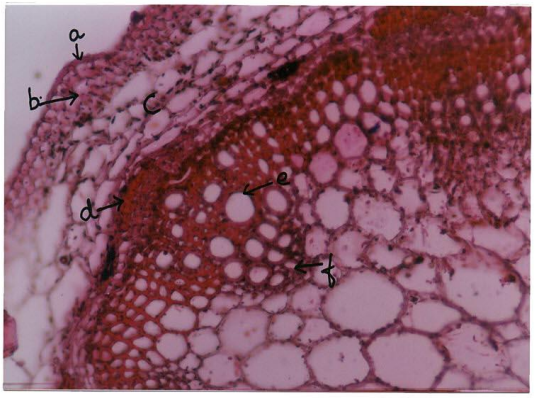


Figure- 3 TS of stem (10 x)
a. Epidermis
b. Hypodermis
c. Cortex
d. Pericyclic fibres
e. Metaxylem
f. Protoxylem

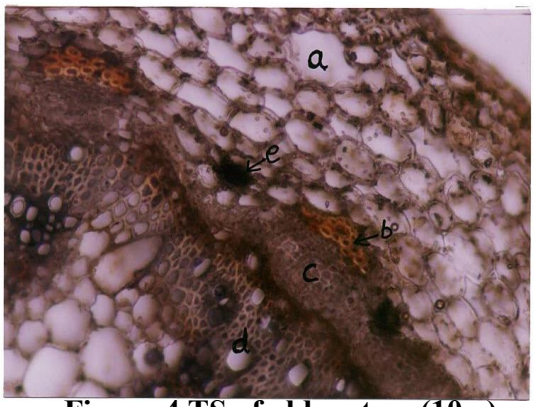


Figure-4 TS of older stem (10 x)
a. Cortex
b. Pericycle
c. Phloem
d. Xylem
e. Resin canal

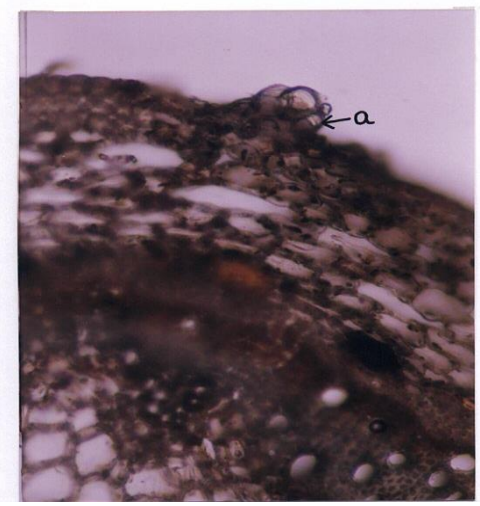


Figure- 5 TS of stem (40 x)
a. Multicellular gland

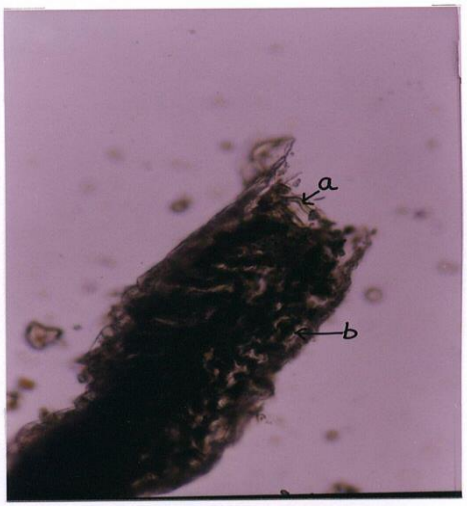


Figure-6 TS of lamina of leaf (10 x)
a. Pallsade parenchyma
b. Spongy parenchyma

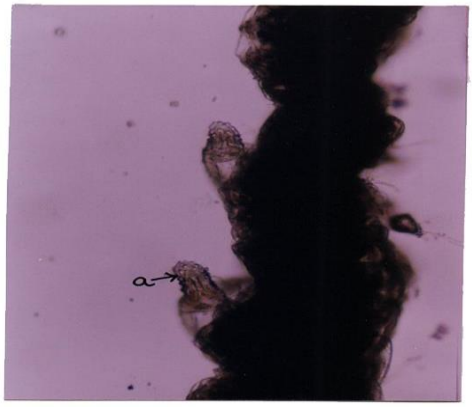


Figure- 7 TS of lamina of leaf (10 x)
a. Glandular hairs

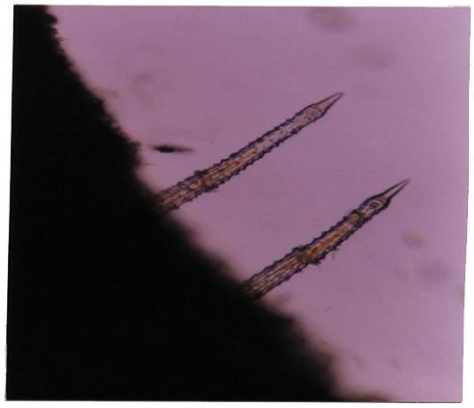
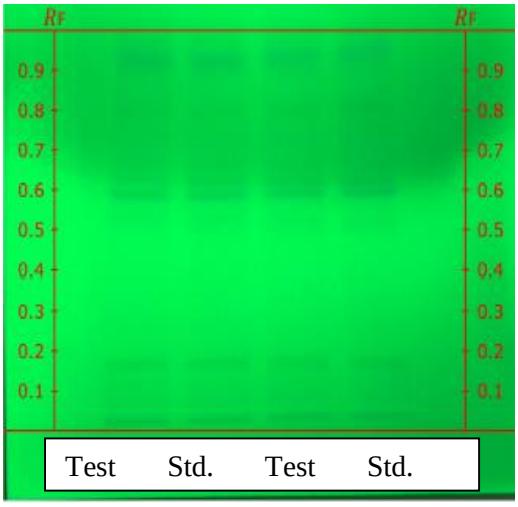
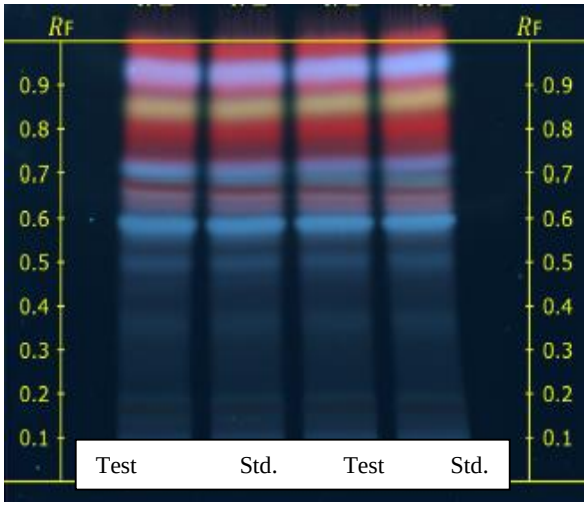


Figure- 8 Glandular hairs of the leaf (10 x)

HPTLC Chromatogram and analysis:



Chromatogram at 254 nm



Chromatogram at 366 nm

Table-01 Physicochemical characters of *Eclipta alba* powder

Sr. No.	Parameters	Observation
1	Foreign matter	0.78 % w/w
2	Acid-insoluble ash	8.57 % w/w
3	Water soluble extract	25.02 % w/v
4	Alcohol-soluble extractive	14.43 % w/v
5	Loss on drying at 105 °C	4.2 % w/w
6	Total Ash value	17.43 % w/w

Table-02 Total Tannin Content determine by using UV Spectroscopy technique

Sr. No.	Absorbance	Total Tannin content mg/g equivalent to Tannic acid.
01	0.345	320
02	0.351	322
03	0.339	318
Mean ± SD		320 ± 2

Table-03 Total Flavonoid Content determine by using UV Spectroscopy technique

Sr. No.	Absorbance	Total Flavonoids content mg/g equivalent to Quercetin.
01	0.198	130
02	0.197	128
03	0.199	132
Mean ± SD		130 ± 2

Table-04 Heavy Metal determination by Perkin Elmer ICP-MS

Sr. No.	Heavy Metals			
	Lead (Pb) (PPM)	Cadmium (Cd) (PPM)	Arsenic (As) (PPM)	Mercury (Hg) (PPM)
01	0.95	0.35	0.05	0.004
02	3.15	0.62	0.07	0.009
03	1.45	0.54	0.10	0.007

Conclusion

Authentication of herbs by physiological and morphological studies through HPTLC, ICP-MS, UV Spectroscopy & PCR evaluation of *Bhringaraja* (*Eclipta alba* Hassk.) for whole plant provided specific parameters that will be useful in scientific evaluation, identification and authentication of the particular species and its medicinal products. This study could serve as a

constructive reference to allow further in-vivo analysis which can be conducted to evaluate the extent of protective effects of *Eclipta alba* against chemically induced cellular damage. The present investigations revealed that *Eclipta alba* contain significant amount of phenols and flavonoids while heavy metals in a regulatory limit and hence can be globally used for manufacturing of herbal preparation.

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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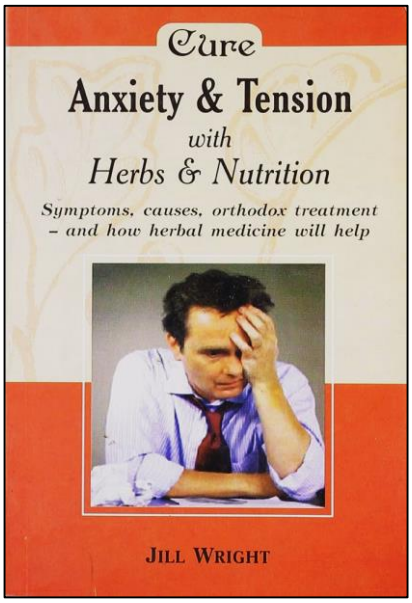
Book Review

Anxiety & Tension with Herbs & Nutrition



JILL WRIGHT

An imprint of B. Jain Publishers (p) Ltd.

This book consists of Seven Chapters alongwith chapter on sources and resources, list of herbs within their applications and general index. The author expresses that herbalism is the oldest, most widely practiced form of medicine and the most popular alternatives to modern drugs. He highlights the subject:

1. Understanding anxiety and tension
2. What conventional medicines can offer
3. Using herbs to treat anxiety and tension
4. Directory of useful herbs
5. Growing and making your own herbal remedies
6. Using nutrition to improve stress resistence
7. Cese histories and sources and resources and list of herbs within their applications and genral index.

He explains how anxiety can cause variety of physical symptoms viz., pain in the chest, frequent bowel movement, constipation, tendency to strain muscles, headaches, indigestion, skin problems including psoriasis and even acne, cold hands and feet as well as breathing problems such as asthma.

Muscles aches, fatigue and depression can also be caused from chronic tension.

Herbal medicine is the leading alternative to orthodox drug treatment. It is especially favourable to conditions such as anxiety and tension which may have a number of dymptoms and respond well to wholistic treatment. The advice contained in the book is meant to be for general use only.

Dr. I. P. Saxena
Editor (UJPAH)

Forthcoming Events

- **International Conference on Applied Bioscience and Biotechnology (ICABB) - Tokyo, Japan**
  January 05, 2026
- **19th World Drug Delivery Summit - Paris, France**
  January 29-30, 2026
- **International Conference on Agricultural Biotechnology Research (ICABR) - Melbourne, Australia**
  February 02, 2026
- **9th World Congress on Traditional and Complementary Medicine- Rome, Italy**
  February 16-17, 2026
- **International Conference on Medicinal Plants, Pharmacognosy, Phytochemistry and Natural Products (ICMPPPNP) - Rome, Italy**
  March 02, 2025
- **Congress on Innovative Chemistry - Dubai, United Arab Emirates**
  April 29, 2025
- **International Conference on Medicinal Plants, Pharmacognosy, Phytochemistry and Natural Products (ICMPPPNP) - Honolulu, United States**
  May 04, 2025
- **3rd International Conference on Pharmacognosy- Madrid, Spain**
  May 28-29, 2026
- **International Conference on Food and Agricultural Biotechnology (ICFAB) - New York, United States**
  Jun 01, 2026
- **International Conference on Molecular Biology, Biochemistry and Biotechnology (ICMBBB) - Ljubljana, Slovenia**
  June 08, 2026
- **25th International Conference on Medicinal and Pharmaceutical ChemistrySAN-DIEGO, USA**
  June 08-09, 2026
- **6th International Conference on Pharmaceutical Chemistry - Paris, France**
  June 15-16, 2026
- **International Conference on Natural Products (ICNP) - London, United Kingdom**
  June 29, 2026
- **26th World Congress on Pharmaceutical Sciences and Innovations in Pharma Industry-Bangkok, Thailand**
  July 27-28, 2026

About flowers on the cover page

Rosmarinus officinalis

Kingdom: Plantae (Plants)
Subkingdom: Tracheobionta (Vascular Plants)
Superdivision: Spermatophyta (Seed Plants)
Division/Phylum: Magnoliophyta (Flowering Plants/Angiosperms)
Class: Magnoliopsida (Dicotyledons)
Order: Lamiales
Family: Lamiaceae (Mint Family)
Genus: *Rosmarinus* (Historically) / *Salvia* (Current Phylogenetic View)
Species: *officinalis* (Historically) / *rosmarinus* (Current)
Binomial Name: *Rosmarinus officinalis* L. (Traditional) / *Salvia rosmarinus* (Modern)

Rosmarinus officinalis (Rosemary) belongs to the Kingdom Plantae, Order Lamiales, and Family Lamiaceae (Mint Family), with recent studies suggesting reclassification into the genus *Salvia*, making its current scientific name *Salvia rosmarinus*. It's an evergreen shrub known for its aromatic, needle-like leaves, used in cooking and traditional medicine for its antioxidant, anti-inflammatory, and antimicrobial properties, found in numerous PDF research articles online.

Rosmarinus officinalis (Rosemary) is used in traditional medicine for boosting memory, improving digestion, reducing pain (muscle/joint), fighting fatigue, and promoting hair growth, thanks to potent antioxidants like carnosic acid and rosmarinic acid, offering anti-inflammatory, neuroprotective, antimicrobial, and stress-relieving properties for conditions from headaches to arthritis, and even showing potential in blood sugar control and anti-aging.

Moringa oleifera

Kingdom: Plantae (Plants)
Division/Phylum: Magnoliophyta (Flowering Plants)
Class: Magnoliopsida (Eudicots)
Order: Brassicales (or Capparales in older systems)
Family: Moringaceae (Moringa Family)
Genus: *Moringa*
Species: *Moringa oleifera* Lam.

Moringa oleifera, the Drumstick Tree, belongs to the Kingdom Plantae, Order Brassicales, Family Moringaceae, Genus *Moringa*, and Species *oleifera*, a classification supported by multiple PDF sources detailing its botanical description, often referencing APG IV or traditional systems like Capparales. Key identifying features include its unique tripartite winged seeds, parietal placentation in flowers, and elongated, three-valved pods, distinguishing it within its genus. **Family Moringaceae:** Contains only the genus *Moringa*, known for its distinct features like parietal placentation and winged seeds.

Moringa oleifera (Moringa) is used medicinally for its potent antioxidants, vitamins, and minerals, supporting blood sugar control, heart health (lowering cholesterol/BP), brain function, and fighting inflammation, infections, and oxidative stress, with traditional uses for liver, digestive, skin, respiratory issues, and as a general tonic, though root/bark should be used cautiously.

DNA bar coding of *Moringa oleifera*

Results of DNA barcoding of investigated moringa germplasm using DNA subway by employing "MUSCLE" tool for sequences of the *matK* gene & *ITS* gene
Source:DOI:10.37273/chesci.cs205507081 Chem Sci Rev Lett 2024, 13 (51), 92-99 Article cs20550708

Murraya koenigii

Kingdom: Plantae
Division: Magnoliophyta (Angiosperms)
Class: Magnoliopsida (Dicotyledons)
Order: Sapindales (Sometimes listed)
Family: Rutaceae (Citrus family)
Genus: *Murraya*
Species: *M. koenigii* (L.) Spreng.
Key Takeaways from PDF Sources:
Synonym: Also known as *Bergera koenigii*.
Common Names: Curry leaf, Sweet Neem, Kadhi Patta.
Family Significance: Rutaceae family includes many genera with medicinal plants, and *M. koenigii* is a key member.

Murraya koenigii (Curry Leaf) belongs to the plant kingdom, featuring in the family **Rutaceae**, genus *Murraya*, and is a species within the flowering plants, known for its aromatic leaves used in cooking and traditional medicine, with its scientific name being *Murraya koenigii* (L.) Spreng.. You can find detailed taxonomic breakdowns in PDFs from research journals like those on ResearchGate and the World Journal of Pharmaceutical Research.

Murraya koenigii (curry tree) has numerous medicinal uses, including treating digestive issues like diarrhea and vomiting, and acting as an anti-inflammatory, antioxidant, and antidiabetic agent. It has also shown potential in reducing cholesterol, fighting certain types of cancer cells, and providing benefits for skin and eye health. Traditional uses also include its application as a tonic and to allay body heat, inflammation, and itching.

Nardostachys jatamansi

Kingdom: Plantae (Plants)
Clade: Tracheophytes (Vascular Plants)
Clade: Angiosperms (Flowering Plants)
Clade: Eudicots
Clade: Asterids
Order: Dipsacales
Family: Caprifoliaceae (Valerian Family)
Subfamily: Valerianoideae
Genus: *Nardostachys* DC.
Species: *N. jatamansi* (D.Don) DC.
Description: A small, perennial, hairy herb with fragrant rhizomes (underground stems) covered in reddish-brown fibers.
Habitat: High-altitude regions of the Himalayas (India, Nepal, China).
Traditional Use: Calming the nervous system, inducing sleep, managing stress, anxiety, epilepsy, and mental weakness (Sanskrit: *medhya* for brain tonic).
Active Compounds: Essential oils, jatamansone, jatamansin, nardostachone, sesquiterpenes, lignans.
Conservation: Considered endangered (Critically Endangered by IUCN) due to overharvesting.

Jatamansi (*Nardostachys jatamansi*) is an Ayurvedic herb known for calming the nervous system, reducing stress/anxiety, improving memory, and aiding sleep; it also supports hair health (growth, shine) and skin (acne, eczema) due to antioxidant/anti-inflammatory properties, and helps with digestive issues, hypertension, and liver health, but consult an expert before use, especially if pregnant or with existing conditions.

Instruction to Contributors

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    b. The w/w yield of prepared extracts in terms of starting crude material.
    c. Complete formulation details of all crude drug mixtures.
    d. 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
Dehradun

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 and 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