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ANTIOXIDANT AND ANTIMICROBIAL HERBS

Eclipta alba
(Bhringraj/ Bhangra)

Hemidesmus indicus
(Anantamool)

Ocimum basilicum
(Sweet Basil)

Wedelia chinensis
(Pilabhangra)

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PROF. DR. ARUN KUMAR TRIPATHI

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Editorial

The rise of AI has led to a decline in physical and mental exercise, while paradoxically increasing mental stress, anxiety, tension, and depression. Morphine was discovered in 1805 as a sedative to alleviate stress. In the past, meditation and hard work were effective solutions; laborers would work during the day and rest at night. Today, we are revisiting natural remedies and exploring herbal solutions for stress relief. Some popular stress-relief techniques include deep breathing, yoga, meditation, and spending time in nature. Additionally, prioritizing sleep, maintaining a balanced diet, and connecting with loved ones are crucial for reducing stress. With the advent of technology, stress level recording has become increasingly important for monitoring health status. As we look to the future, Artificial General Intelligence (AGI) is expected to emerge. Mark Zuckerberg is personally leading the charge at Meta, recruiting a team to develop AGI. Amidst these technological advancements, it's essential to strike a balance between innovation and well-being. By embracing natural solutions and stress-relief techniques, we can promote overall health and wellness."

Ayurveda, the ancient Indian system of medicine, has long been revered for its holistic approach to health and wellness. Rooted in natural remedies and personalized healing, Ayurveda harnesses the therapeutic potential of plants, aligning seamlessly with the principles of phytochemistry—the scientific study of bioactive compounds found in flora. The convergence of these two fields offers exciting possibilities for the future of medicine, blending tradition with rigorous scientific validation.

Phytochemistry delves into the chemical properties of medicinal plants, uncovering active constituents that contribute to their efficacy. Alkaloids, flavonoids, terpenoids, and polyphenols—among others—hold immense therapeutic potential, influencing everything from antioxidant activity to antimicrobial and anti-inflammatory effects. While Ayurveda has employed these botanicals for centuries, phytochemical research provides the analytical tools necessary to quantify their potency, enhance formulations, and ensure consistency in herbal medicine

This journal seeks to bridge the gap between Ayurvedic wisdom and contemporary scientific methodologies, fostering interdisciplinary research that advances natural medicine. By synergizing traditional knowledge with phytochemical innovations, we can unlock novel therapeutic applications, promote sustainable healthcare practices, and reaffirm nature’s role in healing. As we navigate this exciting frontier, we invite researchers, clinicians, and scholars to contribute their findings, ensuring that Ayurveda and phytochemistry continue to evolve—honoring ancient wisdom while embracing the precision of modern science.

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

Dr. S. Farooq
Chief Editor

Content

Dual-Phase Antidiabetic Efficacy of Cultivated *Cordyceps sinensis*: Mechanistic In-Vitro Validation and Therapeutic In-Vivo Outcomes

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Abstract- Cultivation is an effective conservation approach that can balance market demand and species protection. Wild and cultivated *Cordyceps sinensis* were investigated to understand possible variation in potential anti-diabetic-related bioactivity. Diabetes was induced in Wistar rats by the administration of a HFD for 15 days/STZ (35 mg/kg b.w., i.p.). *Cordyceps sinen-sis* at dose of 50 and 100 mg/kg b.w., p.o. was administered to diabetic rats for 28 days in HFD/STZ-induced Type 2 diabetic rats The effect of carbohydrate molecule of cultivated *C. sinensis* (CS) on blood glucose and body weight was studied in Type 2 diabetic rats. All these effects were compared with glibencl-amide (5 mg/kg b.w., p.o.) as a reference antidiabetic drug. HPLC and HPTLC analysis of water extracts of wild and laboratory adopted *C. sinensis* revealed similar properties and there was not any difference observed. The concentration of carbohydrate molecule in cultivated *Cordyceps sinensis* was 5% and the purity of extracted carbohydrate molecule from cultivated *Cordyceps sinensis* powder by HPLC was observed very high carbohydrate molecule of *Cordyceps sinensis* shows significant *In-vitro* antidiabetic activity. The administration of CS (50 and 100 mg/kg b.w., p.o.) resulted in a significant decrease in blood glucose level, body weight and significant increase in serum insulin level when compared to diabetic control. The results suggest that *Cordyceps sinensis* possesses a promising effect on the HFD/STZ -induced Type 2 diabetes.

Keywords: *Cordyceps sinensis*, Cultivation, Carbohydrate molecule, HPLC, HPTLC, Diabetes, *in-vitro, in-vivo* Streptozotocin, High Fat Diet, Cholesterol, Triglyceride, SGOT and SGPT.

Introduction

Diabetes is a group of comorbid diseases characterized by chronic hyperglycaemia, inadequate insulin secretion, and elevated blood glucose levels. It is characterized by disturbances of carbohydrate, protein, and fat metabolisms, secondary to absolute or relative lack of the hormone insulin (Bhutkat et al. 2012). The number of people in the world with diabetes has increased dramatically over recent years. It is also predicted that by 2030, India, China, and the United States will have the largest number of people with diabetes (Wild et al., 2004). The two principal forms of diabetes

are i) insulin-dependent (type 1) and ii) non-insulin-dependent (type 2). Non-insulin-dependent diabetes accounts for 90% of all cases worldwide. Thus, management of the blood glucose level is a critical strategy in the control of diabetes complications.

The current treatment for type 2 diabetes includes insulin and oral hypoglycemic drugs such as biguanides, thiazol-idinediones, sulfonylurea derivatives, and α-glucosidase inhibitors. These medi-cations have side effects, e.g. osteo-porosis, obesity, and sodium retention by thiazolidinedione. Incidences of severe hypoglycemia by sulfonylurea and biguanide (metformin) put patients at risk of developing lactic acidosis (Hamza et al., 2010). Further, for most diabetic patients, the oral monotherapy with lifestyle changes is not sufficient and requires various oral combinations or the addition of insulin (Stumvoll et al., 2005). Thus, there is an increasing need to identify and explore more effective anti-diabetic agents with fewer side effects. As a result, herbals have received attention as sources of antioxidants and hypogly-caemic and anti-hyperlipidemic agents. *Cordyceps sinensis* has been known and used for many centuries in traditional Chinese medicine (TCM). Most of the people in the Indian Himalayan region called it “keerajari.” Traditionally it is well known as herbal Viagra (Kobayasi 1982 and Mizuno 1999). The Latin word "conjunction" aptly describes this club-shaped fungus, whose elongated stroma and fruiting body emerge from the mummified remains of insect larvae, typically those of the Himalayan ghost moth (*Thitarodes armoricanus*, formerly *Hepialus armoricanus*). *C. sinensis* has two parts: larva and stroma. The larva is 10-15 mm in length and 50 mg in weight, and the stroma is 5-10 mm in length and 60 mg in weight (Bhandari *et al*., 2010; Holliday *et al.*, 2004; and Mizuno, 1999). *Cordyceps sinensis* (now *Ophiocordyceps sinensis*) contains a diverse array of bioactive compounds, such as nucleosides (e.g., cordycepin and adenosine), ergosterol and its derivatives, mannitol, peptides, poly-saccharides, proteins, polyamines, and amino acids, which contribute to its medicinal properties (Schmidt et al., 1995; Benowitz et al., 2002; Bok et al., 1999; Gong et al., 2001; Holliday et al., 2004).*Cordyceps sinensis* contains a significant amount of polys-accharides, comprising 3 to 8% of the total dry weight (Li et al., 2001, 2002).

Cordyceps sinensis (caterpillar fungus) or yarsagumba is an exceptional and incredible mushroom that grows in the pastures above 3,500 meters to 5,000 meters in the Himalayan region of Nepal, Bhutan, Tibet, China, and India. It is also referred to as "Himalayan Viagra" or "Himalayan Gold" due to its significant medicinal and commercial value (Kinjo and Zhang, 2001; Mayaram U., 2011). Numerous scientific studies and research reveal that it has antibiotic properties and is used for the treatment of lung and respiratory infections, pain, sciatica, and backache. It also enhances vitality and increases the physical stamina of the body. (Wang et al. 2000). Cultivation is an effective conservation approach that can balance market demand and species protection. This study investigated polysaccharide profiles (via HPLC/ HPTLC) in wild vs. cultivated *Cordyceps sinensis* and evaluated cultivated *C. sinensis* ’s antidiabetic effects in a high-fat diet/STZ-induced diabetic rat model.

Material and Methods

Cultivation and Identification of *Cordyceps sinensis*

The *Cordyceps sinensis* culture was obtained from the Forest Research Institute, Dehradun, and initially grown on potato dextrose agar (PDA) slants at 28°C for 5 days. For liquid culture, mycelia were inoculated into potato dextrose broth (PDB) and incubated at 27°C for 4 days. Subsequent sub-culturing involved streaking mycelia onto fresh PDA plates, followed by incubation at 28°C for 5 days. Fungal colonies were examined microscopically after culturing on concavity slides and staining with phenol cotton blue to observe mycelial morphology and conidiophores (Chen et al., 2006).

Animals and Ethical Approval

Male Wistar albino rats (180–240 g) were procured from NIPER (Mohali) and housed at Shoolini University's animal facility (Solan, HP) under controlled conditions (25 ± 2°C; 45 ± 5% relative humidity) with *ad libitum* access to food and water. The high fat diet were provided to the rats induced with diabetics (Table-1). After a 7-day acclimatization period, experiments were conducted in compliance with CPCSEA guidelines. The rats were divide into five groups and each group comprised of 6 rats (Table-2). The Institutional Animal Ethics Commi-ttee (IAEC) approved all protocols (Approval No. SUBMS/ADM/900-1).

Comparative Analysis of wild and cultivated *Cordyceps sinensis* using chromatographic techniques

a. **High Performance Thin Layer Chromatography (HPTLC)** Methanolic extracts of wild and cultivated *C. sinensis* (1 g each, air-dried) were prepared by refluxing with 10 mL methanol for 10 min, followed by filtration. The extracts (10 µL) were applied band wise to HPTLC plates (Merck 60 F254) using a CAMAG Linomat-5 applicator (8 mm from the lower edge, 35 mm from the left edge). Separation was performed in a CAMAG Twin Trough Chamber (10 × 10 cm) with dichloromethane: methanol (95:5, v/v) as the mobile phase (migration distance: 80 mm). Bands were visualized under UV light at 254 and 366 nm.

b. **High Performance Liquid Chromatography (HPLC)** Aqueous extracts were prepared by ultra-sonicating 5 g of dried *C. sinensis* powder (wild and cultivated) in 70 mL double-distilled water for 30 min, followed by dilution to 100 mL. After membrane filtration (0.2 µm), samples (20 µL) were injected into an HPLC system equipped with a C18 column (Luna C18, 150 × 4.6 mm). The mobile phase consisted of disodium hydrogen phosphate buffer (pH 6.0) and methanol (85:15, v/v) at a flow rate of 1.0 mL/min. Detection was performed at 260 nm (Guo et al., 1998).

c. **Extraction of Polysaccharides from *Cordyceps sinensis***

Cultivated *C. sinensis* powder (10 g) was sequentially decolorized with 95% ethanol and extracted overnight with 75% aqueous ethanol. The extract was centrifuged (6000 rpm, 30 min), and the supernatant was concentrated under reduced pressure. After dialysis, the non-dialyzable fraction was precipitated with 95% ethanol, followed by centrifugation. The precipitate was washed with acetone (3×) and dried at 65°C to constant weight (Zhang et al., 2005).

d. Estimation of Carbohydrates Derived from *C. sinesis* by HPLC

i. Standard Preparation-Sucrose (100 mg) was dissolved in 70–80 mL purified water in a 100 mL volumetric flask and sonicated for 10 min. The volume was adjusted to 100 mL with purified water. The solution was filtered through a 0.2 µm membrane and transferred into 1.5 mL vials for HPLC analysis.

ii. Sample Preparation -Carbohydrate ex-tract (50 mg) from *C. sinensis* was dissolved in 7–8 mL purified water in a 10 mL volumetric flask and sonicated for 10 min. The volume was made up to 10 mL with purified water, filtered (0.2 µm), and aliquoted into 1.5 mL vials.

iii. HPLC Analysis- Samples (20 µL) were injected into a SHIMADZU HPLC system (LC-2010 HT) equipped with a Luna C18 column (150 × 4.6 mm, Phenomenex). The mobile phase consisted of acetonitrile: water (75:25, v/v) at a flow rate of 1.25 mL/min. Detection was performed at 193 nm.

e. *In-Vitro* Antidiabetic Assay (α Glucosidase Inhibition Assay)

α-Glucosidase inhibitors competiti-vely inhibit intestinal α-glucosidase, delaying carbohydrate digestion and reducing postprandial hyper-glycemia. The assay was conducted as follows:

i. Reaction Setup

a. Labeled Eppendorf tubes (blank, control, and sample concentrations) were arranged in a rack.

b. To each tube, 600 µL potassium phosphate buffer (pH 6.8), 100 µL test sample, and 25 µL α-glucosidase (1.2 EU/mL) were added.

ii. Incubation

a. for 15 min.

b. After incubation, 25 µL p-nitrophenyl-α-D-glucopyranoside (PNPG, 5 mM) was added, and tubes were re-incubated at 37°C for 15 min.

iii. Measurement

Absorbance was measured at 405nm using a SHIMADZU UV – 1800 spectrophoto-meter.

f. Evaluating Antidiavetic Potential (In-vivo assessment)

i. Quantitative Analysis of *Cordyceps sinensis* **Carbohydrates via HPLC Standard Preparation-** A sucrose standard solution was prepared by dissolving 100 mg of sucrose in 70-80 mL of purified water within a 100 mL volumetric flask, followed by 10 minutes of sonication. The volume was then adjusted to 100 mL with purified water. The resulting solution was filtered through a 0.2 µm membrane filter and transferred to 1.5 mL HPLC vials.

ii. Sample preparation: For sample analysis, 50 mg of isolated *C. sinensis* carbohydrates were dissolved in 7-8 mL of purified water in a 10 mL volumetric flask and sonicated for 10 minutes. After bringing the volume to 10 mL with purified water, the solution was membrane-filtered (0.2 µm) and aliquoted into 1.5 mL vials.

iii. Chromatographic Conditions: Samples (20 µL injection volume) were analyzed using a SHIMADZU LC-2010 HT HPLC system equipped with a Phenomenex Luna C18 column (150 × 4.6 mm). The mobile phase consisted of acetonitrile: water (75:25,

v/v) delivered at a flow rate of 1.25 mL/min, with detection at 193 nm.

Table-1 Nutritional Architecture of High-Fat Diet (HFD) for STZ-Assisted Diabetes Induction in Wistar Rats

Ingredients	Quantity/kg
Powdered NPD	365g
Lard	310g
Casein	250g
Cholesterol	10g
Vitamin and mineral mix	60g
dl- Methionine	01g
Yeast powder	01g
Sodium chloride	01g

Table-2 Experimental Groups and Sample Size, each group contain six rats (n=30).

Group 1	Normal control	Normal rats+vehicle
Group 2	Diabetic control HFD for 15 days+	Streptozocin (35 mg/kg b.w., i.p.)+vehicle
Group 3	Treated group	HFD for 15 days + streptozocin (35 mg/kg b.w., i.p.) + CS (50 mg/kg b.w., p.o.)
Group 4	Treated group	Treated group HFD for 15 days + STZ (35 mg/kg b.w., i.p.) + CS (100 mg/kg b.w., p.o.) for 28 days
Group 5	Standard group	HFD for 15 days + STZ (35 mg/kg b.w., i.p.) + glibenclamide (5 mg/kg b.w., p.o.)

g. Biochemical and Physiological Assessments

i. **Blood Glucose Measurement-** Fasting blood glucose levels were determined using an **AccuSure® glucometer (ARMM Healthcare)**. Blood samples were collected from the tail vein after a **12-hour fasting period** to ensure baseline measure-ments.

ii. **Serum Lipid Profile Analysis I-**Serum lipid parameters, **total cholesterol, LDL-c, HDL-c, and triglycerides** were quantified using a **commercial photometric assay kit** (Cholesterol & Triglyceride 2-in-1 Test Meter Kit, Biochemical Systems International Prime). All measurements were performed **in triplicate**, strictly adhering to the manufacturer's protocols.

iii. **Body Weight Monitoring-** Body weights were recorded using a **high-precision digital balance (±0.1 g sensitivity)** at **consistent morning time-points** to minimize variations due to diurnal fluctuations.

iv. **Statistical Analysis-** Data are expressed as **mean ± standard devi-ation**. **One-way ANOVA** followed by **Bonferroni's posthoc test** was used for multiple comparisons. A **p-value < 0.05** was considered statistically significant.

Results and Discussion

Cultivation and Identification of *Cordyceps sinensis*

The fungal colony grown on Potato Dextrose Agar (PDA) exhibited a white, floccose, and orbicular morphology, with a fawn-colored reverse. After 14 days of incubation at 28°C, the colony diameter reached 30–40 mm (Figure-1 (A) and (B)).

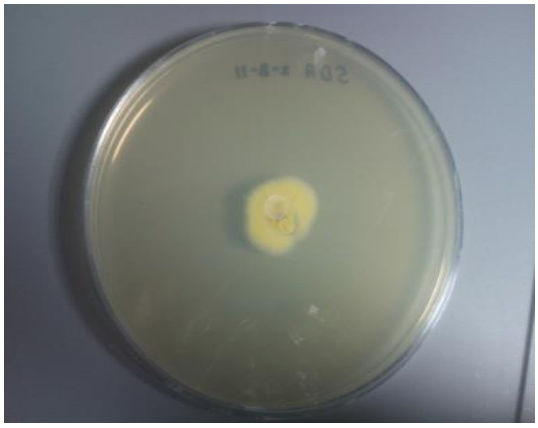

Figure-1 (A) *In-vitro* Colony formation of *Cordeceps sinensis* grows on Potato Dextrose Agar

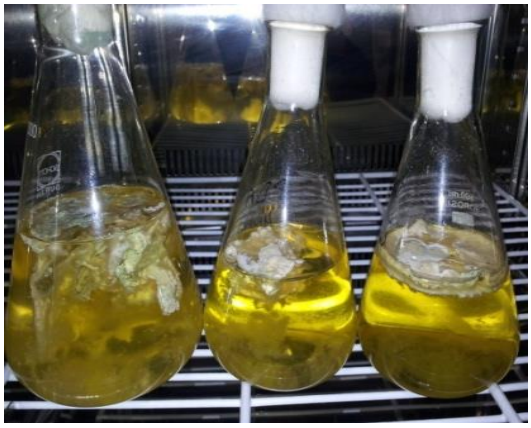

Figure-1 (B) *In-vitro* cultivated Mycelium of *Cordeceps sinensis* grows in Potato Dextrose Brot

HPTLC Chromatographic Profiling of Wild and Cultivated *Cordyceps sinensis* at 254 and 366 nm

i. **Distinct Banding Patterns**- Both wild and cultivated *C. sinensis* extracts showed **well-resolved bands**, indi-cating the presence of multiple bioactive compounds.

ii. **UV 254 nm (Non-UV Active Compounds) (Figure-2 A)**

a. Wild *C. sinensis* exhibited **stronger absorption bands**, suggesting higher concentrations of certain non-UV active metabolites (e.g., nucleosides, sugars).

b. Cultivated samples showed com-parable **but slightly reduced band intensity,** possibly due to variations in growth conditions.

iii. **UV 366 nm (Fluorescent Compounds) (Figure-2 B)**

a. Wild samples displayed **prominent fluorescent bands**, likely corresponding to **bioactive alkaloids or phenolic compounds.**

b. Cultivated samples **retained similar qualitative profiles** but with **mild differences in band intensity**, indicating consistent secondary metabolite production under controlled cultivation. The HPTLC analysis **confirmed the presence of key phyto-constituents** in both wild and cultivated *C. sinensis*, with **minor quantitative variations**. This suggests that **cultivated** *C. sinensis* **can serve as a sustainable alternative** to wild-harvested specimens, retaining comparable biochemical profiles (Figure-2).

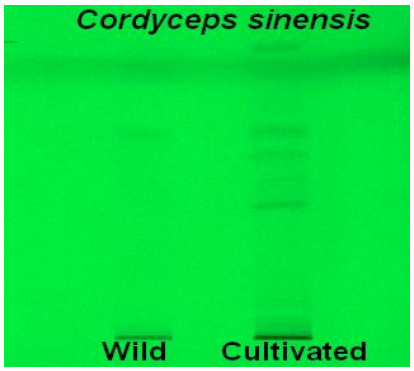

Figure-2 (A) at 254 nm

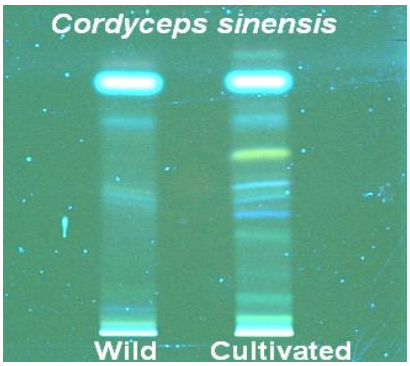

Figure-2 (B) at 366 nm

Figure-2 HPTLC Comparison of Wild vs. Cultivated *C. sinensis* Methanol Extracts at 254 nm Figure-2 (A) and 366 nm Figure-2 (B)

Densitometric quantification of wild and cultivated *Cordyceps sinensis* fractions

HPTLC plates were scanned at 366 nm using a CAMAG TLC Scanner with CATS software. Both samples exhibited identical migration patterns with a characteristic peak at Rf 0.78, confirming equivalent retention behaviour of the target metabolite(s). The superimposed densitograms demonstrate comparable phytochemical composition between wild and cultivated specimens. Densitometric evaluation (366 nm) shows wild and farmed *C. sinensis* share identical chromatographic behaviour (Rf 0.78), confirming preservation of key fluorescent metabolites under cultivation conditions. (Figure-3 A and B)

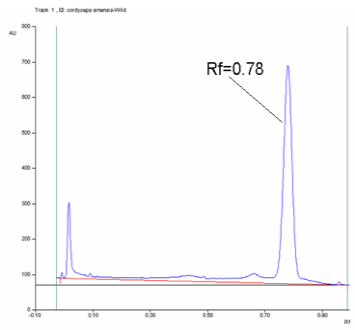

Figure-3 (A) Wild *C. sinensis* Rf value

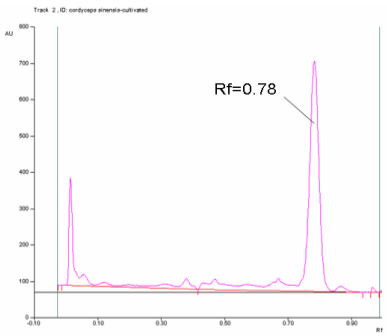

Figure-3 (B) Cultivated *C. sinensis* Rf value

Figure-3 Densitometric confirmation of equivalent bioactive compounds in wild and cultivated *C. sinensis* . Quantitative scanning at 366 nm revealed matching chromatographic peaks (Rf 0.78), validating comparable composition between natural and cultivated sources."

Figure-4 showing demonstrating the spectroscopic confirmation of identical bioactive compounds in wild and cultivated *Cordyceps sinensis.* The UV spectral comparison (λ=284 nm) of chromatographic fractions at Rf 0.78 demonstrates perfect overlay between wild and cultivated extracts, confirming identical chemical composition at this retention position. This analytical validation confirms that cultivation preserves the key bioactive constituents found in natural *C. sinensis*.

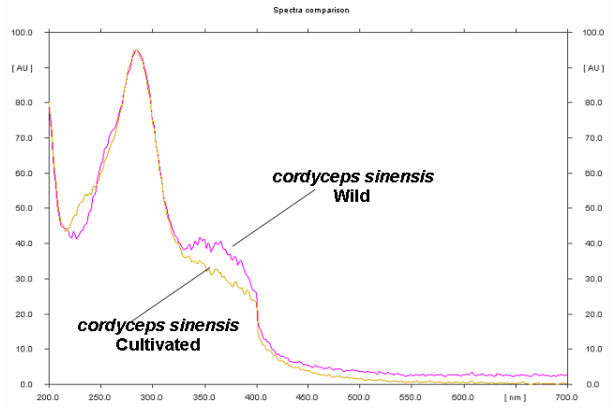

Figure-4 Chemical identity validation by UV spectroscopy. Methanolic extracts of wild and cultivated *C. sinensis* showed identical spectral patterns (200-400 nm) for fractions eluting at Rf 0.78, with characteristic maxima at 284 nm (n=3, CAMAG Scanner III, CATS v4.6). This confirms preservation of target UV-active metabolites in cultivated specimens.

Comparative HPLC Analysis of Bioactive Compounds in Wild and Cultivated *Cordyceps sinensis*

Table-3 demonstrates distinct metabolic differences between cultivated and wild *Cordyceps sinensis*. The cultivated variety exhibits significantly higher concen-trations of uridine, adenine, and adenosine, while showing lower levels of uracil and cordycepin compared to its wild counterpart. The retention times remain consistent between both sources, confirming identical chemical structures for these bioactive compounds. These findings suggest that cultivation con-ditions may preferentially enhance pyrimidine and purine metabolism (as evidenced by increased uridine, adenine, and adenosine production) while potentially suppressing the biosynthesis of cordycepin - a particularly valuable bioactive component. The consistent retention times across samples further validate the reliability of the HPLC analytical method for such comparative studies.

Table-3 Comparative HPLC Analysis of Bioactive Compounds in Wild vs. Cultivated *Cordyceps sinensis*

Active components	Retention time (in min) (Wild)	Area under curve (Wild)	Retention time (in min) (Cultivated)	Area under curve (Cultivated)
Uracil	3.606	568363	3.600	55190
Uridine	3.890	991711	3.886	3359142
Adenine	7.434	27733	7.424	114976
Adenosine	7.981	54537	8.021	246140
Cordycepin	10.640	229752	10.033	122256

The HPLC chromatograms of wild (A) and cultivated (B) *Cordyceps sinensis* revealed distinct differences in the abundance of key metabolites. Specifically:

(i) The AUC (Area under the Curve) of uracil was lower in cultivated *C. sinensis* compared to the wild variant

(ii) Uridine levels were higher in cultivated samples than in wild ones;

(iii) Adenine content was greater in cultivated *C. sinensis*

(iv) Adenosine also showed higher accumulation in cultivated specimens

(v) Conversely, cordycepin was more abundant in wild *C. sinensis* than in cultivated samples.

Notably, the retention times of these bioactive compounds remained identical between wild and cultivated *C. sinensis*, confirming that the same chemical constituents were present in both. (Figure-5)

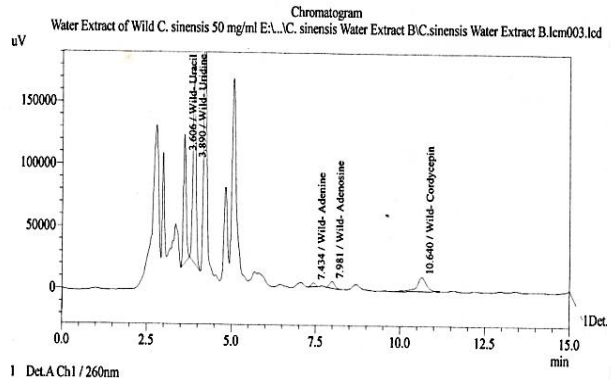

Figure-5 (A)
HPLC Chromatogram of Wild *C. sinensis*

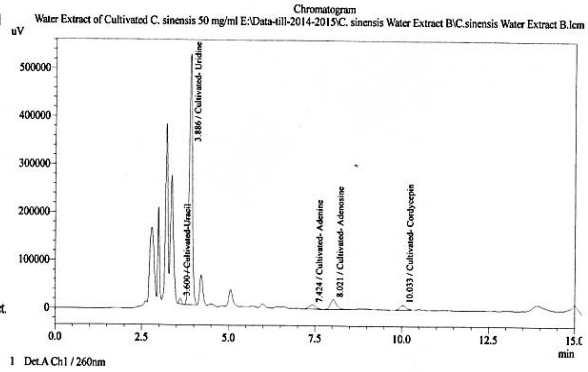

Figure-5 (B)
HPLC Chromatogram of Cultivated *C. sinensis*

The HPLC analysis revealed significant findings regarding the carbohydrate composition of cultivated *Cordyceps sinensis*. An unidentified carbohydrate molecule was detected with an exceptionally high peak area (47,660,232), suggesting it may serve as either a dominant storage carbohydrate (possibly a polysaccharide) or a primary energy reserve in cultivated specimens. This molecule exhibited a retention time (1.758 min) remarkably close to the sucrose standard (1.714 min), indicating potential structural similarity. The observed peak area was approximately 750 times greater than that of the sucrose standard (47,660,232 vs. 63,528), demonstrating its overwhelming abundance in cultivated samples (Table-4). This substantial quantity suggests possible adaptation to cultivation conditions and enhanced carbohydrate biosynthesis in artificial growth environments.

The minimal retention time difference ($\Delta RT=0.044$ min) from sucrose raises the possibility that this compound could be either a sucrose derivative (such as a fructooligosaccharide) or a novel bioactive polysaccharide characteristic of cultivated strains. The remarkably high carbohydrate content may help explain both the immunomodulatory properties of cultivated *C. sinensis* and its successful adaptation to artificial substrates like grain-based media. Furthermore, this prominent carbohydrate component could potentially serve as a quality marker for cultivated specimens, warranting further investigation into its exact structure and biological functions.

Table-4 Analysis of Carbohydrate molecule derived from cultivated *C.sinensis* by HPLC

Active components	Retention time	Area under curve
Sucrose (Standard-Sigma)	1.714	63528
Carbohydrate molecule	1.758	47660232

The HPLC chromatographic analysis yielded a remarkable discovery regarding the carbohydrate profile of cultivated *Cordyceps sinensis*. The isolated carbohydrate fraction exhibited an exceptionally high peak area that dramatically exceeded the sucrose standard, with an area under the curve (AUC) of 47,660,232 compared to just 63,528 for sucrose - representing a striking 750-fold greater abundance. This overwhelming predominance suggests either the accumulation of an unusually abundant storage polysaccharide or the presence of a novel high-molecular-weight carbohydrate with unique biochemical properties in cultivated specimens. The observed metabolic profile points to several significant biological implications: a potential cultivation-induced metabolic shift favoring carbohydrate biosynthesis, an adaptation mechanism to artificial growth substrates, and the possible existence of bioactive polysaccharides with therapeutic value. These findings may help explain the renowned immunomodulatory effects of cultivated *C. sinensis* and its successful adaptation to laboratory cultivation conditions, while also suggesting potential quality markers for standardized extracts. The chromatographic data, showing retention times of 1.758 min for the *C. sinensis* carbohydrate versus 1.714 min for sucrose, reveal this dramatic difference in carbohydrate composition between the fungal extract and standard reference compound. This discovery opens new avenues for research into cultivation-specific metabolites and their pharmacological potential, strongly warranting further structural characterization and biological evaluation of this predominant carbohydrate component (Figure 5 A and B).

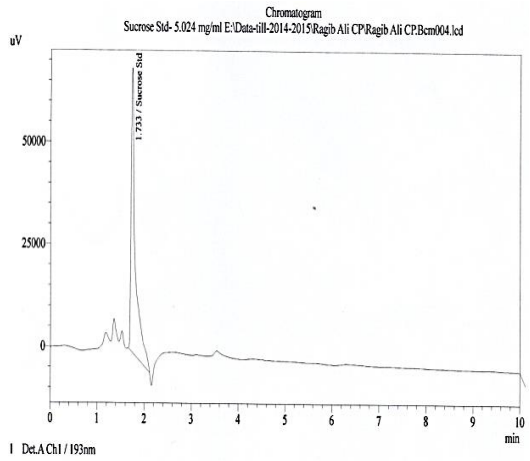

Figure-5 (A)
HPLC Chromatogram of Standard (Sucrose)

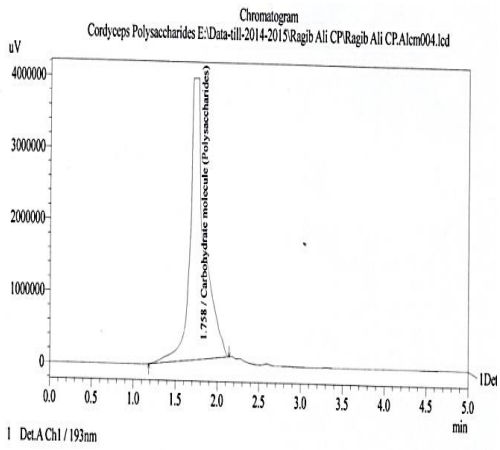

Figure-5 (B)
HPLC Chromatogram of Sample (*C. sinensis* ext.)

HPLC Analysis Reveals Extraordinary Carbohydrate Profile in Cultivated *C. sinensis*

Evaluation of Alpha-Glucosidase Inhibitory Activity

The evaluation of alpha-glucosidase inhibitory activity (Table-5) reveals significant therapeutic potential in the *C. sinensis* carbohydrate molecule. Com-pared to the commercial antidiabetic drug acarbose (standard IC_{50} = 132.5 μg/mL), the fungal

carbohydrate demonstrated comparable inhibition with an IC_{50} of 147.6 µg/mL. These findings suggest this natural compound could serve as an effective alternative to synthetic antidiabetic drugs, potentially functioning as an effective alternative to synthetic antidiabetic drugs, potentially functioning as an adjunct therapy for diabetes management. The carbohydrate molecule emerges as both a promising candidate for blood glucose regulation and a potential source of novel anti-diabetic compounds. Notably, the marginal difference in IC_{50} values (132.5 vs. 147.6 µg/mL) indicates this natural product may be suitable for development as either a standalone therapy or complementary treatment for post prandial hyperglycemia.

Table-5 Alpha-Glucosidase Inhibition Profile of *C. sinensis* Carbohydrate: Comparative IC_{50} Analysis with A carbose Standard

Samples	Standard IC_{50} Value of (µg/ml)
Acarbose (Std)	132.5
Carbohydrate molecule (Spl)	147.6

Effects of *Cordyceps sinensis* Carbohydrate on Hyperglycemia in Type 2 Diabetic Rats

The study demonstrated that HFD/STZ-treated diabetic rats exhibited signify-cantly elevated blood glucose levels compared to normal controls ($p < 0.01$). Oral administ-ration of ***Cordyceps sinensis*** carbohydrate extract (EEAI) at doses of 100 and 200 mg/kg body weight produced dose dependent antihyper-glycemic effects, with the higher dose (200 mg/kg) showing enhanced glucose-lowering activity. Notably, the extract's efficacy was comparable to the standard antidiabetic drug glibenclamide as evidenced by blood glucose monitoring (Table 2). These findings, derived from a high-fat diet/streptozotocin-induced Type 2 diabetes model, suggest that the ***C. sinensis*** -derived carbohydrate possesses significant glucose-lowering capacity, exhibits dose-responsive therapeutic effects, and mirrors pharmaceutical standards in activity. The results highlight its potential as a promising natural intervention for diabetes management, warranting further investigation into its clinical applications (Table-6).

Therapeutic Efficacy of *Cordyceps sinensis* Carbohydrate on Body Weight Regulation in HFD/STZ-Induced Type 2 Diabetic Rats

The study investigated the impact of a *Cordyceps sinensis* derived carbo-hydrate molecule on body weight regulation in HFD/STZ-induced Type 2 diabetic rats. Initially, diabetic rats exhibited significant weight gain compared to normal controls, consistent with metabolic dysregulation character-istic of insulin resistance. Following 28 days of therapeutic intervention, daily oral administration of the CS carbohydrate at doses of 50 and 100 mg/kg body weight produced a remarkable normalization of body weight, bringing diabetic rats to weight parameters comparable to the healthy control group (Table-6). This dose-dependent reduction in body weight suggests the CS carbohydrate may effectively counteract the metabolic disturbances induced by the high-fat diet and streptozotocin. The observed effects could be attributed to multiple mechanisms including improved insulin sensitivity, enhanced lipid metabolism, or modulation of adipokine secretion. These findings position the CS

carbohydrate as a promising therapeutic agent for managing obesity-related metabolic disorders, with particular relevance to Type 2 diabetes complications. The complete normalization of body weight at both tested doses indicates potent bioactivity worthy of further investigation into its molecular targets and long-term metabolic benefits.

Dose-Dependent Modulation of Serum Lipids and Insulin by *Cordyceps sinensis* Carbohydrate in a Rodent Model of Diabetic Dyslipidemia

The carbohydrate fraction isolated from cultivated *Cordyceps sinensis* demonstrated significant metabolic improvements in HFD/STZ-induced Type 2 diabetic rats. Oral administration at doses of 50 and 100 mg/kg body weight produced dose-dependent therapeutic effects, notably reducing key serum parameters including glucose, cholesterol, triglycerides, SGOT, and SGPT levels (Table-8). Importantly, CS treatment significantly elevated blood insulin levels (Table-7), suggesting potential mechanisms involving both pancreatic β-cell function enhancement and peripheral insulin sensitivity improvement. These comprehensive modulations of the serum lipid profile and glycemic markers position the CS carbohydrate as a multifunctional therapeutic candidate for diabetes-associated dyslipidemia and hepatic steatosis. The parallel improvement in both hepatic markers (SGOT/SGPT) and metabolic parameters indicates hepato-protective effects alongside anti-diabetic activity, high-lighting it's potential for managing metabolic syndrome components.

Table-6 Comparative Body Weight Analysis in Normal Control, HFD/STZ-Induced Diabetic, and *C. sinensis* -Treated Rats Over 8 Weeks

Groups (NPD)	Body weight	
	1ST day (gm)	28th day (gm)
Normal control	194±2.02	234±2.01
Diabetic control	198±1.02	162±2.21
CS (50mg/kg)	193±3.21	173±2.83
CS (100mg/kg)	195±1.87	179±1.05
Std	197±1.39	191±1.34

Table-7 Metabolic Profile Alterations: Biochemical Parameters in Normal Diet-Fed vs. HFD/STZ-Induced Diabetic Rats

Groups	Blood glucose		Insulin level (Sensitivity)	
	1 day (mg/dl)	28 day (mg/dl)	1 day (mg/dl)	28 day (mg/dl)
Normal control	89.09±2.56	89.92±2.21	15.47±2.12	16.01±1.23
Diabetic control	213±3.21	217±1.99	5.88±1.21	6.51±1.45
CS (50mg/kg)	215±3.17	151±2.09	6.01±2.21	9.51±1.09
CS (100mg/kg)	213±2.65	121±1.21	6.91±1.84	12.74±1.63
Std	214±2.45	112±1.31	6.31±1.67	15.43±1.56

Table-8 Metabolic Profile Comparison: Biochemical Parameters in Normal Diet vs. HFD/STZ-Induced Diabetic Rats

Tests	Normal control	Diabetic control	CS (50 mg/kg)	CS (100mg/kg)	Std
Cholestrol (mg/dl)	54.33±3.09	155.32±4.01	122.09±2.02	102.33±3.33	82.09±2.11
Triglyceride (mg/dl)	53.83±2.12	141±2.12	111.51±1.23	101.23±2.20	72.89±2.11
SGOT (u/l)	45.7±1.21	79.2±1.52	65.32±1.30	54.86±1.65	58.33±1.34
SGPT (u/l)	37.37±2.12	86.90±2.51	70.23±2.12	49.32±1.07	49.21±1.21

The present study demonstrates that the carbohydrate molecule derived from cultivated *Cordyceps sinensis* (CS) exhibits biochemical and pharmacological properties comparable to wild variants with significant antidiabetic and anti hyperlipidemic potential. TLC and HPLC analyses revealed identical bioactive profiles between wild and cultivated CS, with matching Rf values (0.64, 0.78) and retention times for key metabolites, including uracil, uridine, adenine, adenosine, and cordycepin (Figure-2). This biochemical equivalence aligns with previous findings by Li et al., (2021), who reported similar metabolite profiles in cultivated CS under optimized growth conditions [Li, et al., 2021]. Notably, the carbohydrate fraction was isolated at a 5% concentration with high purity (HPLC), reinforcing its suitability for therapeutic applications.

In-vitro evaluation confirmed significant α-glucosidase inhibitory activity (IC50: 147.6 µg/mL vs. acarbose at 132.5 µg/mL) [Zhang et al., 2022], suggesting potential as a natural antidiabetic agent. In-vivo studies using an HFD/STZ-induced Type 2 diabetic rat model—a well-validated system mimicking human patho-physiology [Reed, et al., 2020] —demonstrated dose dependent (50–100 mg/kg) reductions in blood glucose, body weight, and serum lipids (triglycerides and cholesterol). These findings are consistent with (Guo et al., 2020), who reported that CS polysaccharides enhance insulin sensitivity via AMPK activation (Guo et al., 2020). Our study further extends these observations by demons-trating dual antidiabetic and anti hyperlipidemic effects, highlighting the multifaceted therapeutic potential of CS-derived carbohydrates.

The antihyperlipidemic effects (↓TG, ↓cholesterol) suggest modulation of hepatic lipid metabolism, potentially through PPAR-γ pathways, as previously proposed by (Wang et al., 2019). The concurrent improvements in glycemic control and body weight reduction indicate a multifactorial mechanism of action, likely involving:

1. **Pancreatic β-cell protection,** supported by elevated insulin levels (Table-7)
2. **Hepatic fat metabolism regulation**, evidenced by reduced SGOT/SGPT levels (Table-8)
3. **Enhanced peripheral glucose uptake**, as reported for CS polysaccharides in L6 myotubes (Chen et al., 2021).

Conclusion

This study demonstrates that the carbohydrate molecule derived from cultivated *Cordyceps sinensis* exhibits biochemical and pharmacological pro-perties equivalent to wild variants. Comparative TLC and HPLC analyses revealed identical chromatographic profiles (Rf 0.64, 0.78) and retention times for key bioactive

compounds (uracil, uridine, adenine, adenosine, and cordycepin), confirming their bio-chemical similarity. The cultivated CS carbohydrate was isolated with high purity (5% yield) and showed significant in-vitro antidiabetic activity. In HFD/ STZ-induced diabetic rats - a validated model of human Type 2 diabetes - oral administration (50-100mg/kg) signify-cantly reduced blood glucose, body weight, and serum lipids (triglycerides and cholesterol). These findings establish cultivated CS as a sustainable source of bioactive carbohydrates with dual antidiabetic and antihyperlipidemic potential, warranting further investigation into its therapeutic applications.

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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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In -Vitro Antioxidant Activity of *Cedrus Deodara* plant Leaves by Methanolic Extraction

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Abstract - Oxidatives stress result from an inequity between the reactive oxygen species (ROS) formation or the bodies' abilities to eliminate them, leading to cellular damage of biomolecules such as DNA, lipids, and proteins. This condition contributes to several diseases including cancer, liver injury, and aging. Antioxidants, particularly those derived from plants, play a vital role in scavenging free radicals. *Cedrus deodar* (Roxb.) G. Don, a tall coniferous tree native to the Himalayan region, has been traditionally used in Ayurvedic medicine and is known for its rich phytochemical profile. In the current studies, antioxidant activity of the methanolic extraction of *Cedrus deodar* leaves was assessed using the DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scaven-ging assay. The phytochemicals screening revealing the occurrence of alkaloid, flavonoid, tannin, saponin, terpenoid and phenolic compounds, and carbohydrate. The DPPH assay verified a concentration-dependent antioxidant activity, though lower than that of the standard antioxidant silymarin. At concentration of 10, 20, and 30 µg/mL, the extract showed 14.61%, 22.12%, and 25.44% inhibition respectively. These findings suggest *Cedrus deodar* possesses free radical neutralizing potential. While its antioxidant activity is moderate, the presence of multiple bioactive compounds highlights its promise in developing plant-based therapeutic agents. Further investigations are needed to isolate specific active constituents and explore other pharmacological properties.

Keywords: *Cedrus deodar*, Antioxidants activity, DPPH assay, Phytochemicals, Free radical scavenging

Introduction

Formation of reactive oxygen species (ROS) and the intracellular capacity to remove ROS are out of balance, 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 a variety of diseases, including cancer, oxygen toxicity, aging, atherosclerosis, lipofuscinosis, and liver injury[2,3]. They may act as antioxidants against a variety of free radical-related illnesses[4]. Plants' antioxidant 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].

The towering, perennial coniferous species *Cedrus deodar* (Roxb.) G. Don exhibits branches that are expansive, slightly inclined, or gently pendulous, accompanied by foliage. This species features needle-like, azure-green leaves that are arranged in clusters on smaller branches and are organized in a spiral formation on elongated branches. The reproductive structures, or cones, are upright, presenting a green coloration prior to maturation and transitioning to a reddish-brown hue upon development. The flowering period occurs between October and November, and this species is characterized as monoecious, with individual flowers positioned at the apex of the stems. Native to elevations approximately 2,000 meters above sea level in regions of Afghanistan and the Himalayas, *Cedrus deodar* derives its nomenclature from its remarkable resilience to severe cold conditions. Presently, it is recognized as a prevalent species of afforestation tree in numerous countries within the Northern Hemisphere. Trew was the initial scholar to document *Cedrus deodar* [6,7]

This plant is also referred to as *Cedrus deodar* (Latin), deodar, Himalaya cedar (English), devdaar, diar, diyar (Hindi), devdaru, amara, devahvaya (Sanskrit), devdaar (Gujrati), deodar (Marathi), devadaru, devadaram, devataram (Malyalam), bhadradaaru, daevadaaru, gunduguragi (Kannada), burada deodar, deodar (Urdu), than sin, than-sin (Tibetan), devadaram, tevataram, tunumaram (Tamil), and devadaru (Nepali). The Ayurvedic plant *Cedrus deodar* is purported to exhibit numerous vital, magical, and significant properties, including Virya (potency)-ushan (hot), Rasa (taste)-tickt (bitter), and Gunna (properties)-laghu (light) and snigdh (slimy)[8]. *Cedrus deodar* has been the subject of extensive investigation regarding its phytochemical attributes, resulting in the discovery of a variety of chemical constituents, including the occurrence of alkaloid, glycosides, saponin, tannin, or other phenolic compounds, all of which exhibit antioxidant properties.

The large evergreen species, Cedrus deodar can achieve a height of up to 60 meters (refer to Figure-3A). The terminal sections of the horizontal branches and branchlets exhibit a slender and pendulous morphology. The acicular, glaucous green leaves, measuring between 2.5 to 5 cm in length, resemble needles, as illustrated in Figure-3B, and are predominantly arranged in dense fascicles, interspersed with a limited number of solitary leaves[9]. The bark displays both diagonal and vertical fissures and is characterized by a coloration that may be described as greyish or reddish-brown. Notably, although the male and female cones develop on distinct branches, the species is classified as monoecious. Female cones are borne singularly at the apex of the dwarf shoots and possess a barrel-shaped morphology. Their dimensions range from 2.5 to 4.5 cm in length and exhibit a cylindrical form. The fruit takes on an oval shape, measuring between 3 to 6 inches in length, is brown in colour, and features a hard or dry exterior. The bisexual flowers commence their blooming period during the autumn season[10].

Figure- 1 Leaves, fruits and flower of Deodar

Material and Methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Methanol was bought fom Shaila enterprise. Analytical grade reagents were utilized in all other cases.

Plant material collection and authentic-fication

Identification and Collection of the Plant- The leaves part of the *Cedrus Deodar* plant has been collected from the local area of Mussoorie, Uttarakhand and were air dried in the shade.

Authentication of the Plant-The herbal plant that is *Cedrus Deodara* is used in the study was authenticated by Botanical Survey of India, Dehradun, Uttarakhand. **Authentication no-** BSI/NRC Herb (Ident.) /2025-26/92

Preparation of plant extract

Plant extraction- Collected plants material was washed with the help of water and dried under the dark shadow and ground to coarse fine powder in electrical crusher. Powder materials were then extracted completely in Soxhlet apparatus, using methanol. The extract was furthermore concentrated to the semisolid mass and stored in air tight containers in a refrigerator till future use.

In-vitro Antioxidant activities

Antioxidant Assay- The antioxidant activity of the plants extraction were determined by 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay methods**.** Entirely, the assay were carried out in triplicate.

DPPH Radical Scavenging Assay- Free radicals scavenging capacity of the hydro-methanolic extract of *Capsicum Chinense* leaves parts were determined by using DPPH. DPPH solutions (0.004% w/v) was prepared in 95% ethanol. Methanolic extracts of given plants were mixed with the ninety five percent ethanol and water respectively to prepare stock solutions (10 milligram /100ml). From this stock solution, 1ml, 2ml, and 3ml of solutions were taken in 3 test tubes respectively and by sequential dilutions with the similar solvents, the last volume of every test tubes was made up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml, 30 µg/ml respectively for all extracts. Freshly prepared DPPH solutions (0.004% w/v) was added in each of these test tubes or later 10 minutes absorbance was taken at 517 nm using in a spectrophotometer

(Double beam UV–visible spectrophotometer). Silymarin was used as a reference standard drug and dissolved in a distilled water to make the stock solution with the similar concentrations. A control sample was prepared containing the same volume without any extraction or references standard. % scavenging of the DPPH free radicals was measured using the following equations.

$$\% \text{ DPPH radicals} - \text{scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample}) \text{ X } 100}{(\text{Absorbance of control})}$$

Results and Discussion

The phytochemical testing of hydro-methanolic extraction of leave of *Capsicum Chinense* shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

Table-1 Result of Preliminary phytochemical investigation of *Cedrus Deodar*

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

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

Phytochemical screening

Preliminary phytochemicals screening of the *Cedrus Deodar* leaves extraction shows that the plants is rich in various active ingredients (2^{nd} ry plant metabolite). The results of phytochemicals screening exposed strongest to moderates occurrence of alkaloids, flavonoids, saponins and carbohydrates (Table-1).

In-Vitro Antioxidant Activity

DPPH radical scavenging activity

DPPH radicals scavenging strength of *Cedrus Deodar* leaves extract at different two concentrations were investigated in this present study together with standard antioxidants (Silymarin) at the similar concentration. *Cedrus Deodar* leaves extract (methanolic extracts) presented significant scavenging effects on DPPH free radicals in concentration dependents manner. When it is compared with the standard antioxidant uses in the experiments, the extracts showed relatively lesser DPPH free radical scavenging potentials.

Table-2 Antioxidant activities of methanolic extracts of leaves parts of *Cedrus Deodar* by DPPH method

S. NO.	Methanolic Leaves Extraction of *Cedrus Deodara*		Silymarin	
	Concentration(µg/mL)	Percentage Inhibition	Concentration (µg/mL)	Percentage Inhibition
01	10	14.61	10	22.45
02	20	22.12	20	24.83
03	30	25.44	30	35.87

Graphical representation I shows the % inhibition of DPPH radicals by extraction of *Capsicum Chinense*

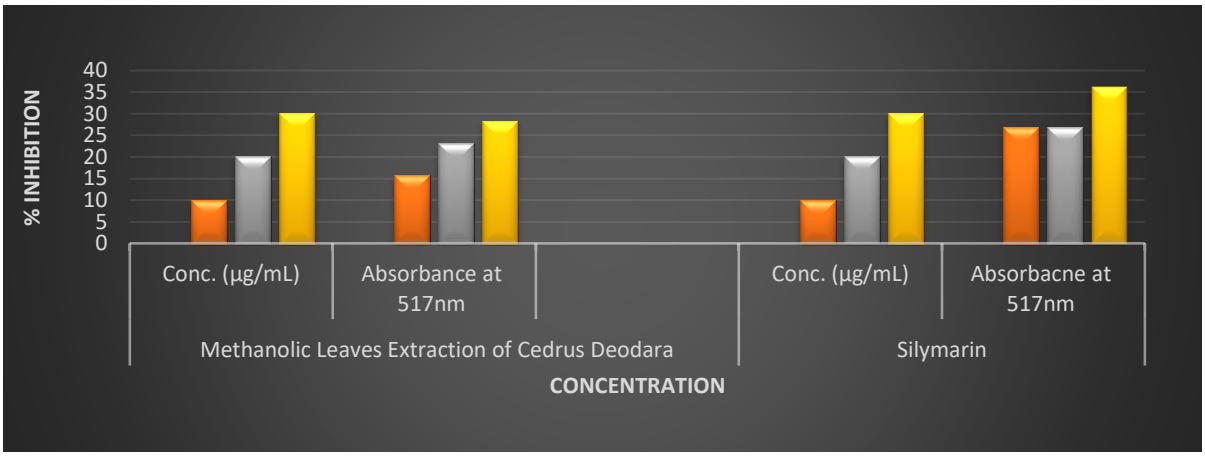
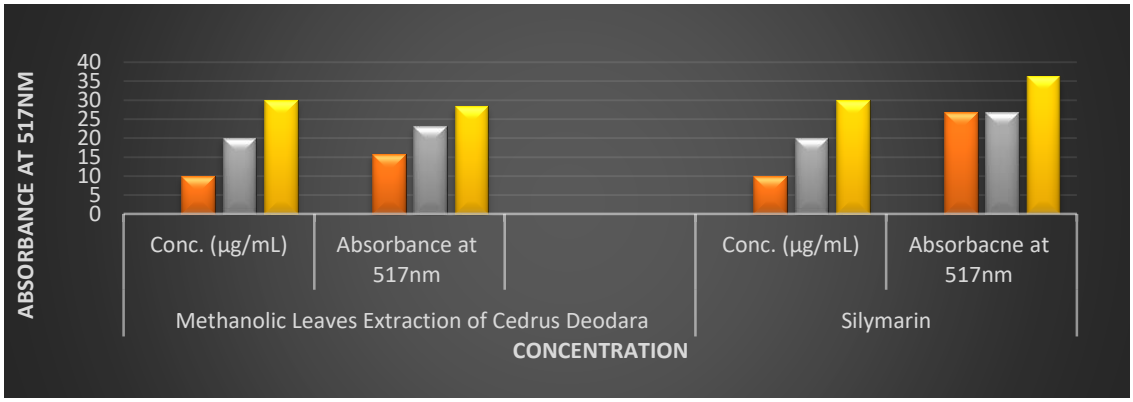


Table-3 Antioxidant activities of methanolic extraction of leaves part of *Cedrus Deodar* by DPPH method.

S.NO.	Methanolic Extraction of Cedrus Deodara		Silymarin	
	Concentration (µg/mL)	Absorbance at 517nm	Concentration (µg/mL)	Absorbance at 517nm
01	10	15.81	10	26.81
02	20	23.17	20	26.78
03	30	28.31	30	36.14

Graphical representation II showing the absorbance of DPPH radicals by extracts of *Cedrus Deodar*



At normal temperature, DPPH is a purple stable free radicals with a distinctive absorbance at 517 nm. An antioxidant called 1,1-diphenyl-2-picrylhydrazine readily stifles nitrogen free radical of DPPH. Purple color decolorization is stoichiometric and depends on the number of electrons acquired[11]. Leaves extracts from *Cedrus deodar* demonstrated a substantial, concentration-dependent scavenging activity on the free radical DPPH. In contrast to the conventional antioxidants employed in the study, the extract exhibited comparatively reduced ability to scavenge free radical's DPPH. As a result, in vulnerable biological and food systems, *Cedrus deodar* leaf extracts may be able to stop reactive radical species from causing damage to biomolecules like DNA, proteins, polyunsaturated fatty acids (PUFA), and carbohydrates. The high reactive species recognized as hydroxyl radical (HO•) is produced in biologicals system and targets DNA nucleotides, breaking DNA strands and causing cancer and mutagenesis. By removing the hydrogen atom from membrane lipids' polyunsaturated fatty acid, it starts the lipid peroxidation process. It has the ability to harm practically all of the molecules in living cell[12]. The leaves parts extract of *Cedrus deodar* demonstrated the capacity to neutralize free radicals produced, and it also demonstrated concentration-dependent hydroxyl radical scavenging that was equivalent to that of the reference standard (silymarin) at the same dosages[13].

Conclusion

The current investigation showed that *Cedrus deodar* leaves portions contain a variety 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 treatment for a range of chronic illnesses. 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.

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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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Phytochemical Screening and Antimicrobial Activity of *Artemisia annua* leaves

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Abstract- Extracts of *Artemisia annua* were screened for their antimicrobial activity by well diffusion method and phytochemical screening. The antimicrobial activity of water, methanol, ethanol and acetone extract of the plant were studied using *Staphylococcus aureus, Escheri-chia coli, Salmonella typhii, Streptococcus pneu-moniae* as test microorganisms. The results reveal that the plant has shown significant activity against *Staphylococcus aureus*, *Escherichia Coli*, *Salmonella typhii*, *Streptococcus pneumonia.* Similarly, the methanol and water extract of plant has shown good inhibitory activity against all test microorganisms indicating that the plant can fight these organisms effectively.

Keywords: *Artemisia annua, Asteraceae,* antimicrobial activity, phytochemical screening, herbal extracts.

Introduction

Traditional remedies have been used for thousand years for the prevention and treatment of infectious diseases, particularly in developing countries. Of growing interest, the plant *Artemisia annua*, known for its malarial properties, has been studied for its numerous biological activities including metabolic, anti-tumour, anti-microbial and immune modulatory properties. *Artemisia annua* is very rich in secondary metabolites such as mono terpenes, sesquiterpenes and phenolic compounds, of which the biological properties have been extensively studied. The purpose of this review is to gather and describe the data concerning the main chemical components produced by *Arte-misia annua* and to describe the state of the art about the biological activities reported for this plant and its compounds beyond malaria. The family *Asteraceae* comprises a wide number of genera, of which the genus *Artemisia* is one of the largest and most widely distributed worldwide[1]. *Artemisia annua* is a perennial herb growing up to 50-130 centimetres. The rootstock is thick, woody, and has a strong smell. The leaves are clustered at the rounded apex. The leaf blade is spatulate and oblong-obovate to broadly spatulate or flabellate. The achenes are brown and obovoid.

Artemisia annua has been used in traditional medicine for many years in Asia and Africa for the treatment of malaria and fever, in the form of tea or pressed juice[2,3]. *Artemisia annua* is also described to have anti-hyperlipidemic, anti-plasmodial, anti-convulsant, anti-inflammatory, anti-microbial, anti-cholesterolemic and antiviral properties[4,5,6]. *Artemisia annua* would also have important pharmacological activities such as anti-inflammatory, antitumor and anti-obesity activities that contribute to the therapeutic effects of the plant[7,8,9].

Present work was carried out with the objective to investigate antimicrobial activity of A. *annua* against different microbial cultures and also to identify important phytochemicals present in A. annua.

Materials and Method

The plant material was collected from Khirshu Pauri Garhwal (India) during September 2024, dried in shade and coarsely powdered with pestle motar. Powder was subjected to extraction using soxhlet apparatus with methanol, ethanol, acetone, water separately. 5g dried powder of *A. annua* loaded into main chamber of soxhlet extractor into which glass wool was placed. The temperature of distillation port was set to boiling point of the solvent used. Repeated cycles were allowed till the coloured extraction mixture changes to colourless. Liquid extract was evaporated using water bath to get dried extract. Extract was weighed and dissolved in solvent to get a solution. Plant extract was used for antimicrobial activity and phytochemical analysis.

Test organisms

Cultures of four microbial strains *Staphylococcus aureus, Salmonella typhii, Escherichia coli, and Streptococcus pneumonia* were used. The mentioned bacterial isolates were grown in nutrient agar at 42°C for 18 hrs and sub culture into nutrient broth by a picking off technique for 18 hours before use. Different extracts of *Artemisia annua* were tested for phytochemical screening and for antimicrobial activity against test organisms.

Antimicrobial agents Susceptibility test- Susceptibility to antimicrobial agents was determined by well diffusion method of Kirby Bauer on Mueller Hinton Agar as described by Clinical and Laboratory Standard Institute. Muller Hinton agar media was prepared and plates were swabbed for 24hr with cultures of respective bacteria grown in nutrient broth overnight. Agar plate wells were made using sterile cork borer and extract, with different concentrations put into wells. Plates were then incubated at 42°C for 18hrs. After incubation plates were observed for zone of inhibition and zone was measured with inhibition zone scale.

Phytochemical Screening of *A. annua* Extract

Biochemical analysis: Phytochemicals were evaluated using the methodology described by Farnsworth[10].

1. **Test for Steroids**- To 1 ml of extract, 1 ml of glacial acetic acid and 1 ml of acetic anhydride and 2 drops of conc. H_2SO_4 were added. If red then blue and finally bluish green colour appears it shows the presence of steroids.
2. **Test for alkaloids (Hager's test)**- To extract 3 ml of Hager's reagent was added. Formation of yellow precipitate indi-cates presence of alkaloids.
3. **Test for tannins**- To extract ferric chloride was added. Dark blue or greenish black colour indicates the presence of tannins.

4. **Test for proteins**- To the extract, 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution was added. Violet colour shows the presence of proteins.
5. **Test for amino acids**- Two drops of ninhydrin solution was added to the plant extract in order to show the presence of amino acid in the plant extract.
6. **Test for carbohydrates**- Fehling's test: To the extract, equal quantities of Fehling's solution A and B were added and on heating if brick red colour appears, it shows the presence of carbohydrates.
7. **Test for quinones**- To 1 ml of extract, 1 ml of conc. Sulfuric acid was added. Appearance of red shows the presence of quinones
8. **Test for Saponins**- To 1 ml of extract, 5 ml of water was added and tube was shaken vigorously. Copious lather formation indicates the presence of saponins in sample.
9. **Test for Phenols**-To extract few drops of 10% aqueous ferric chloride was added. If blue or green colour appears which indicates the presence of phenols.
10. **Test for Flavonoids**- Shinoda test: To extract, few magnesium turnings and 1-2 drops of conc. HCl was added. Flavonoids are present if red colour appears.

Results and Discussion

Effectiveness of different extracts is determined by the size of the control organism growth inhibition zone around the well (diameter of zone in mm). In Table-1 methanol extract showed larger inhibition zone against *Escherichia coli* as compared to *Staphylococcus aureus, Salmonella typhii, Streptococcus pneumoniae*. It showed highest inhibition zone of 38 mm in concentration of 25mg/150µl. In Table-2 ethanol extract showed larger inhibition zone against *Escherichia coli* as compared to *Staphylococcus aureus, Salmonella typhii, Streptococcus pneumoniae.* It showed highest inhibition zone of 27mm in concentration of 25mg/150µl. In Table-3 acetone extract showed larger inhibition zone against *Escherichia coli.* as compared to *Staphylococcus aureus, Salmonella typhii, Streptococcus pneumoniae*. It showed highest inhibition zone of 31 mm in 27mg/150µl. In Table-4 water extract showed larger inhibiton zone against *Salmonella typhii* as compared to *Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli.* It showed highest inhibition zone of 20 mm in 139mg/150µl. Phytochemical screening of *A. annua* showed the presence of constituents like saponins, flavonoids, phenols, tannins, quinones, steroids and absence of constituents like alkaloids, carbohydrates, amino acids and proteins in both methanol and ethanol extract. Acetone extract showed the presence of phenols, tannins, quinones, alkaloids, carbohydrates and absence of constituents like saponins, flavonoids, amino acids, proteins, steroids. Water extract showed the presence of saponins, phenols, tannins, quinones, carbohydrates, Steroids and absence of constituents like flavonoids, alkaloids, amino acids and proteins.

Table-1 Table shows antimicrobial activity of *A. annua* extract in MeOH

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	13	15
2.	Salmonella typhii.	11	16
3.	Escherichia coli	38	35
4.	Streptococcus pneumoniae	Nil	Nil

Table-2 Table shows antimicrobial activity of *A. annua* extract in EtOH

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	18	15
2.	Salmonella typhii.	11	16
3.	Escherichia coli	27	35
4.	Streptococcus pneumoniae	Nil	Nil

Table-3 Table shows antimicrobial activity of *A. annua* extract in Acetone

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	13	12
2.	Salmonella typhii.	Nil	Nil
3.	Escherichia coli	15	15
4.	Streptococcus pneumoniae	17	18

Table-4 Table shows antimicrobial activity of *A. annua* extract in Water

S.No.	Name of micro-organism	Inhibition Zones at concentration (25mg)	Inhibition Zones at concentration (30mg)
1.	*Staphylococcus aureus*	14	15
2.	Salmonella typhii.	Nil	Nil
3.	Escherichia coli	15	20
4.	Streptococcus pneumoniae	19	17

The extracts showed apparent effect in methanol extract and ethanol extract and moderate effect against water and lesser effect with acetone solvent. The phytochemical evaluation of plant is achieved through biochemical testing and HPLC analysis. Through biochemical testing, the important constituents present in plant extract are Flavonoids, Phenols, Saponins, Tannins, Alkaloids, and Quinones and through HPLC analysis the important Flavonoids present in plant extract are Quercentin, Rutin, Kaempferol, and Gallic acid[7]. Due to presence of these important phytochemicals, the plant (*Artemisia annua*) possesses the activity like antimicrobial, antioxidant and antimalarial. As the plant possesses such important activities the herbal extract of plant may be used as medicine against microbial infection

Conclusions

It can be concluded from the results that *Artemisia annua* plant leaves possess antimicrobial activity against test microorganism and also possess important phytochemicals. This means that the compound responsible for antimicrobial activity is present in each extract at different concentrations. The chance to find antimicrobial activity was apparent in methanol and ethanol extracts. The

phytochemicals present in the extracts may be responsible for 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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Phytochemical Analysis of Leaves Extract of Plant *Skimmia Laureola* Obtained from the High-Altitude Region of Uttarakhand, India

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Abstract- The medicinal shrub *Skimmia laureola* (Rutaceae) is recognized for its health benefits, yet little is known about its chemical composition and bioactivity. Ethyl acetate and methanol were utilized in this study to extract the dried leaves, which were then evaluated using both qualitative and quantitative methods. Simple chemical tests detected alkaloids, flavonoids, and phenolic compounds. According to quantitative analysis, each gram of ethyl acetate extract had a total alkaloid content of 7.11 ± 4.67 mg atropine equivalents, total flavonoid content of 4.16 ± 3.13 mg quercetin equivalents, and total phenolic content of 1.45 ± 1.21 mg gallic acid equivalents. The abundance of alkaloids and flavonoids in *S. laureola* is associated with its reported antiinflamma-tory and antimicrobial properties, providing a scientific basis for its traditional uses. This study established a foundation for the potential application of *S. laureola* extracts in the pharmaceutical and nutraceuticals sectors.

Keywords: *Skimmia laureola*, Total flavonoid, Total alkaloid, Total phenolic

Introduction

The pharmaceutical industry has long used plants as a source of new bioactive chemicals, and *Skimmia laureola* has great potential. Flavonoids, alkaloids, and essential oils are abundant in this medicinal plant, adding to its potent therapeutic effects[1]. Traditional medicine has used this plant for its therapeutic properties for ages, establishing the foundation for contem-porary pharmacological study[2].

Skimmia laureola

It is a species in the Rutaceae family that has a long and illustrious history that begins in the Himalayan region [3]. *S. laureola's* distribution throughout Japan and the Philippine Islands demonstrates its tolerance to a variety of Asian environments[4]. In India, western and eastern Himalayan regions, which include states like Jammu & Kashmir, Himachal Pradesh and Uttarakhand are home to the majority of the *Skimma genus*, especially *Skimmia anquetilia* and *Skimmia laureola*[5]. Although they are mostly found between 1700 and 3100 meters above sea level, certain populations can be found at lower elevations in shady ravines, indicating their capacity to adapt to different

micro-climates[6]. Morphologically, a crucial identifier is simple, alternating, gland-dotted leaves that emanate an aromatic scent when crushed[7]. Its taxonomic distinctiveness is enhanced by the thyrsiform inflorescences (type of flower cluster where the main stem produces side flower groups one after another, from bottom to top, like a raceme) and polyandry of its flowers[8]. They produce drupaceous berries with 1–5 wrinkled pyrenes, a fruit structure atypical for Rutaceae, aiding in taxonomic differen-tiation[9]. Male and female plants are often dioecious, with male specimens historically favored for ornamental cultivation [10].

Chemical Composition

An article published by *Darmwal et. al 2024* performed the (GC-MS), it is found on analysis of the essential oil extracted from *S. laureola* leaves, has identified 28 constituents. The composition is dominated by monoterpenes which account for over 93.5% of the total oil, whereas sesquiterpenes only make up roughly 0.3%[11].

Primary Components- Linalyl Acetate (50.5%) is an important substance with potential medical uses, such as antispasmodic action and fragrant qualities[12]. Linalool (13.1%) is known for its antibacterial and sedative qualities[13]. Geranyl Acetate (8.5%) and Cis-p-menth-2-en-1-ol (6.2%) [14]. These substances contribute to the scent of the oil and may also possess other bioactive properties[15]. The plant has variability in composition[16], like the composition of essential oils[17] variance is based on environmental factors and geographic location[18]. Linalyl acetate, for instance, has been demonstrated to exhibit antispasmodic effects, it has significant antibacterial and antifungal activity against various pathogenic strains, such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*[19]. Some Quinoline alkaloids like oxirane and coumarins like scopoletin glucoside inhibit calcium channels and lower pro-inflammatory mediators like histamine[20]. Its effectiveness in a variety of inflammatory diseases is explained by this multi-target mechanism[21].

Medicinal Use- The plant possesses many properties that can be used to treat numerous diseases, making it most widely used in research and pharmaceutical uses.

Antinociceptive Property- The essential oil of *S. laureola* leaves (SLO) exhibits peripheral analgesic effects by inhibiting prostaglandin synthesis and blocking pain signal transmission[22] acetic acid-induced writhing tests, SLO (200 mg/kg) reduced abdominal contractions in mice by 67.2%, comparable to aspirin (64.8%)[23]. The oil also increased pain latency in hot plate tests (55.6% at 200 mg/kg), suggesting central analgesic pathways[24].

Antipyretic Activity- SLO has shown promising effects in modulating fever responses through its action on the hypothalamus, where it suppresses the levels of prostaglandin E2 (PGE2)[25]. PGE2 is a significant mediator of the febrile response, and its reduction can lead to notable decreases in body temperature during hyperthermic conditions[26].

Anthelmintic Activity- The anthelmintic activity of *Skimmia laureola* has been extensively studied, especially its essential oils and extracts, which show promising results against parasitic worms[27]. In-vitro studies demonstrated significant efficacy against *Haemonchus contortus* adult worms, with essential oils at 50 µL/10 mL causing rapid immobilization and death within

hours[28]. Ethyl acetate extracts induced paralysis in earthworms within few minutes and death within few minutes afterwards at 100 mg/mL, comparable to albendazole[29].

In - Vitro Anti-inflammatory Mechanisms

Human red blood cell (HRBC) membranes are stabilized by *S. laureola* leaf extracts, verified in vitro anti-inflammatory test. At 400 mg/kg, methanol extracts show a 67.53% suppression of hemolysis, outperforming the fractions of ethyl acetate and chloroform[30]. Alkaloids like skimminan which interfere with arachidonic acid pathways, are responsible for the concentration-dependent anti-inflammatory effect (up to 90.70% inhibition at 400 mg/mL ethyl-acetate extract)[31].

Material and Methods

There are quantitative and qualitative tests performed on the leaf extract of *Skimmia laureola,* and during the test, various chemical reagents and instruments are required which are mentioned below.

Chemicals

The chemicals used in this research study were methanol and ethyl acetate, which were obtained from the chemical store facility of Sardar Bhagwan Singh University, Dehradun, India.

Plant Sample Collection and Identification

S. laureola plant was collected in August 2024 from the national forest department nursery, which was present in high altitude region of Deoban, situated in Chakrata, Uttarakhand, India. The plant was, then taxonomically identified by the Botanical Survey of India.

Sample Extraction

Plant leaves of *S. laureola* were shade-dried at room temperature. The fully dried leaves were powdered in an electric blender. About 50g of powder of *Skimmia laureola* leaves was inserted in a Soxhlet apparatus, and the solvent extract was siphoned. Afterwards, the solvent obtained was recovered using a distillation assembly to prevent wastage of solvent.

Extraction Yield Determination

The formula shown in **equation-1** helps to determine how much extract material is obtained from given amount of plant material this metric is commonly used to find efficiency of extraction solvent and the method used

$$\%\ \boldsymbol{Yield} = \frac{\boldsymbol{weight\ of\ dry\ extract\ after\ solvent\ evaporation}}{\boldsymbol{weight\ of\ dry\ leaves}} \times \mathbf{100}$$

Preliminary Phytochemical Screening

Phytochemical screening was conducted on methanol and ethyl acetate extracts to identify the presence of metabolites, including proteins, amino acids, alkaloids, phenols and flavonoids.

Total Phenolic Content

First, an ethyl acetate extract is made from dried and finely ground plant material to find the total phenolic content of *Skimmia laureola*. The Folin–Ciocalteu reagent, is then added to a determined portion of the extract in test tubes so that it can react with the phenolics of the plant extract[32]. To ensure full color development, the mixture is left to incubate for approximately half an hour at room temperature. Then, a UV-Vis spectrophotometer is used to measure the absorbance of the blue complex at about 760 nm. Using a standard, such as gallic acid, a calibration curve is created that helps to link known concentrations to their absorbance measurements[33].

Total Flavonoid Content

One often used technique for determining the total flavonoid content (TFC) in extracts of *Skimmia laureola* is the colorimetric assay with aluminum chloride. This process involves combining aluminum chloride with a methanolic and ethyl acetate extract of dried, finely crushed plant material. The aluminum chloride then combines with flavonoids to create a stable compound that has a distinctive yellow hue[34]. A UV-Vis spectrophotometer is used to measure the absorbance of the colored complex at about 415 nm after it has been incubated at room temperature for a while, usually around 30 minutes. The TFC can then be expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g) by creating a calibration curve with quercetin as a standard[35].

Total Alkaloid Content

An acid-base extraction followed by a colorimetric estimate is a commonly used technique to ascertain the total alkaloid content in *Skimmia laureola* extracts. An acidic solvent is initially used to extract the dried and finely crushed plant material to aid in solubilizing the plant's alkaloids. After filtering, the alkaloids are precipitated by adding an appropriate reagent, such as Dragendorff's reagent or bromocresol green, which forms a colored complex with the alkaloids after the extract's pH has been adequately adjusted[36]. A UV-Vis spectrophotometer is then employed to detect the color's intensity at a wavelength of approximately 470 nm. To quantify the number of alkaloids in the extract, a calibration curve is created using a recognized alkaloid standard, like atropine[37].

Results

On conducting qualitative and quantitative tests, all the information is converted into tables, and the formulas and chemicals used are mentioned in the respective sections.

Qualitative

They are analytical procedures that are designed in a way to detect the presence or absence of a particular class of compounds in your plant extract. **Table-1** shows the result by performing various phytochemical constituents present in the plant extract.

Table-1 Phytochemical compounds of *Skimmia laureola* identified in screening tests

	Tests	Methanol	Ethyl Acetate	Color
Alkaloid	Dragendorff's	++	++	Reddish brown ppt
	Wagner	++	++	Brown/reddish ppt
	Mayers	++	++	Cream white/yellow ppt
	Hager	++	++	Creamy white ppt
Flavonoid	Shinoda	+	+	Pink to crimson colour
	Zn hydrochloride	+	+	Magenta
Phenol	Ferric chloride	-	-	Green ppt
	Iodine sol	-	-	Transient red colour
Protein and amino acids	Million reagents	-	-	White ppt
	Ninhydrin test	-	-	Deep Blue

(+) Depicts the presence, (−) Depicts the absence, + low, ++ high

Quantitative

These tests are conducted to determine the quantity of specific classes of compounds present in plant extracts by measuring the absorbance against a series of known standards. These tests provide an accurate and precise measurement of the compounds' concentrations.

Discussion and Conclusion

S. laureola's plenty of alkaloids and flavonoids are linked to reported anti-inflammatory and antimicrobial properties, and also has some anti-anxiety effect, giving its traditional uses a scientific basis. These encouraging findings indicate that SLO is an excellent source of bioactive compounds and natural antioxidants.

As per the study conducted, the results of qualitative test are shown in **Table-1** and for quantitative the standard curves of alkaloid, flavanoid and phenolic content are shown in **Figure-1, 2** and **3** and the result obtained of plant material are mentioned in **Table-2, 3** and **4**. To support the advancement of plant-based therapeutics, future research will concentrate on separating individual compounds, evaluating their biological activities in animal and laboratory models, and investigating sustainable cultivation techniques. This study laid out the foundation for the possible use of *S. laureola* extracts in pharmaceutical and nutraceutical applications.

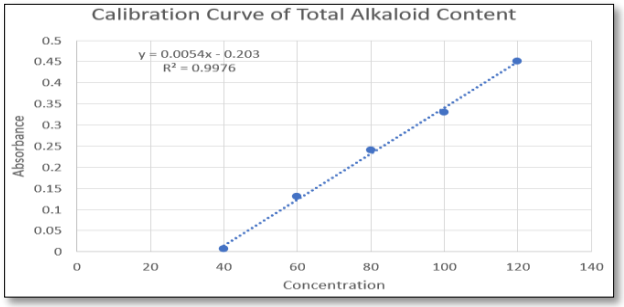

Figure-1 Calibration curve of TAC

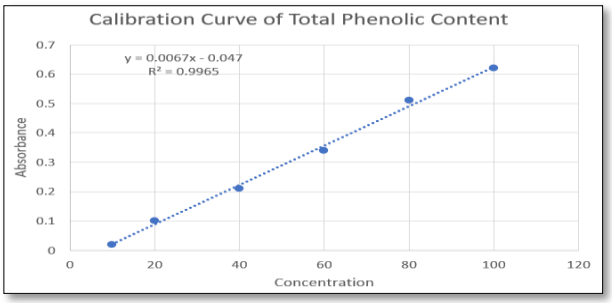

Figure-2 Calibration curve of TPC

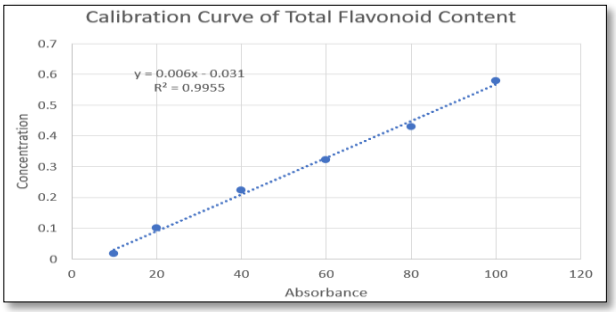

Figure-3 Calibration curve of TFC

Table-2 Total alkaloid content of solvents

S. No.	Extracts	Total Alkaloid Content (% w/w)
1.	Ethyl Acetate	2.67 %
2.	Methanol	2.34 %

Table-3 Total flavonoid content of solvents

S. No.	Extracts	Total Flavanoid Content (% w/w)
1.	Ethyl Acetate	1.49 %
2.	Methanol	1.32 %

Table-4 Total phenolic content of solvents

S. No.	Extracts	Total Phenolic Content (% w/w)
1.	Ethyl Acetate	0.86 %
2.	Methanol	0.83 %

Conduct in future bioassay-guided fractionation to isolate and characterize individual phytochemicals responsible for the specialized activities, such as anticancer, antidiabetic, neuroprotective, or cardio-protective effects in targeted bioassays[38].

Perform detailed mechanistic studies at the cellular and molecular levels to identify specific enzymatic or signaling pathways modulated by *S. laureola* constituents[39].

Evaluate the in-vivo pharmacological efficacy of standardized extracts in relevant animal models of inflammation, infection, and oxidative stress[40].

Investigate potential synergistic or antagonistic interactions between *S. laureola* compounds and other herbal or conventional pharmaceuticals[41].

Apply genomics and metabolomics approaches to elucidate the biosynthetic pathways underlying key secondary metabolites and their regulation[42].

Explore endophytic and rhizospheric microbial associations that may enhance phytochemical production or confer additional bioactivities[43].

Formulate nanocarrier-based delivery systems (e.g., nanoparticles, liposomes) to improve the solubility, stability, and bioavailability of active constituents[44].

Implement conservation strategies and germplasm repositories to protect wild populations and support sustainable harvesting practices[45].

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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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Evaluation of Antimicrobial Activity of *Hemidesmus indicus* Root Extracts

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Abstract-This study investigates the antimicrobial potential of various solvent extracts of *Hemidesmus indicus* (Indian sarsaparilla) roots. Extracts were prepared using methanol, hexane, and distilled water. The antibacterial and antifungal efficacy of these extracts was assessed against *Salmonella typhi, Staphylococcus aureus, and Candida albicans* using the agar well diffusion method. Results showed that methanolic extracts exhibited the highest antimicrobial activity. These findings support traditional medicinal uses of *H. indicus* and point to its potential in developing plant-based antimicrobial agents.

Introduction

Medicinal plants have played a vital role in traditional healing systems and continue to offer promising leads in pharmaceutical research. *Hemidesmus indicus* (family: Asclepiadaceae), commonly referred to as Indian sarsaparilla or "Nannari," is a perennial climber found throughout India and neighbouring countries. Its roots are aromatic, woody, and have long been used in Ayurveda for treating a range of disorders including venereal diseases, skin infections, and urinary tract infections[1]. The plant is rich in bioactive compounds such as tylophorine (an alkaloid with anti-inflammatory properties), coumarins, essential oils, starch, tannic acid, and triterpenoid saponins. Modern pharmacological studies have suggested that extracts from *H. indicus* roots possess antimicrobial, anti-inflammatory, anti spasmodic, and antioxidant properties[2].

H. indicus serves as an alternative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases, urinary infections, negative emotions and impotence. It also prevents abdominal distention, arthritis, rheumatism, gout and epilepsy. According to practitioners of traditional Indian medicine, Ayurveda, this root can be administered in the fourth and ninth month of pregnancy to prevent mis-carriage. They also claim its efficacy in treating ulcers, fever, loss of appetite, Gastritis, Anorexia nervosa cough, excessive thirst Menorrhagia, Diarrhea and Diabetes. It is also believed that the extracts from this root help in increasing semen count, purifies blood, neutralizes poisons, works as a diuretic and emetic, and has anti-inflammatory properties. Some experimental studies have displayed the beneficial effect of the extract of this root. The alkaloid content present in it is Tylophorine and is anti-inflammatory, antispasmodic and anti-anaphylactic in nature. The other

compounds present in it are coumarin, essential oil, starch, tannic acid and triterpenoid saponins. The roots and leaves of the plant possess medicinal properties[3].

Given its widespread ethnopharmacological use and growing interest in herbal medicine, this study aims to evaluate the antimicrobial activity of different solvent extracts of *H. indicus* roots against selected bacterial and fungal strains.

Material and Methods

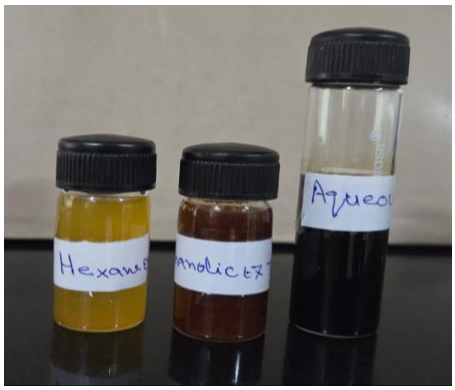
Chemicals and Reagents

Conical flasks, beakers, spatula, glass funnels, measuring cylinders, autoclave, top-loading Mettler balance, mortar and pestle, test tube holder, analytical balance, capillary tubes, and filter paper were used. All solvents (methanol, hexane, distilled water) were of analytical grade.

a. Hexane S wt – 50g
Hexane = 200ml
Wt.-15ml(10.4217gm)

b. Methanol S wt -50g
Methanol = 200ml
Wt.-15ml(14.8150gm)

c. Aqueous S wt -50gm
Purified water = 200ml
Wt.-20ml(22.0985gm)



Procurement of Plant Material

Root samples of *H. indicus* were procured from Himalaya Wellness Company, Dehradun. The specimen was authenticated by Dr. Maya Ram Uniyal (Specification No. SPEC/HD/202/03). The roots were washed, air-dried, shade-dried for seven days, and ground to a 60-mesh powder. Some whole roots were preserved for morphological examination.

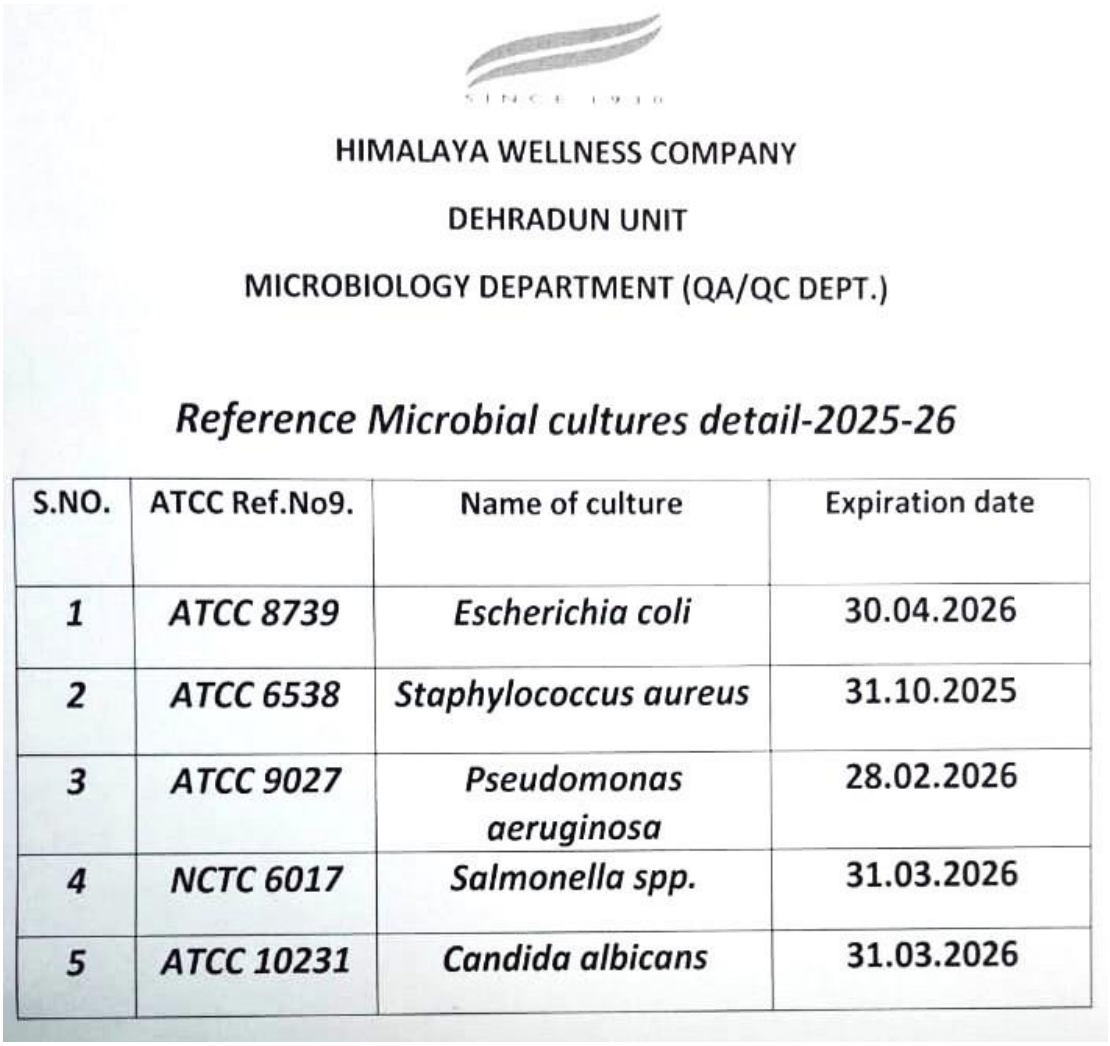
Preparation of Extracts[8&9]

Fifty grams of powdered root material were extracted sequentially with hexane, methanol, and distilled water using a Soxhlet apparatus for 6-8 hours each. Extracts were filtered, concentrated using a rotary evaporator, and stored at 4°C.

Antimicrobial Assay[10]

The agar well diffusion method was used to assess antimicrobial activity against Salmonella typhi, *Staphylococcus aureus*, and Candida albicans. Muller-Hinton Agar and Sabouraud Dextrose Agar were used for bacterial and fungal cultures, respectively. Extracts (100 µL at 100 mg/mL) were loaded into wells. Oxacillin (200 mg) served as a positive control. Solvents were used as negative controls. Details of cultures procured is given in Table-1.

Table-1 References of Microbial cultures details-2025-26



HIMALAYA WELLNESS COMPANY

DEHRADUN UNIT

MICROBIOLOGY DEPARTMENT (QA/QC DEPT.)

Reference Microbial cultures detail-2025-26

S.NO.	ATCC Ref.No9.	Name of culture	Expiration date
1	ATCC 8739	Escherichia coli	30.04.2026
2	ATCC 6538	Staphylococcus aureus	31.10.2025
3	ATCC 9027	Pseudomonas aeruginosa	28.02.2026
4	NCTC 6017	Salmonella spp.	31.03.2026
5	ATCC 10231	Candida albicans	31.03.2026

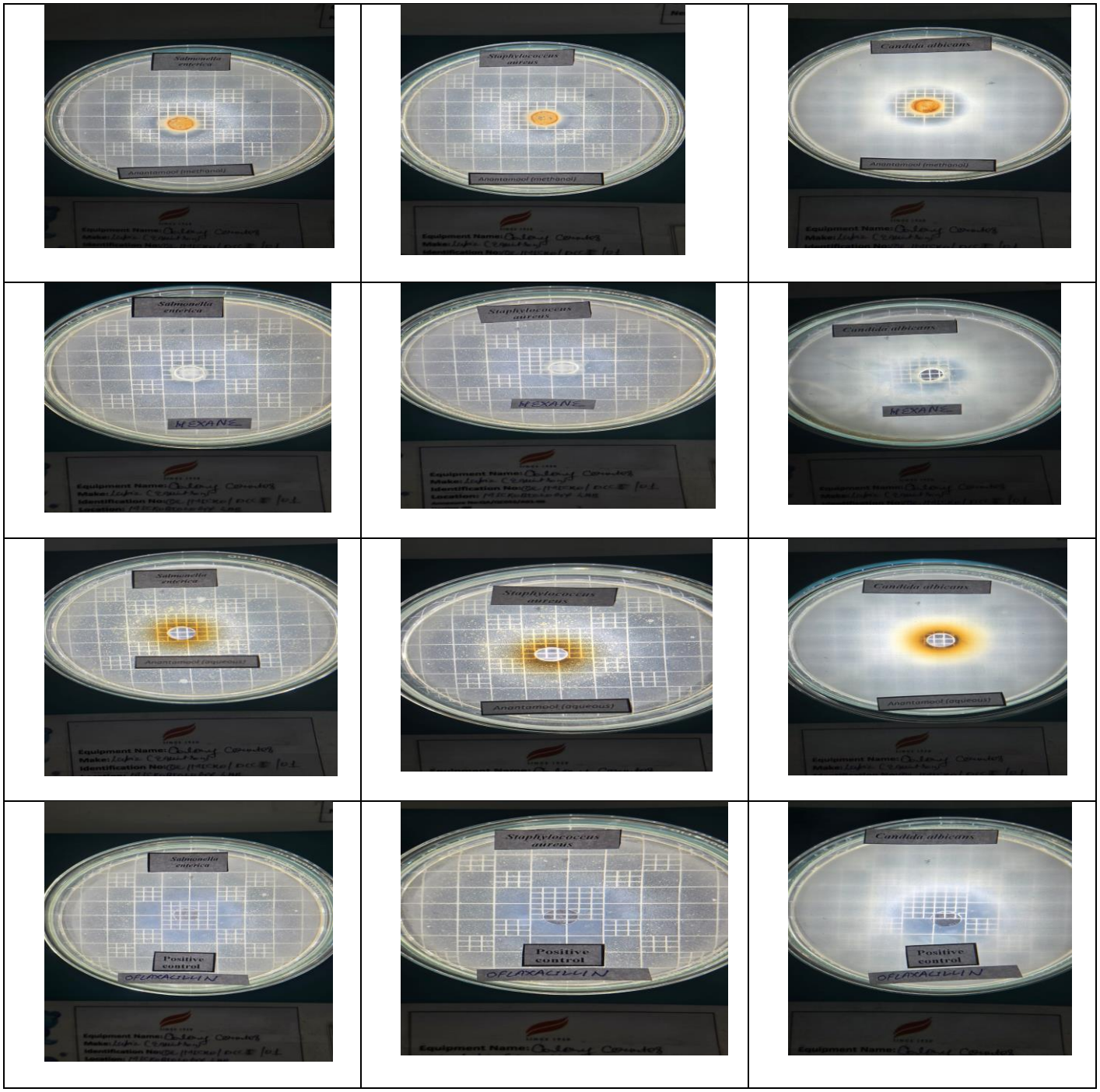
Results and Discussion

The methanol extract showed the strongest antimicrobial activity . Zones of inhibition were measured and are summarized in the Table-2.

Table-2 Zones of inhibition were measured

Extract/Solvent	***Salmonella typhi***	***Staphylococcus aureus***	***Candida albicans***
Methanol Extract	20 mm	21 mm	25 mm
Hexane Extract	14 mm	15 mm	19 mm
Aqueous Extract	ND	11 mm	12 mm
Positive Control	35 mm	36 mm	29 mm
Negative Control	ND	ND	ND

ND = No detectable inhibition zone



The results confirm that the methanolic extract of *H. indicus* roots has significant antimicrobial activity, particularly against *Candida albicans* and gram-positive *Staphylococcus aureus*. This suggests that active constituents responsible for antimicrobial effects are more efficiently extracted in methanol. Hexane extracts showed moderate activity, while aqueous extracts demonstrated minimal efficacy[4-6].

These findings align with traditional uses of *H. indicus* and reinforce previous studies reporting antimicrobial potential. The absence of activity

in negative controls confirms that the effects are due to plant-derived compounds, not solvents.

Conclusion

Hemidesmus indicus root extracts, especially those prepared in methanol, exhibit notable antimicrobial activity. These results support its traditional applications in treating infections and indicate potential for development into plant-based antimicrobial formulations. Future research should focus on phytochemical isolation and in vivo testing to validate efficacy and safety.

Acknowledgement

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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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Comparison of Phytochemical and Antimicrobial activities of *Amaranthus cruentus* (Red Amaranthus) grown in Hydroponic system and in traditional soil system

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Abstract- Hydroponics has emerged as one of the most popular agricultural production methods today. However, whether hydroponically produced plants of are of comparable quality to that of soil grown plants is still unclear and a lot of research is going on this matter. This study is also a part of research on phytochemical, and antimicrobial activity of ethanol and aqueous extract of plant *Amaranthus cruentus* grown in hydroponic system and traditional soil system. *Amaranthus cruentus is* naturally gluten-free and a good source of calcium, zinc, copper, vitamin B6, folate, and an excellent source of fiber, iron, magnesium, phosphorus, and manganese. Growth data revealed that it appears morphologically far better in hydroponic system as compared to that of soil system. In a hydroponic setup, dry weight was increased by seven times. Flavonoid and Mineral elements content is found to be higher in hydroponic systems. The diameter of inhibition zone of *Amaranthus cruentus* grown in hydroponic system is higher as compared to that of soil system. While, Minimum inhibitory concentration (MIC) is less in case of hydroponic system as compare to soil system. This study concludes that the hydroponic system for growing of plants is good choice.

Keywords: Hydroponics, Soil grown plants and Nutrients,

Introduction

A wide variety of indigenous and minor crops has been utilized for daily consumption since ancient times. They are not only important ingredients of unique gastronomic dishes but also traditional functional food to maintain wellness. In order to elucidate such a phenomenon as well as to seek highly effective plants, a number of plant extracts and isolated compounds have been tested for their bioactivity by various *in-vitro* model systems. Information on the biological functions and active constituents of each plant species may contribute to the improvement of food habits and public health in tropical countries. Furthermore, it is expected that the wide use and extension in the utilization of such local agricultural products would increase and stabilize the income of farmers in the rural areas.

Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic

drugs to which many infectious micro-organisms have become resistant. Plants have provided a source of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold namely they provide key chemical structure for the development of new antimicrobial drugs and also as a phytomedicine to be used for the treatment of disease.

Amaranthus cruentus Linn. collectively known as amaranth or pigweed is an annual flowering plant in the family Amaranthaceae that yield staple amaranth grain[1-4]. It is a tall annual herb with clusters of dark pink flowers. The plant can grow up to 2m in height, and blooms in summer to fall (Flora of Tamil Nadu, VOL. II, 1987). The present study was undertaken to assess antimicrobial and phytochemical property of *Amaranthus cruentus*.

Amaranthus cruentus commonly used as a leaf vegetable. Some time they are grown as ornamentals, for fodder, and for making dye. Dried plant is burnt for the preparation of potash. It is given to lactating mothers for treating constipation, anemia, kidney complaints. Roots are boiled with honey and given to infants for their laxative effect, its aqueous extract is used to treat pains in the limbs, as a tape worm expellant and wound dressing and tumors. It has antioxidant properties.

Material and Methods

Hydroponic set up

Amaranthus cruentus was grown using the ebb and flow method in a hydroponic system. The technique made use of PVC pipes with 3-inch diameter pores. A steady flow of nutritional solutions was maintained in the growth channels. The plants were planted in net pots with coco-peat and lacca balls as the support medium, and the roots were hung in a nutritious solution running through the channels. The reservoir's feeding solution was pumped out. The flow was directed into the other channel, which was equipped with an end cap and spout at the decreasing end of the increasing channel. Before returning to the reservoir, the nutrient solution passed through all of the system's channels (closed system). To prevent contamination of the nutrient solution, a reservoir with a surface area of 0.053 m^2 was placed beneath the growing system and covered[10-13].

The *Amaranthus cruentus* plant was chosen as the source material for this objective. This experiment was divided into two groups, one was carried out in the soil of HWC field, and the other was in the laboratory of Himalaya Wellness Company, Faridabad. *Amaranthus cruentus* seeds were purchased from a local nursery and verified by Dr. M.R. Uniyal, Ex. Advisor Medicinal Plant, U.P., Govt. Three replicates of 20 seeds were placed in a tray filled with cocopeat and moistened with water. Similarly, seeds were grown in a greenhouse in the soil as well. At 3, 4 leaves stage three replicates of 10 good plants were transferred in a hydroponic setup and exposed to an external environment. In the hydroponic setup, we have used modified Hoagland solution as a nutrient media with pH 6.9. 10 L nutrient media were added every week and maintained the EC with nutrient media and pH with ortho-phosphoric acid and lastly, phytochemical and antimicrobial activity analysis was done.

Preparation of plant extract

The crude plant extract was prepared using the Soxhlet extraction technique. About 20 g of powdered plants material was evenly packed into a thimble and extracted with 250 ml of

solvents. As a solvent, acetone was used. The extraction procedure is repeated for another 24 hours or until the extractor's syphon tubes solvent becomes colourless. The extracts was then placed in a beaker and cooked on a hot plate at 30℃-40℃ until the solvent had evaporated completely. The dried extract was kept at 4℃ in the fridge for future study.

Phytochemical Analysis

Quantification of total phenolic compounds- The Folin Ciocalteu reagent technique was slightly modified to detect the quantity of phenol in the aqueous extract. 2.5 ml of 10% Folin- Ciocalteu reagent and 2 ml of 2% Na_2CO_3 solution were added to 1 ml of plant extract. The resultant mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid (1 mg/ml) was utilized as a standard. All of the tests were performed triplicate. The findings were computed and represented as gallic acid equivalent (mg/g of extracted substance) using the standard curve.

Quantification of flavonoid- To determine flavonoid content, the aluminium chloride colorimetric method was used with some modifications. 1 ml of the sample plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water for 30 minutes at room temperature. At 420 nm, the absorbance was measured. As a standard, 1 mg/ml of quercetin was used. All of the tests were carried out in triplicate. The flavonoid content was calculated using the standard curve and expressed as quercetin equivalent mg/g of extracted compound.

Anti-Microbial Activity

Strains of tested organisms

The bacterial strains used in the study were obtained from the Microbiology Laboratory, Department of Quality control and Quality assurance, Himalaya Wellness Company, Faridabad, Haryana. Two Gram positive bacteria that is *S. saprophyticus*, *E. faecalis* and four Gram negative bacteria that is *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Proteus vulgaris* were used for this study.

Antibacterial activity

The antibacterial efficacy of both methanolic leaf extracts of *Amaranthus cruentus* was tested by agar well diffusion method. The collected clinical isolates were grown in Muller Hinton broth (Himedia, Mumbai, India) at 37°C for 24 hrs. with constant agitation in a shaker. The cultures from broth were aseptically swabbed on sterile Muller Hinton agar (Himedia, Mumbai) plates using sterile cotton swabs. The wells of 6 mm were punched in the inoculated plates using a sterile borer. Aliquots of 100 µl of methanolic leaf extracts (50 mg/ml in dimethyl sulphoxide) were transferred into labelled wells. The wells were also filled with 50 µl positive (Amikacin, 10 mg/ml in dimethyl sulphoxide) and 50 µl negative (dimethyl sulphoxide only) controls. The plates were incubated at 37°C for 24 h in upright position and the zones of inhibition were recorded. The activity assays were conducted in triplicate.

Determination of MIC by Microlitre plate assay

The microtitre plate was prepared in aseptic conditions. A stock solution of test sample (10% w/v) was prepared in dimethyl sulphoxide. A volume of 100 µl of test material was filled in first row of the plate. To

all other wells 50 µl of sterile nutrient broth was filled. The test material was transferred to the next well to attain serial dilutions. To each well, 30 µl of resazurin indicator solution (0.02%) was added. Finally 10 µl of bacterial suspension (1x108 CFU/ml) was added to each well. The plate was set with positive control and a column with all solutions except test compound and negative control; a column contains10 µl of sterile nutrient broth except test compound and bacterial suspension. The plates were prepared and incubated at 37°C for 24 h. The colour change from purple to pink indicated a positive response. The lowest concentration at which colour change was noted was the minimum inhibitory concentration (MIC) values for the test material and bacterial strain[13-16].

Results and Discussion

Our data have clearly shown that flavonoids and total phenol are more in hydroponically grown plants (Figure-1), whereas in soil cultivated plants, it is less. The phenolic compounds are among the most numerous and widespread groups of plant metabolites. They have biological properties such as anti-apoptosis, antiaging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardio vascular protection, endothelial function improvement, and inhibition of angiogenesis and cell proliferation. Natural antioxidants are primarily found in plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols, and so on. Flavonoids are hydroxylated phenolic substances that plants produce in response to microbial infection and have been shown to be antimicrobial *in-vitro* against a wide range of microorganisms. In the case of phenolic compounds, soil cultivated plants possess more. Polyphenols, saponins, tannins, and oxalates are phytochemicals found in *amaranth* grain that are not considered nutrients but may be antinutrient factors. Cooking reduces these compounds' content and anti-nutrient effect.

The results of inhibitory effect of methanolic leaf extracts of *Amaranthus cruentus*. hydroponically grow and soil grow are shown in Table-1. The results show that different bacterial species exhibit different sensitivities towards the extract. The extract was found to be inhibitory to all bacterial isolates but with variable extent. The order of activity against selected bacteria was *E. coli* > *P. vulgaris* > *P. aeruginosa* > *K. pneumoniae* > *S. saprophyticus* > *E. faecalisand*. Different plant metabolites have shown effective antibacterial activity against uropathogens including drug resistant strains. In the present study, the *A. curentus (L)* leaf methanolic extract effectively inhibited all bacteria tested. The zone inhibition values of the extract against tested bacteria ranged from 14.2±0.55 to 18.3±0.55 mm in hydroponically grow methanolic extract while soil grow show range from 13.3±0.55 to 17.5±0.55mm. Amikacin showed inhibition zones that ranged from 15.8±0.57 to 23.2±0.57 mm. The both methanolic extracts exhibited maximum activity against E. coli (hydroponic 18.3±0.55 mm and soil grow 17.5±0.55) followed by P. vulgaris (16.5±0.57 mm and 16.0+.057) and then against *P. aeruginosa* (15.6±0.57 mm and 15.3±0.57 mm). The results in the present study indicate that the antibacterial activity varies according to type of bacteria used for the study. The least activity was exhibited by *E. faecalis* with the smallest zone (14.2±1.15 mm and 13.8±1.15 mm) and inhibited at lowest concentration 1.25 (mg/ml) (Table 2). The antibacterial activity of tested *A. cruentus*

was compared with the standard drug amikacin. The cell growth was evaluated using resazurin, an oxidation-reduction indicator. A change in colour from blue to pink indicated the growth of bacteria, and the minimal inhibitory concentration (MIC) was noted as lowest concentration of the test compound that prevented this change in colour. The MIC of methanolic leaf extract ranged from 5.5 to 0.37 mg/ml. The results from MIC indicated that E. coli was the most sensitive microbe to the *A. cruentus* leaf extract grown in hydroponic and soil system being negatively affected at lowest concentration tested 0.37 (mg/ml) and 0.37 (mg/ml) respectively.

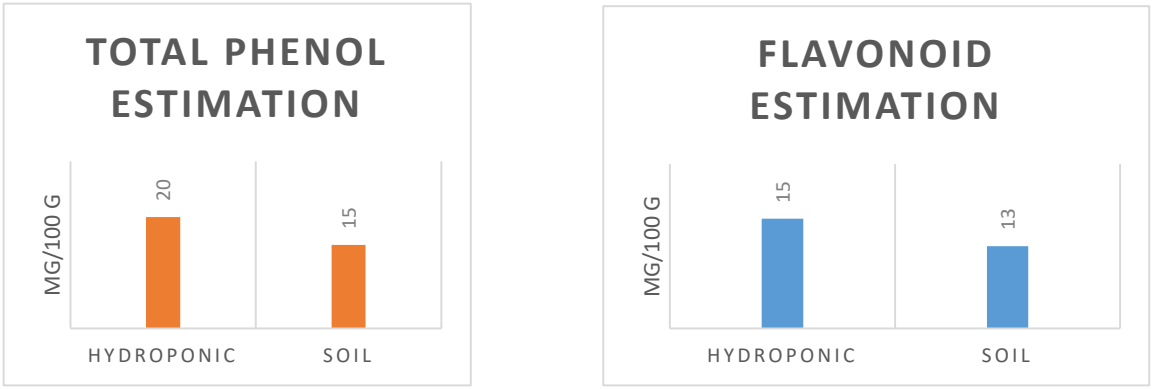

Figure-1 Comparison of flavonoids and phenolic compounds in *Amaranthus cruentus* plant extracts grown in soil and hydroponic system.

Table-1 Comparison of antimicrobial activity of *Amaranthus cruentus* plant extracts grown in soil and hydroponic system.

Sl No.	Microorganism	Zone of Inhibition Hydroponically grown (mm)	Zone of Inhibition soil grown (mm)	Zone of inhibition of Amikacin* (mm)
1	*S. saprophyticus*	15.4±0.57	14.3±0.57	20.2±0.57
2	*E. faecalis*	14.2±1.15	13.8±1.15	15.8±0.57
3	*E. coli*	18.3±0.57	17.5±0.57	23.2±0.57
4	*P. aeruginosa*	15.6±0.57	15.3±0.57	17.5±0.57
5	*K. pneumoniae*	14.9±1.15	14.5±1.15	19.2±0.57
6	*P.vulgaris*	16.5±0.57	16.0±0.57	20.3±1.15

*Standard antibiotic

Table-2 Minimum inhibitory concentration (MIC) of *Amaranthus cruentus* plant extracts grown in soil and hydroponic system.

Serial No.	Microorganism	MIC mg/ml Hydroponically grown	MIC mg/ml soil grown
1	*S. saprophyticus*	5.0	5.5
2	*E. faecalis*	2.3	2.5
3	*E. coli*	0.37	0.38
4	*P. aeruginosa*	1.24	1.26
5	*K. pneumoniae*	0.60	0.62
6	*P.vulgaris*	0.60	0.62

Conclusion

The comparative study of Amaranthus curentus conclude that every domain like phytochemical and antibacterial studies in hydroponic as well as in soil reveals that hydroponics technology is better for harvesting of *this* plant not only because of improved quality, but also because of higher yield, system ease of operation, and water efficiency. Small-scale farmers should use an open field hydroponic system instead of a controlled environment hydroponic system because it requires less capital. Further research into hydroponic production and optimization of its ability to assure acceptable product quality and the selection of suitable types to deliver better hydroponic products is also worthwhile.

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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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Estimation of Wedelolactone in *Eclipta alba* and *Wedelia chinensis* and Comparative Antimicrobial Activity against Pathogenic Bacteria

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Abstract- *Eclipta alba* (L.) Hassk. and *Wedelia chinensis* (Osbeck) Merr., both belonging to the Asteraceae family, are well known in traditional medicine. Wedelo-lactone, a coumestan derivative, is considered a key bioactive constituent due to its potent anti-inflammatory, hepato-protective, analgesic, antibacterial and antidiabetic properties. The present study aimed to estimate wedelolactone content and evaluate the comparative antimicrobial activity in both plant extracts. HPLC and HPTLC methods were developed for evaluation of wedelolactone in both the plants E*clipta alba* and *Wedelia chinensis*. Furthermore, the study investigated the in vitro antimicrobial activity of the crude extract of both plant against pathogenic bacteria, (*Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa* and a pathogenic yeast *Candida albicans).* A comparative study of the wedelolactone content and antimicrobial activity suggests a potential correlation between the concentration of this bioactive compound and its antimicrobial effects.

Keyword: *Eclipta alba*, *Wedelia chinensis*, HPLC, HPTLC, Antimicrobial activity, Anticandidal activity

Introduction

Wedelolactone is a coumestan compound found in both *Eclipta alba* (Bhringraj) and *Wedelia chinensis* (Pilabhangra). It's a naturally occurring compound with various pharmacological properties, including anticancer, anti-inflammatory, anti-diabetic, and anti-obesity effects, as well as protective effects on various organs. Both plants are recognized for their medicinal value, the concentration of bioactive compound like wedelolactone can vary in different parts of the plant.

Eclipta alba is an annual herb, has a short, flat or round stem, deep brown in color belonging to family Asteraceae. The plant is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation [(1)]. There are also reports of clinical improvement in the treatment of infective hepatitis [(2)]. *Eclipta alba* leaves showed antihyperglycemic activity [(3)]. The roots of Eclipta alba were found effective in wound healing [(4)].In ayurvedic medicine, the leaf extract is considered a powerful liver tonic, rejuvenative, and especially good for the hair[(5,6)]. In Ayurveda, the plant is considered as a rasayana for longevity and rejuvenation. Dried leaves of E. alba are source of coumarins like wedelolactone and its derivative, dimethyl wedelol actone, isodemethylewedelol actone and strycholactone[(7)].

Wedelia chinensis is a perennial herb belonging to family Asteraceae. The leaves are oval in form, succulent,[8] Flowers are 4-5 cm in diameter, yellow, tubular, and found in terminal or axillary heads. Various pharmacological activities were found in W. Chinensis such as antioxidant, anti- inflammatory, analgesic, anticancer, antibacterial, antifungal, hepatoprotective and androgen suppressing activities[9]. The presence of Wedelolactone and dimethyl Wedelolactone in Wedelia Chinensis promote hair growth [10].

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [11]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections [12]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs[13]. The investigation into the antimicrobial activity of *Eclipta alba* and *Wedelia chinensis* against pathogenic bacteria is thus crucial for validating their traditional uses and exploring their potential as sources for developing new, effective and less resistance – prone antimicrobial therapeutics. The antimicrobial efficacy is particularly relevant in combating common bacterial pathogens, including both gram positive such as *Staphylococcus aureus* and Gram negative bacteria like *Escherichia coli, Pseudomonas aeruginosa* and fungi (*candida albicans)* which are frequently implicated in human infection.

This study will investigate the wedelolactone content in *Eclipta aba* and *Wedelia chinensis* plants and their antimicrobial activity against various pathogens. It aims to validate traditional uses of these plants and explore their potential as antimicrobial agents by quantifying wedelolactone and assessing their antibacterial and anticandidal effects.

Material and Methods

Collection and Authentication

The plants *Eclipta alba* and *Wedelia chinensis*, were collected from herbal garden of Himalaya wellness company, Dehradun, UK, India and were identify by the Department of Pharmacognosy, Himalaya wellness Company, Dehradun, UK.

Sample Preparation for HPLC

5g of the coarsely powdered *Eclipta alba* and *Wedelia chinensis* leaves were refluxed under examination with 30 ml of methanol on a water bath for 30 minutes, cooled and filtered respectively. The residue refluxed further with methanol till the last extract turned colorless. It was cooled down and filtered. All the filtrates were combined and concentrated to 100 ml[14].

Standard preparation- 0.01% w/v solution of wedelolactone was added in methanol. This was our Standard solution.

Mobile phase Preparation- 65 volumes of 0.1% v/v phosphoric acid were prepared by diluting 1 ml of phosphoric acid to 1000ml with water. These 65 volumes of 0.1% v/v phosphoric acid was mixed with 35 volumes of acetonitrile.

HPLC Conditions

Column- C18 Shemadzu column 250= 4.6mm particle size 5µ.

Flow rate – 1ml per minute

Detection- at 351 nm

Volume of injection- 20 µl of sample and standard solution.

Method of Analysis

Stabilize the instrument with the mobile phase till the baseline is satisfactory. Inject the standard solution and record the chromatogram. The % RSD between the results should be less

than 2%. Similarly inject the sample solutions in duplicate and record the chromatogram.

Calculation

$$\frac{\text{Area of sample}}{\text{Area of Standard}} \times \frac{\text{Area of sample}}{\text{Area of Standard}} \times \text{Purity}$$

HPTLC Procedure

Material and Reagents

HPTLC Plates- Precoated silica gel 60F254 plates E. Merck.

Wedelolactone Standard- Accurately weigh a suitable amount of wedelo-lactone standard and dissolve it in methanol to prepare a standard solution (1mg/ml).

Sample preparation- Extract the plant material using an appropriate solvent (e.g., methanol, hexane and aqueous). Concentrate the extract for further analysis like HPTLC and antimicrobial activity.

HPTLC instrumentation- Applicator (e.g., Camag; Linomat), developing chamber (twin through glass chamber) **and documentation system.**

Mobile phase- The solvent system used was toluene: acetone: formic acid (11:6:1 v/v) for developing the chromate-gram[(15)].

Antimicrobial Activity

In the antimicrobial activity, the microorganisms used for the study are:

1. ***Escherichia coli*** *- ATCC 8739*
2. ***Pseudomonas aeruginosa*** *– ATCC 9027*
3. ***Staphylococcus aureus*** *- ATCC 6538*
4. ***Candida albicans.*** *- ATCC 10231*

These cultures were maintained on nutrient agar slants at first being incubated at 37^0C for about 18-24 hours and then stored at 4^0C as stock for antimicrobial activity. Fresh culture was obtained by transferring a loop full of cultures into nutrient broth and then incubated that 37^0C overnight. To test antimicrobial activity, the well diffusion method was used.

The agar well diffusion method [(16)], is used to determine the antimicrobial activity of plant extracts. This method involves creat-ing wells in an agar plate inoculated with a microorganism, filling these wells with plant extracts, and observing the formation of zones of inhibition (clear areas) around the wells, indicating the presence of antimicrobial compounds.

Results and Discussion

Antimicrobial activity for different extracts of *Wedelia chinensis* and *Eclipta alba,* along with wedelolactone tested against four microorganism: *S.aureus* (Gram – positive bacteria) *E.coli* and *P.aeruginosa* (Gram – negative bacteria) and *C.albicans* (Pathogenic Yeast).

Table-1 Antimicrobial activity of *Wedelia chinensis, Eclipta alba and wedelolactone.*

Microorganism	Wedelia chinensis in(mm)			Eclipta alba in(mm)			Wedelolactone (1mg/ml)	Positive control
	ME	HE	AE	ME	HE	AE		
S. aureus	**16**	**11**	**13**	**ND**	**ND**	**ND**	**14**	**36**
E. coli	**18**	**13**	**12**	**17**	**10**	**ND**	**15**	**37**
P. aeruginosa	**10**	**13**	**12**	**10**	**ND**	**ND**	**ND**	**39**
C. albicans	**25**	**17**	**18**	**17**	**20**	**ND**	**17**	**35**

Note: ME- Methanol extract; **HE**- Hexane extract; **AE**- Aqueous extract; **ND**-Not Detected

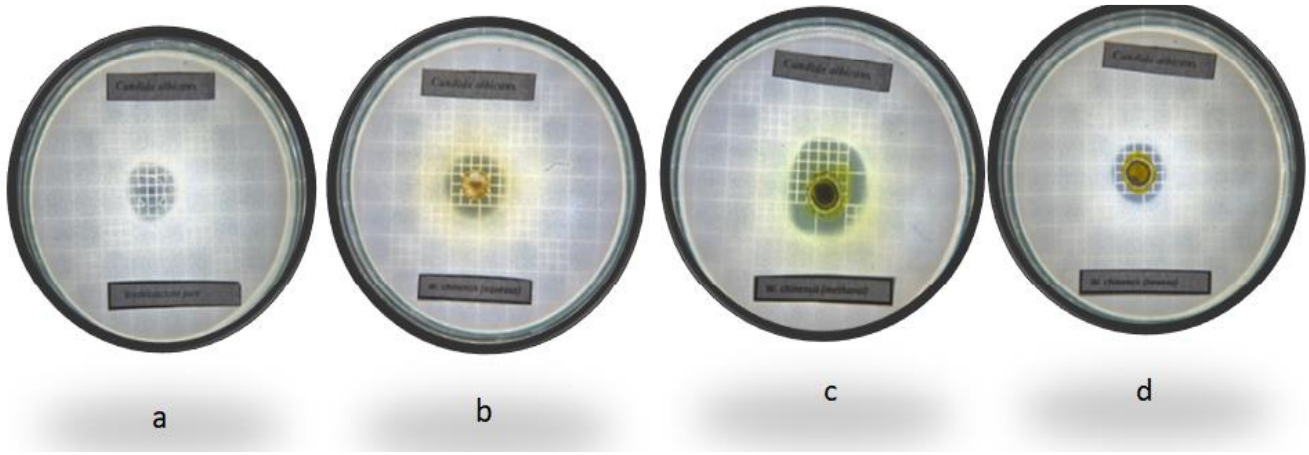

Figure-1 Anticandidal activity against *Candida albicans* of
a) Wedelolactone **b)** HE of W.chinensis **c)** ME of W.chinensis **d)** AE of W.chinensis

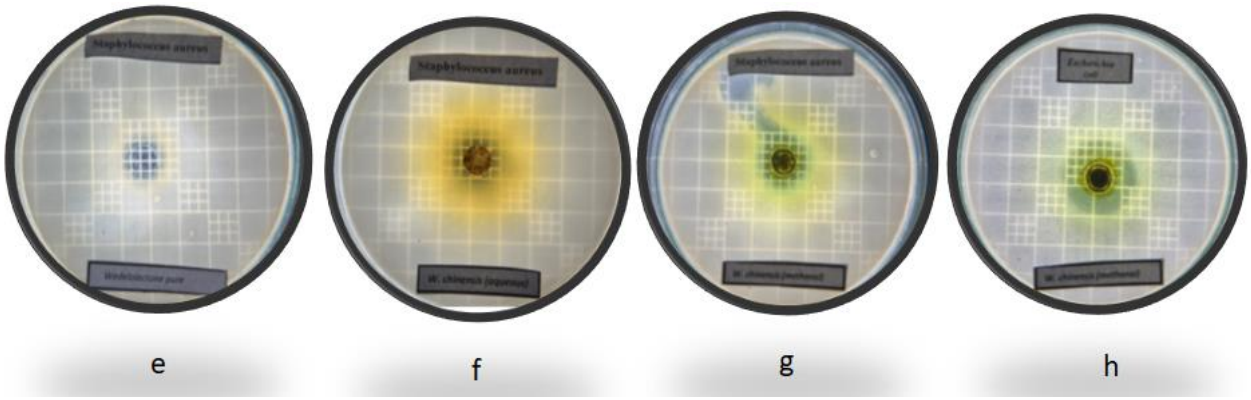

Figure- 2 Antibacterial activity of Wedelia chinensis
e) Wedelolactone against *S.aureus* **f)** AE against *S.aureus* **g)** ME against *S.aureus* **h)** ME against *E.coli.*

Different extracts of both plants were evaluated for their antimicrobial potential against microorganisms. In this study using well agar method (Table-1) summarizes and revealed the results obtained and listed the only plant species that presented some activity against at least one microorganism. Both plants showed antimicrobial activity. *E. alba* and *Wedelia chinensis* are the best drug for treating liver cirrhosis and infective hepatitis; wedelolactone is considered as the active principles responsible for the use of these drugs in liver disorders [(17)].

The varying activity across different extracts (methanol, hexane, aqueous) highlights the importance of extraction solvent. Methanol extract generally appear to be more effective, suggesting that the active antimicrobial compound in these plants are more soluble in methanol. The lack of activity from *E.alba* aqueous extract is particularly notable and indicates that its active components might not be water soluble.

C.albicans seems to be particularly more susceptible to the *Wedelia chinensis* showing zone of inhibition of (25mm) and *Eclipta alba*

showing zone of inhibition of (20 mm) respectively.

The HPTLC analysis was performed on *Eclipta alba* and *Wedilia chinensis*, along a standard of wedelolactone, using two detection wavelength at 254nm and 366nm.

At 254nm a dark band corresponding to the wedelolactone standard was observed at an Rf value of 0.57. In *E.alba* a prominent band was observed at Rf 0.57. This suggests the presence of a compound in *E.alba* with similar UV absorption to wedelolactone. The *W.chinensis* also showed multiple bands, a compound was observed at 0.57 Rf consistent with that of wedelolactone, indicating the presence of this compound in *W.chinensis* as well.

Under 366nm (fluorescence), the wedelolactone standard shows a distinct blue fluorescence at Rf of 0.57. The *E. alba* and *W. chinensis* display a strong fluorescent band at same Rf value identical to wedelolactone. Many fluorescent bands were also visible in both plant extracts, indicating a diverse array of different compounds.

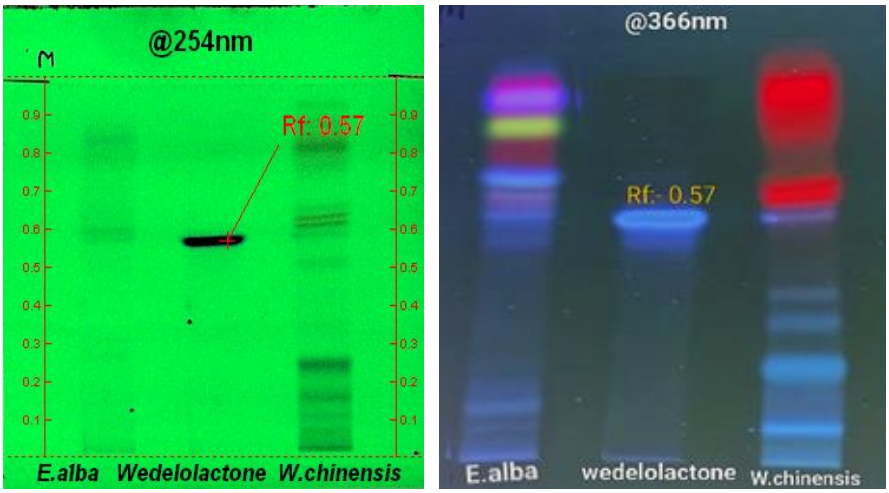

Figure-3 HPTLC Chromatogram of *E. alba, W. chinensis* **and Wedelolactone**

In HPLC, the chromatogram shows a prominent peak at Rt (Retention time) of 7.487 minutes for standard wedelolactone. The sharp and symmetrical nature of the wedelolactone peak indicates good chromatographic separation and efficient column performance this suggests the HPLC methods developed or utilize is suitable for analyzing wedelolactone. The data confirm the presence of wedelolactone in the *Eclipta alba* and *Wedelia chinensis* with a retention time of 7.522 and 7.429 minutes respectively.

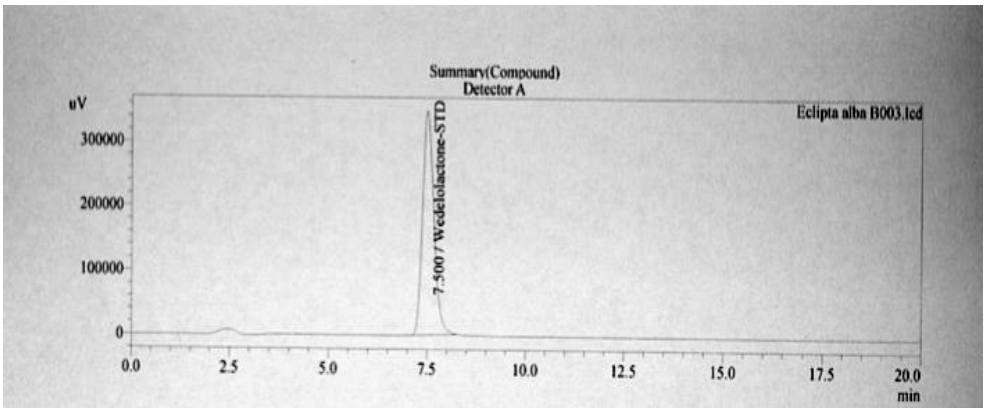

Figure-4 HPLC Chromatogram of wedelolactone standard t Rt-7.487

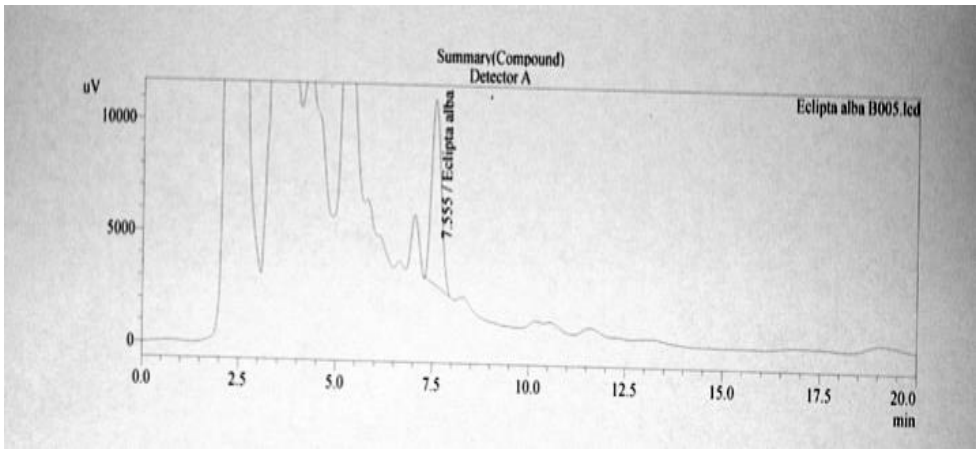

Figure-5 HPLC Chromatogram of Eclipta alba Rt-7.522

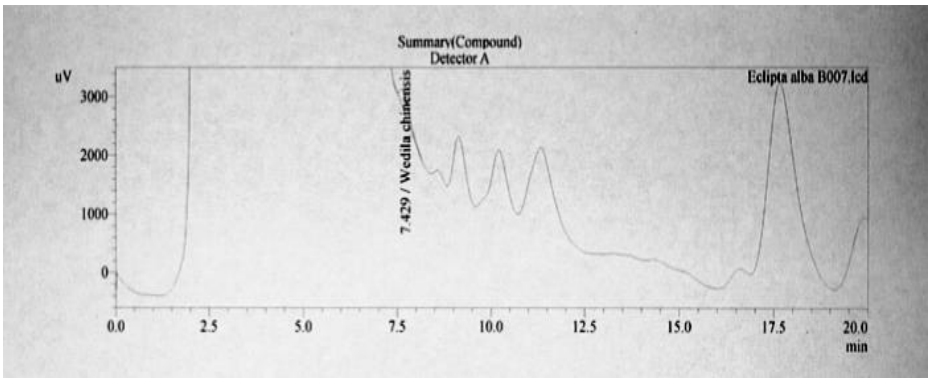

Figure-6 HPLC Chromatogram of *Wedelia chinensis* Rt-7.487

Table-2 Calculation of Wedelolactone in different plant

Sr. No.	Rt (retention time)	Plant Name	Plant part used	Wedelolactone (% w/w)
1	7.522	E.alba	Whole plant	0.040%
2	7.429	*Wedelia chinensis*	Whole plant	0.031%

E.alba contains 0.040% (w/w) of Wedelolactone, which is substantially higher than the 0.031 %(w/w) found in *Wedelia chinensis*. This suggests that *E.alba* is a richer natural source of wedelolactone compared to *Wedelia chinensis* under the conditions of this analysis and using the whole plant.

Conclusion

In the present study we found Wedelolactone content is higher in *Eclipta alba* compared to *Wedelia chinensis*. Wedelolactone is a known bioactive compound with various medicinal properties. This difference in concentration makes *Eclipta alba* the more commercially viable and efficient plant source for the extraction and isolation of wedelolactone. The superior antimicrobial activity of Wedelia chinensis likely stems from the synergistic effects of other phytochemicals present in its extract revealed the complexity of natural product, where the collective action of multiple compounds often dictates biological activity rather than the concentration of a single marker compound. Methanol is more suitable solvent for extraction.

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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Suitability Evaluation of Phytochemical Screening and Anti-nutritional, Phytochemistry and Antibacterial Activity of *Prunus armeniaca* fruits L.

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Abstract- *Prunus armeniaca* commonly known as Apricot Wild Himalayan Cherry is a medium sized deciduous tree, belonging to the family Rosaceae, Subfamily: Amygdaloideae. It is one of multipurpose species which is used as a medicinal plant in Himalayan region. The plant is acclaimed for significant medicinal importance in the traditional system of medicines and used in several Ayurvedic formulations. Its efficacy has been also recognized by the modern system of medicine. The plant has been extensively investigated for its phytochemical constituents and a considerable number of chemical constituents of diverse classes including steroids, terpenoids, flavonoids, polyphenolics, glycosides, etc. have been reported from different parts of the plant. Phytochemistry studies have revealed antibacterial, diuretic, BPH protective, antioxidant activities of the plant. However, despite wide-ranging chemical composition, and traditional medicinal investigation of the plant is limited. This review is an attempt to present a comprehensive summary of traditional uses, ethnomedicinal value, phytochemical, pharmacological, and toxicological aspects of the plant.

Keywords: *Prunus armeniaca, Traditional uses, antibacterial, diuretic value, Phytochemistry and Pharmacology.*

Introduction

Free radicals are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implicated in the process of ageing and several degenerative diseases[1]. Oxidative stress result in wide range of pathophysiological disorders like arthritis inflammation, atherosclerosis, diabetes, liver injury, coronary heart problems, Alzheimer & Parkinson diseases and cancer[2]. Antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS and therefore enhance the immune defense and reduce the risk of degenerative diseases[3]. Polyphenols are responsible for much of the antioxidant activity of fruits and vegetables[4].

Prunus armeniaca is the most commonly cultivated apricot species. The apricot is native to China and Japan but is also cultivated in the warmer temperate regions of the world. The apricot (*Prunus armeniaca L.*), a member of Rosaceae family is a rich source of carbohydrates, fibre, vitamins and minerals. Apricot trees are not ubiquitous since they can only grow in certain regions where environmental conditions are favorable. It is one of the important temperate fruits grown in India with production of 16,739 tonnes from an area of 4,886 ha[5]. In India, it is commercially cultivated in Himachal Pradesh, Jammu &

Kashmir and Uttaranchal. In Himachal Pradesh, it is grown in the districts of Shimla, Mandi, Kullu, Chamba, Sirmour, Kinnaur and Lahaul-Spiti and occupies about 423 hectares of area with an annual production of 1450 metric tons[6]. Apricot kernels contain a substantial amount of dietary protein, along with significant amounts of oil and fiber[7]. Apricot kernels, depending on the variety, contain the toxic cyanogenic glycoside amygdalin, which is responsible for the bitterness of apricot kernels[8]. The diglucoside amygdalin was the first member to be isolated of a class of natural products now known as cyanogenic glucosides. Cyanogenic glucosides are present in more than 2,500 different plant species, including many important crop plants[9].

Prunus armeniaca fruit is common in temperate and is the 3rd most economically traded fruit globally after plum and peach. The *Prunus armeniaca* fruit or apricot originated from the "golden fruit" from its nutritional properties and medicinal values. It belongs to family Rosaceae. *Prunus armeniaca* consists high levels of phytochemicals namely flavonoids, carotenoids, antioxidants and phenolics. It has a spectacular yellow to orange colours with reddish random overlay, a heavy fragrance and a marked flavour. *Prunus*

armeniaca fruit can be consumed fresh, dried, or processed into jam or juice[10].

Common Name- Apricot, Khubani, Jaradaalu, Sakkare Badami, Chuli, Urumana, Maghz badam Shirin, Malhei[11].

Synonym- Amygdalus armeniaca, Armeniaca ansu, Armeniaca vulgaris, Prunus ansu, Armeniaca holosericea, Armeniaca armeniaca, Prunus tiliifolia, Prunus xanthocarpos[11].

Taxonomy

Kingdom: Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Rosales
Family : Rosaceae
Genus : Prunus
Species : *armeniaca*[12].

Figure - 1 *Prunus armeniaca*

Description

The apricot tree is a tiny one, growing to a height of 8 to 12 meters. It has a trunk that may reach a diameter of 40 cm and a dense, spreading canopy. The oval-shaped leaves have a pointed tip, rounded base, and finely serrated edge. They are 5–9 cm in length and 4–8 cm in width. The 5-petaled, 2- 4.5 cm-diameter blooms are produced singly or in pairs in the early spring, prior to the leaves. The fruit is a drupe that resembles a little peach, with a diameter of 1.5–2.5 cm (bigger in some contemporary varieties), with colors ranging from yellow to orange. The side that receives the greatest sun exposure frequently has a red tint. It typically has a pubescent surface. The one seed is encased in a tough, rocky shell that is sometimes referred to as a "stone." The shell is smooth and grainy except for three ridges that run along one side[13].

Distribution

Currently, the primary growing zones for apricots are a strip that runs from Turkey through Iran, the Hindu Kush, the Himalayas, China, and Japan. Nonetheless, the Mediterranean region provides the majority of the world's apricot crop. Turkey and Iran are the world's biggest producers, contributing 21.6% and 14.7% of global apricot output. These countries are followed by Pakistan, Uzbekistan, Italy, Algeria, Japan, Morocco, Egypt, and Spain. The fruit, which is high in nutrients and health benefits, is also grown in mountainous regions of northeastern Ladakh, Uttar Pradesh and Himachal Pradesh in India[14, 15].

Traditional uses

Uses Beta carotene is abundant in apricots, which supply 30% of the daily required amount. They are also a good source of fiber, potassium, and vitamin C. As an example, apricot peel, fruit, and kernel all provide a variety of health benefits, such as lowering cholesterol and promoting regular bowel movements. Additionally, apricot kernels and kernel oil have shown benefits in other situations as well, such as Tinnitus and obits media[16, 17].

Materials and Methods

Plant materials

Fruits of *Prunus armeniaca* free from diseases were collected from the authentic source i.e. the Botanical Garden of Forest Research Institute (FRI), Dehradun. Uttarakhand, India and were identified and authenticated by Systematic Botany Section of Department of Botany, FRI. A voucher specimen of the collected material is preserved in the Chemistry Division for future reference. The collected Fruits of *Prunus armeniaca* were cleaned properly under running tap water to make them free from dust. Fruits of *Prunus armeniaca* seeds were separated from the preserve fruits by pressing and berries pulp portion was dried at room temperature and used for further extraction.

Preparation of extracts Exhaustive

Fruits of *Prunus armeniaca* were dried berries pulp and powdered of (100g). It was successively extraction of fruits with n-hexane, chloroform, ethyl acetate, acetone and methanol. Fruits of *Prunus armeniaca* keeping it for 24 hrs with intermittent shaking. All extractive values were determined on dry extract weight and ground berry pulp taken weight. The exhaustive extraction was done at ambient temperature by shaking the contents at a regular interval of time till discolored solvents were obtained indicating completion of extraction. The extracts so obtained were distilled and then dried on water bath. The percentage yield of dried extracts was calculated with reference to the dried plant material initially taken[18].

The result showed crude extracts were transfer into airtight sample bottles and kept at 4^0C.

Phytochemical screening

The extracts of *Prunus armeniaca* fruits obtained by exhaustive extraction with different solvents were subjected to qualitative phytochemical screening to detect the presence and/or absence of different group of chemical constituents such as alkaloids, flavonoids, phenolics, tannins, steroids, saponins, carbohydrates, glycosides, proteins and free amino acids (FAA), etc. The extracts were tested qualitatively by standard methods using special reagents that produce characteristic colour changes with different categories of chemical constituents[19-21]. Fruits of *Prunus armeniaca* were prepared and subjected to phytochemical screening to detect the presence

and or absence of various phytochemicals constituent.

Phytochemistry

Apricot contains compounds such as polyphenols, phenolic acids, coumarins, tannins, lignins, phenols, and flavonoids; vitamins, minerals, carbohydrates, fibers, and phytochemicals, such as glycosides, carotenoids, polyphenols, phenolic chemicals, aldehydes, sugars, terpene alcohols, and flavonoids; terpenoid chemicals, geraniol, and nerolidol; cyanogenic glycosides etc[22].

Anti-nutritional factors in Fruits of *Prunus armeniaca*

Iron, calcium, and phosphorus content in ragi grains are exceptionally high as compared to other cereals. However, bio availability of these minerals may be at stake, due to the presence of anti-nutritional factors like, phytic acid and tannins (polyphenols). Tannins and phytic acid bind the mineral as well as proteins and reduce their digestible contents. These anti-nutritional factors could be reduced by conventional processing techniques like germination, fermentation and dehulling[23].

Results and Discussion

Extracts values

Prunus armeniaca fruits of the extract values in n-hexane, chloroform, ethyl acetate, acetone, and methanol are presented in the Table-1.

Table-1 Extract values of *Prunus armeniaca* fruits with different solvents

Solvents	Extract Yield (% w/w)
n-Hexane	2.31
Chloroform	3.34
Ethyl acetate	7.54
Acetone	7.62
Methanol	13.02

Qualitative Phytochemical Analysis

The results of qualitative phytochemical analysis are summarized in the Table 2. It revealed the presence of carbohydrate, protein, amino acids, steroids, terpenoids, phenolics, flavonoids, tannins, saponins, glycosides in the *Prunus armeniaca* fruits.

Table-2 Phytochemical screening of Extracts of *Prunus armeniaca* fruits

Phytochemicals	n-Hexane	Chlorofom	EtOAc	Acetone	Methanol
Steroids	+	–	+	–	+
Terpenoids	+	+	+	+	+
Phenolics	–	+	+	+	+
Flavonoids	–	+	+	+	+
Saponins	–	–	–	–	+
Tannins	–	–	–	+	+
Carbohydrates	–	–	–	–	+
Glycosides	–	–	–	–	+
Protein	–	–	–	–	+
Amino acids	–	–	–	–	+

(+) Present; (–) Absent

Phenolics and flavanoids were recorded in all extract of *Prunus armeniaca* fruits except n-Hexane. Remarkably, Phenolics and flavonoids were present all the extracts except n-hexane whereas presence of alkaloids was not detected in any of the extracts.

Phytochemical composition

Apricot contains compounds such as polyphenols, phenolic acids, coumarins, tannins, lignins, phenols, and flavonoids; vitamins, minerals, carbohydrates, fibers, and phytochemicals, such as glycosides, carotenoids, polyphenols, phenolic chemicals, aldehydes, sugars, terpene alcohols, and flavonoids; terpenoid chemicals, geraniol, and nerolidol; cyanogenic glycosides, such as amygdalin, quercetin-3-glucosides, kaempferol-3-rutinoside; neochlorogenic acid, rutin, cynidin-3-glucosides, pcoumaric acids, ferulic acid, epicatechin, epigallocatechin, and Catechin; and terpene chemicals, geraniol and nerolidol. Hexanal, ethanol, hexyl acetate, 1-hexanol, (Z)-3-hexenol, (E)-2-hexenol, and (Z)-3-hexenol-1-ol, catechin, 2-(3,4- dihydroxy phenyl)-3,5,7-trihydroxy-4H-chromen4-one, chlorogenic acid, 3,4,5-trihydroxy benzoic acid, and 3-(3,4 dihydroxy phenyl)-2-propenoic acid, fiber, lipids like sterols and fatty acids, minerals including phosphorus, selenium, magnesium, zinc, iron, potassium, and calcium[22].

Significance of Nutritional composition

Fruits of *Prunus armeniaca* are nutritionally superior to other fruits. It serves as an excellent source of carbohydrate (71%), proteins (6.6%) with essential amino acids as well as non essential amino acids like valine, methionine, and tryptophan, minerals (calcium, phosphorus, potassium, and iron) as well as vitamins (thiamine, niacin, and riboflavin), and fats for which they are extensively been researched[23].

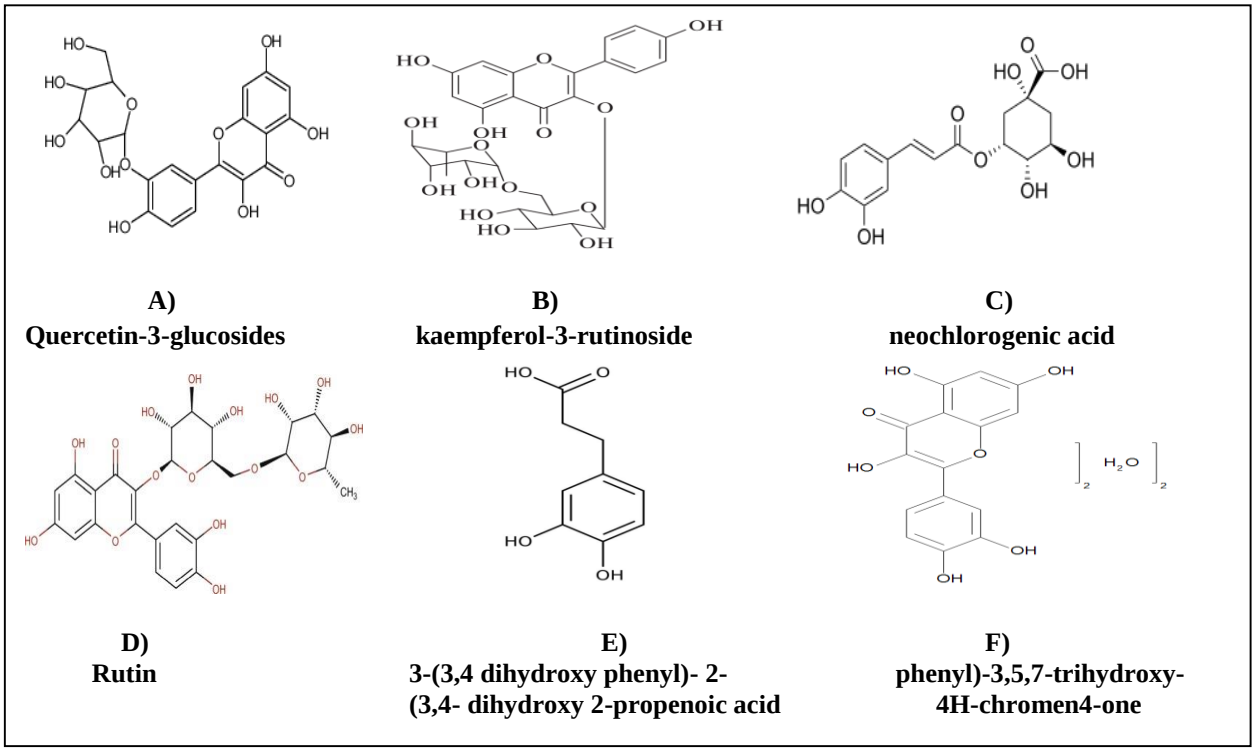

Figure – 2 Chemical structures of major phytochemicals compounds present in *Prunus armeniaca*

Table – 3 General Nutrient Composition of Fruits of *Prunus armeniaca* per 100g

Nutrient composition of Fruits of *Prunus armeniaca*	
Moisture	11.24%
Protein	6.6%
Carbohydrate	71.36%
Fibre	1.12%
Minerals	2.33%
Fat	1.12%
Energy	331.4 cal/100g

Antioxidant activity

The wine made from fruits of *Prunus armeniaca* fruits contains highest flavonoids and antioxidant compounds Total antioxidant activity and polyphenol content were also found to be highest in wines made from the fruits which entail a better option for bio-utilization of this highly valuable wild fruit which are otherwise of no commercial value[24]. The antioxidant properties of the gum were evaluated by DPPH and hydroxyl scavenging activities, reducing power and total phenolic contents which showed the gum possess antioxidant property[25]. Chloroform, ethylacetate, acetone and methanol extracts of *Prunus armeniaca* fruits were evaluated for total phenol, total flavonoid and antioxidant activity. All the extracts exhibited varying degree id antioxidant efficacy in a concentration-dependant manner, Methanol extract however recorded the highest total phenolic content 0.035 mg GAE/g of extract and free radical ± (3.660.232± scavenging (antioxidant) activity (IC50, 55.00 µg/ml). Total phenolic content had positive correlation with antioxidant capacity. The study established *Prunus armeniaca* fruits as rich sources of phenolic compounds and natural antioxidants[26].

Antibacterial activity

Prunus armeniaca fruits reported to have antibacterial activity against both gram positive and gram-negative bacteria. Antibacterial activity of ethanolic extract of *Prunus armeniaca* fruits was examined with Gram positive bacteria (Bacillus subtilis and *Staphylococcus aureus*) and Gramnegative bacteria (Escherichia coli and Salmonella typhi) and the extract recorded inhibitory action against all the bacteria[27]**.** Antimicrobial screening of aqueous extract from stem bark of the plant was carried out against various pathogenic microorganisms and results suggest that the aqueous extract could be a potential source to obtain new antimicrobials and effective herbal medicines to combat the problem of ever emerging microbial resistance[28].

Diuretic activity

The seeds of *Prunus armeniaca* fruits are traditionally known for treatment for urinary disorders, polyurea and the treatment of stone in the kidney[29]. Puddumin-A a flavonone glucoside from *Prunus armeniaca* fruits showed the increased diuretic activity. Effects of fruits extract were investigated on prostate and urinary disorders. Three different fractions of methanolic extract were evaluated of activity against prostate disorder on rat. The Fraction III treated group shows the lessened effect of

testosterone on the prostate gland enlargement comparing to that of group I and group II treated group. The fruits extract of *Prunus armeniaca* fruits showed the capability to reduce the testosterone induced prostate weight of rat[30] .

BPH protective activity

Protective effect of bark of *Prunus* species including *Prunus armeniaca* fruits was evaluated against benign prostatic hyperplasia (BPH) and the results indicated a meaningful inhibitory effect of testosterone induced BPH by the bark of different species of *Prunus* in the and *Prunus armeniaca* fruits[31] .

Conclusion

In the ongoing study, efficacy of berries in relation to its chemical composition is established. In this study the extreme free radicals production generally leads to oxidative stress. *Prunus armeniaca* fruits have natural anti-oxidants and anti-bacterial, Diuretic activity, BPH protective activity, nutritional activity in the ability to protect organisms from damage caused by free radical-induced oxidative stress. *Prunus armeniaca* fruits have been traditionally used in folkloric medicine and most of the medicinal properties. Also *Prunus armeniaca* fruits have been attributed to its antioxidant activities. Now considering, this study revealed that *Prunus armeniaca* fruits has higher polyphenolic content and antioxidant activity. Therefore, it can be ventured that *Prunus armeniaca* fruits can also be potentially useful as a natural source of antioxidant or in medicine. As well as phytochemical studies and in-vitro antioxidant assay of fruit *Prunus* species will notably add to the inventory of natural antioxidants.

Therefore, the plant deserves proper attention towards systematic approach for the collection, storage, processing and value addition that could be helpful in the economic development of tribal areas in the Himalayan foot hills where the species is mostly grown.

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Disclaimer Statement

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

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Comparative study of In-Vitro Antioxidant and Thrombolytic activities, In-Vitro Anti-Anemic activity of ethanolic fruit and leaf extracts of Phoenix sylvestris in anemic rats

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Abstract- In order to prevent problems from thrombosis in atherosclerotic arteries that affect the heart, brain, extremities, and stagnant blood in veins and heart chambers, it is crucial to treat patients with haemostatic problems effectively. Using chemical agents that are already on the market presents challenges because there is a possibility of numerous adverse effects. An investigation using medicinal plants was done in an effort to prevent that. This study was conducted to assess the in-vitro anticoagulant and thrombolytic thromboplastin time using human plasma activities by measuring clotting time, prothrombin time, and activated partial collected from healthy volunteers at various concentrations. Additionally, the in-vivo anti-anemic activity of *Phoenix sylvestris* fruit and leaf extracts was evaluated in phenyl hydrazine-induced anemic rats.

There were seven groups of rats, each containing six rats. While the other groups were given phenyl hydrazine 60 mg/kg b.w. for two days to induce anaemia Group I was given normal saline as a control. Group II is the positive control; it only received treatment with phenyl hydrazine, whereas Group III received standard treatment with a vitamin B12 complex. functioned as the norm. Groups VI and VII were treated with ethanolic leaf extract of *P. sylvestris* (250 and 500 mg kg-1 b.w.), whereas Groups IV and V were treated with ethanolic fruit extract at varying doses (250 and 500 mg/kg b.w.). All the treatments were given orally and continued up to 28 days. On 29th day, blood was withdrawn, through tail puncture and subjected to the estimation of RBC, Haemoglobin and percentage haematocrit using haematology analyser.

Both extracts showed a more notable effect on the prolongation of these coagulation parameters and significantly increased coagulation. The fruit extract produced preferential increase in bleeding and clotting time, nevertheless the effect was more perceptible compared to leaf extract. When compared to *P. sylvestris* leaf extract, the fruit extract of *P. sylvestris* significantly increased haemoglobin, red blood cells, and percentage haematocrit; the outcomes were comparable to those of standard vitamin B12 complex.

Thus, the present study provides the pharmacological basis for its medical use in cardiovascular and other thrombotic disorders due to its significant anticoagulant, thrombolytic and anti-anemic activities.

Keywords: *In-vitro* Anti coagul ant, Thrombolytic Activities, *In- vivo* anti-Anaemic Activity, *Phoneix Sylvestris.*

Introduction

Blood in health and disease is the subject of haematology. Anaemia, haemophilia, autoimmunity, throm-bosis, and blood coagulation are among the major blood disorders and diseases that occur in sick patients as a result of RBC destruction. These conditions can lead to mortality[1]. The WHO reports that 2 million people worldwide, ranging in age from children to new borns, suffer from anaemia, accounting for 30% of the total population[2]. Red blood cells (RBCs) are involved in both blood coagulation and thrombotic disorders[3]. Prolonged bleeding and thrombosis occur primarily in anaemic patients due to their action as pro-coagulant and pro-thrombotic blood components[4]. Diabetes, hypertension, coronary artery disease, and ischemic heart stroke are caused by changes in red blood cells both in-vitro and in-vivo in whole blood[5].

A variety of blood disorders include blood coagulation. It's persistent process linked to anaemia condition[6]. Thrombosis, haemor-rhage, and hypertension are among the C.V.S. diseases that result from clotting disorders. Factor deficiencies (II, V, VI, X) are found in the extrinsic pathways. Prothrombin time and clotting time are used to evaluate these deficiencies. When calcium ions are present, thromboplastin stimulates the coagulation system's extrinsic pathway. Deficiency of factors VII and X shows increased prothrombin time or clotting time. The four main anticoagulant medications are citrate, EDTA, heparin, and Warfarin[7.]

Thrombosis is one of the blood disorders causing heavy blood clots due to which occurs alteration in flow of blood in arteries, veins and tissues leading to severe heart problems[8]. Numerous thrombosis types, such as arterial thrombosis, can result in an ischemic stroke and myocardial infarction. Deep vein thrombosis, which is linked to pulmonary embolism, chronic thrombus embolism, post-thrombotic syndrome, and pulmonary hypertension, is caused by venous thrombosis. Anticoagulant therapy which dissolves blood clots by activating plasminogen, which forms plasmin[10], is the standard treatment for thrombosis. Tissue plasminogen activators, streptokinase, and urokinase are additional medications[9].

Anaemic is a blood disorder which can lead to many diseases. There are more than 400 types of anaemic conditions occurring by decrease number of circulating red blood cells are affecting tissue oxygenation[11]. It is diagnosed by decreased Hb less than 13g dl in males and in female's 12g dl[12]. For the synthesis of haemoglobin in our body the process of erythropoiesis is needed which contains several metabolites[13].

Various Treatment includes based on different types of aneamic condition are vitamin B12, immune suppressants, cortico steroids, erythropoietin injection, blood transfusion and osseous marrow transplan-tation[14]. Most of the pharmacological drugs which are used for the treatment of coagulation, thrombosis and aneamic conditions are not that much effective due to their unwanted side effects and risk factors. It was investigated on herbal

medicines that their medicinal properties in plants helps to cure blood disorders.

Traditional plant of *P. sylvestris roxb*, together with 13 other species from the genus phoenix. belongs to family *Are-caceae*. It is widely distributed in India, it is unbranched, tall evergreen ornamental tree occurs at altitude of 1500m. Leaves are 3-4.5 m in length.

Fruits are 15-25mm long and 12mm broad. It possesses some of the medicinal properties which are heart related problems, diarrhea, asthma, lactation, tuber-culosis, cough, gonorrhoea, pains, toothache[15].

So far, much pharmacological work has not been carried out on *P. sylvestris* plant. The present study was carried out to evaluate the *in-vitro* anticoagulant and thrombolytic, *in-vivo* anti-anaemic activities using rats were carried out and evaluated.

Material and Methods

Collection of Plant Material

The fruits and leaves of the *P. sylvestris* plant were collected in September from the Forest Research Institute in Dehradun, Uttarakhand, India. The authenticity of the plant was confirmed by the departmental authorities of FRI.

Preparation of Ethanolic Extract

The various leaf sections and immature fruits of *P. sylvestris* were gathered and cleaned with deionized water. Fruit and seeds were separated, and leaves were chopped into tiny pieces. The dried extract materials were allowed to air dry for a week before being ground into a coarse powder using a mixer grinder. Two liters of ethanol were used to macerate 500 g of dried powder separately for four days. Filtered and collected, the fruit and leaf extract solvents were then further distilled and evaporated. The concentrated drug extract was finally produced, dried under a desiccator and stored in a china dish covered in aluminium foil.

Phytochemical Screening

The phytochemical screening of ethanolic fruit and leaf extract of *P. sylvestris* was carried out for the determination of phytochemical constituents like carbohydrate, vitamins, minerals, proteins, enzymes, sugars, iron, alkaloids, flavonoids, saponins, steroids, tannins and phenols[15,16] .

Acute Toxicity Studies

Oral doses of 50, 100, 200, 400, 800, and 1600 mg kg^{-1} of ethanolic *P. sylvestris* leaf and fruit extracts were given to groups of mice (n = 6); the mice were then monitored for signs of behavioural and neurological damage, and the % mortality was documented[24] hours later. The animals were given their doses. According to OECD guideline 42018, this plant species has not yet been the subject of any pharmacological research. Therefore, doses for acute toxicity tests were taken into consideration based on the other species in the same genus. At 1600 mg kg^{1}, the extracts were shown to be mortality-free.

In-Vitro Anticoagulant Activity

The *In-vitro* anticoagulant activity of ethanolic fruit and leaf extract of *P. sylestris* was determined by prothrombin time[17].

Blood Samples Collection

In order to obtain pure platelet plasma, five millilitres of blood were first drawn from the veins of each of the four healthy volunteers. The sterile syringes were used to remove the blood from each volunteer's right arm, and the samples were then placed individually in containers containing trisodium citrate to

prevent the clotting process. Each person's obtained plasma sample was labelled and used right away to determine the prothrombin time test.

Blood Serum Samples Separations The collected plasma samples were divided into 9 groups as given below in **Table-1**.

Finally, the clotting time was recorded with a stop watch by titling the test tube for every 30 seconds. This time was noted as prothrombin time.

Table-1 Blood Serum Samples

Group	Type	Composition
I	Negative Control	0.2 mL plasma + 0.1 mL 0.9% saline + 0.3 mL $CaCl_2$
II	Test (Fruit Extract)	0.2 mL plasma + 0.05 g/mL fruit extract + 0.3 mL $CaCl_2$
III	Test (Fruit Extract)	0.2 mL plasma + 0.1 g/mL fruit extract + 0.3 mL $CaCl_2$
IV	Test (Fruit Extract)	0.2 mL plasma + 0.3 g/mL fruit extract + 0.3 mL $CaCl_2$
V	Test (Fruit Extract)	0.2 mL plasma + 0.5 g/mL fruit extract + 0.3 mL $CaCl_2$
VI	Test (Leaf Extract)	0.2 mL plasma + 0.05 g/mL leaf extract + 0.05 mL $CaCl_2$
VII	Test (Leaf Extract)	0.2 mL plasma + 0.1 g/mL leaf extract + 0.3 mL $CaCl_2$
VIII	Test (Leaf Extract)	0.2 mL plasma + 0.3 g/mL leaf extract + 0.3 mL $CaCl_2$
IX	Test (Leaf Extract)	0.2 mL plasma + 0.5 g/mL leaf extract + 0.5 mL $CaCl_2$

In-vitro Thrombolytic Activity

The *In-vitro* thrombolytic activity of ethanolic fruit and leaf extracts of *P.sylvestris* were assessed by thrombolytic test. Aliquots (5 mL) of venous blood sample was withdrawn from healthy volunteers which are dist-ributed in eight different preweighed sterile micro centrifuge tube (1 mL/ tube) and incubated at 37^{O}C for 45 minutes. After the clot formation occurs the serum is completely removed without clot disruption with the help of micropipette for determining clot weight of each individual volunteer. The tube clot was weighed once more **(tube clot weight = tube weight with clot – tube alone weight)**. Following the addition of a pre-weighed clot to each sterile micro centrifuge tube, 100µL of an aqueous solution containing various partitions of fruit and leaf crude extracts at varying concentrations (0.05, 0.1, 0.3, and 0.5 g mL-1) was added to the pre-weighed clot. 100µL of distilled water was used as the negative control, and 100µL of streptokinase was added as the standard control. The clots were then incubated at 37^{O}C for 90 minutes to observe clot lysis. Following the release of the incubation fluid, the tubes are weighed once more to measure any weight differences following clot disruption. Finally, the difference obtained in weight taken before and after clot lysis was determined as percentage clot lysis [18].

$$\% \text{ Clot lysis} = \frac{\text{weight of released clot} \times 100}{\text{clot weight}}$$

In-Vivo Anti-Anaemic Activity

Experimental Animals

Wistar male albino rats of either sex (150-180gms) were used for study. Animals were housed in colony cages at ambient temperature of 25 ± 2^{0}C, 12 h light/dark cycle and 50±5% relative

humidity with free access to food and water ad *libitum*. Prior to experimentation, the animals were given at least a week to become used to the laboratory setting. Throughout the experiment, participants went without food but not water for the entire night. Every experiment was run from 9:00 to 16:00 hours during the light period. Every group had five animals in it. Based on the approval of the Institutional Ethics Committee (IEC) and the guidelines provided by REG. No. 1269/a/10/ CPC SEA, animal experiments were conducted.

Table-2 Treatment details of different controls and test groups

Group	Type	Treatment Details
I	Normal Control	0.1% Carboxy Methyl Cellulose (CMC)
II	Anaemic Control	Phenylhydrazine – 60 mg/kg/day for 2 days
III	Reference Control	Vitamin B12 syrup for 28 days
IV	Test Group (Fruit Extract – Low Dose)	*P. sylvestris* fruit extract – 250 mg/kg for 28 days
V	Test Group (Fruit Extract – High Dose)	*P. sylvestris* fruit extract – 500 mg/kg for 28 days
VI	Test Group (Leaf Extract – Low Dose)	*P. sylvestris* leaf extract – 250 mg/kg for 28 days
VII	Test Group (Leaf Extract – High Dose)	*P. sylvestris* leaf extract – 500 mg/kg for 28 days

Evaluation of Anti-Anaemic Activity

In-vivo anti-anaemic activity in rats was evaluated by induction of anaemic. Used phenyl hydrazine (60 mg kg^{-1}) by intraperitoneal administration for 2 days for all groups of animals except normal control group. Animals were divided into 7 groups of 5 each.

Finally, on 29th day treated anaemic rats with different plants extracts of *P. sylvestris* activity was evaluated by withdrawn of blood sample through tail puncture. Blood is taken in blood collecting tubes containing EDTA for prevention of blood clotting. Further, blood was subjected to centrifugation process for plasma separation. Finally blood samples were used for the purpose of estimation of blood parameters like red blood cells (RBC), haemoglobin (Hb) and percentage Haematocrit (% Hct) by using Haematology analyser apparatus[19].

Statistical analysis

All the values are expressed as the mean ± SEM and were analyzed *via* one-way analysis of variance (ANOVA) followed by Dunnett's test, using SPSS 18.0 software. Statistical significance was set at $P < 0.05$.

Results and Discussion

Preliminary Phytochemical of plant extracts revealed the presence of phytochemical constituents like carbohydrates, sugars, alkaloids, flavonoids, saponins, phenols and tannins.

The effects of various ethanolic fruit and leaf extracts of *P. sylvestris* show that dose escalation increases prothrombin time at different concentrations. The ethanolic fruit extract exhibits greater anticoagulant activity than the leaf extract at 500 µg/mL (50:35±0.13), while the leaf extract at 500 ug/ml (41:14±0.02) yields similar results. (**Table-3**).

Table-3 *In-vitro* Anticoagulant Activity of Ethanolic Fruit and Leaf Extracts of *P. Sylvestris*

Drug Treatment	Concentration (µg/mL)	Time of Coagulation (Mins)
Normal control	0.9%NaCl	2:13±0.002
Fruit extract	50	7:15±0.04
	100	13:52±0.08
	300	24:14±0.15
	500	50:35±0.13*
Leaf extract	50	6:12±0.04
	100	10:14±0.06
	300	22:23±0.17
	500	41:14±0.02*

All the values are expressed as Mean ± SEM, n= 5 ,* p<0.001 when compared with Normal Control.

Utilizing varying concentrations, the effects of an ethanolic fruit and leaf extract of *Phoenix sylvestris* were investigated in terms of clot lysis. 500 g/mL of fruit extract demonstrates 84.22% clot lysis, while 500 g/mL of leaf extract demonstrates 79.5 % clot lysis. These are represented in **Table-4.**

Table-4 In-*vitro* Thrombolytic Activity of Ethanolic Fruit and Leaf Extract of *P. Sylvestris*

Drug Treatment	Concentration (µg/mL)	Weight Of Clot Before Lysis	Weight Of Clot After Lysis	Clot Difference	%Clot Lysis
Normal Control (Distilled Water)	100	0.121 ±0.0012	0.0063± 0.0012	0.115 ±0.0017	5.2
Standard Control (Streptokinase)	100	0.118 ±0.0010	0.016± 0.00089	0.102 ±0.00034	86.2
Fruit Extract	50	1.25 ± 0.014	0.60 ± 0.015	0.7± 0.14	80.66
	100	1.12± 0.015	0.49 ± 0.012	0.6± 0.12	81.65
	300	1.22± 0.013	0.53 ± 0.0089	0.62 ± 0.015	83.88
	500	1.18 ± 0.012	0.48 ±0.015	0.57± 0.012	84.22 *
Leaf Extract	50	1.2 ± 0.044	0.36 ± 0.17	0.67± 0.014	71.4
	100	1.46± 0.17	0.66± 0.014	0.81± 0.0189	73.43
	300	1.35± 0.0067	0.61± 0.021	0.90± 0.012	75.66
	500	0.95± 0.017	0.53 ± 0.017	0.42 ± 0.014	79.5 *

All the values are expressed as Mean ± SEM, n=5,* P<0.001 when compared with standard values

Anaemic rats induced with phenyl hydrazine were used to test *Phoenix sylvestris* ethanolic fruit and leaf extracts for their anti-anemic properties. The table-3 displays the results. In

comparison to the leaf extract (500 mg/kg), which had RBC (5.69), Hb (10.8), and %HCT (38.6), the fruit extract of *P. sylvestris* (500 mg/kg) significantly increased the haemato-logical parameters of red blood cells (7.17), hemoglobin (14.7), and percentage heamatocrit (48.53).By comparing fruit and leaf extracts with anaemic control group , fruit extract shows maximum activity with standard control group of vitamin B_{12} syrup. The results mentioned in **Table-5**.

Table-5 Effect of Ethanolic Fruit and Leaf Extracts of *P. Sylvestris* in Phenyl hydrazine Induced Anaemic in Rats.

S. No.	Drug Treatment	RBC	HB(G/DL)	HCT%
1	Normal Control	8.81±0.65	13.72±0.65	48.75±0.03
2	Anemic Control (Phenyl hydrazine-60mg/kg)	2.51 ±0.018	9.76 ±0.18	21.4±0.21
3	Reference Standard (Vitamin B_{12} syrup)	8.43±0.42	13.18±0.73	46.25±0.54
4	Test Group (Fruit Extract-250mg/kg)	5.86 ±0.18	13.6 ±0.13	36.8±0.43
5	Test Group (Fruit Extract-500mg/kg)	7.17 ±0.027	14.7 ±0.13	48.53±0.21
6	Test Group (Leaf Extract-250mg/kg)	4.03± 0.0144	9.3± 0.077	31.61±0.18
7	Test Group (Leaf Extract-500mg/kg)	5.69 ±0.0044	10.8±0.15	38.6±0.41

All the values are expressed as Mean± SEM, n=5,*p<0.001 when compared with standard values

The present study performed to evaluate *In-vitro* anticoagulant, throm-bolytic and *in-vivo* anti-anaemic activity in anaemic rats using *P. sylvestris* of ethanolic fruit and leaf extracts. During study research plant extracts activity assessed by phytochemical screening tests and acute toxicity studies. The phytochemical tests show presence of carbohydrates, alkaloids, tannins, phenols, sugars, flavonoids, sap-onins[15,16]. In acute toxicity studies reveal plant extracts does not have adverse effects and risk factors showing presence of low toxicity profile in animals by administration of ethanolic plant extracts of *P. sylvestris* at various doses. By comparative study it has been clearly shown that ethanolic fruit extract shows maximum effect than leaf extract. It possesses anticoagulant, thrombolytic and anti-anaemic properties. The coagulation occurs due to heavy bleeding and clots[6.] The condition of coagulation is treated by using *P. sylve-stris* plant of leaf and fruit ethanolic extracts exhibits reduce blood clotting on human blood sample. *In-vitro* study based on prolongation of dose at 500µg/mL fruit extract shows maximum anticoagulant activity compared to the leaf extract. *In-vitro* thrombolytic activity revealed that ethanolic extracts of *P. sylve-stris* show maximum blood clot-lysis. condition of thrombosis cause heavy blood clots leading to severe CVS diseases and blood disorders[8]. By using medicinal plant of *P.sylvestris*, we can cure thrombosis by decreasing blood clots and increasing clot lysis. Based on increasing dose concentration at 500 µg ml^{-1} shows maximum *in-vitro* lysis of clot in fruit extract. Mild effect in leaf extract was noted. By comparing fruit extract shows significant activity as compared to leaf extract.

In-vivo anti-anaemic activity using phenyl-hydrazine induced anaemic rats were investigated by administration of Phenyl-hydrazine (i.p) for 2 days treatment at dose of 60mg/kg. There is decreased haematological parameters, leads to destruction of red blood

cells, haemoglobin and percentage haema-tocrit. The decreased parameters attain in normal level[20]. When treated with plant extracts of *P.sylvestris, it* shows increase of RBC, Hb and % haematocrit. From results it is shown that ethanolic fruit extract of *P. sylvestris* have significant effect at 500 mg kg^{-1} dose based on dose prolongation. Ethanolic leaf extract shows minimum activity at dose of 500 mg kg^{-1}. On comparison fruit extract shows better activity than leaf extract.

Conclusion

The findings of the present study suggest that the ethanolic extracts of *Phoenix sylvestris* fruits and leaves possess anticoagulant, thrombolytic, and anti-anemic properties, indicating their potential in the treatment of various blood-related disorders.

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Data Availability Statement

The data are contained within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Disclaimer Statement

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

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Hydroponically Cultivated and Soil Cultivated *Ocimum basilicum* (Italian Basil)- A study on its Yield and Quantity of Antioxidants

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Abstract- Growing of *Ocimum basilicum* in Hydroponic as well as in traditional soil system are well established, while the comparison between these two growing system is not studied in detail. This study is conducted to check the performance of *Ocimum basilicum* in both hydroponic as well as in traditional soil systems. The Italian Basil plants were grown in hydroponic beds with flowing of nutritional water and a set of the same plants was grown in soil pots. The studied parameters include morphological, biochemical and antioxidant levels. The number of leaves increased with age and found more in number about 111.00 and 185.00 with hydroponic system as compared to that of traditional soil system which reduced to 101.00 and 156.00 evaluated on 40 days and 70 days respectively. The total leaf area showed a similar trend representing 3.88 cm and 3.92 cm in the hydroponic system while in traditional soil system it is 3.18 cm and 3.75 cm at 40 and 70 days respectively. The hydroponic system significantly increased the ascorbic acid content of the plants as compared to that of the traditional soil system plants; it represented 3.67 mg/g in hydroponic system when compared to 3.2 mg/g in the traditional soil system evaluated on 70 days. The fresh weight was higher in hydroponic system plants when compared to traditional soil system plants. From the results of this study, it can be concluded that the hydroponic system is the better choice for growing of Italian Basil.

Keywords: *Ocimum basilicum*, Hydroponic system, Antioxidants and Biochemical activities.

Introduction

A suitable method like hydroponic system or Aeroponics system of cultivation would plays an important role in the coming era due to climate change, growing urbanization, water scarcity, war situation in many countries and shrinking land area for traditional agriculture. The current estimated population of world is about 8.23 billion and will reach to 9 billion people in 2037 on the planet.

There is also a rise in the urbanization rate from 50 to 70%, traditional soil agriculture system suffers from the growing consequences of climate change, drought, salinity, and extreme temperature swings. Soilless culture like hydroponic and Aeroponics system eliminate these bad effects and guarantee long-term, sustainable food security for world population. These systems include circulation of water/nutrient solution in such a manner that the solution continues to pass through the roots of plants and provide sufficient nutrients to

grow them. The source of light will be attached on upper side of the system to mimic the sun light and help in the photosynthesis. In hydroponic system a well-known soilless culture system, is the process of cultivating plants without the need of soil, usually in combination with a nutrient solution. Hydroponics system involves submerging the plant roots in nutrient solution. This is important that the solution consists of water and fertilizers carefully dosed to produce the right concentration of macro- and micro-elements required for particular plant growth. The reduced planting area is efficiently utilized in this systems. The nutrient circulate in hydroponic system and consume less water to grow the plants. The hydroponic system in large-scale cultivation runs in a closed circuit, with regular pumping, recycling, and renewal of the nutrient solution and pH is monitored which plays an important role in the growth of plants in this system. Plants grown in a hydroponic system collect nutrients from circulating water and hence no need for soil to grow. This system requires no need for pesticides to grow plants. Number of crops, including ornamentals, seasonal _lowers, cereal crops, radishes, beets, carrots, and potatoes can be grown on inert supporting material rather than traditional soil system. *Ocimum basilicum* is a significant plant that contains _flavonoids, polyphenols, phenolic, and essential oils. The Italian basil plant, which belongs to the Lamiaceae family and genus. It is one of the aromatic plants whose consumption gradually rises. It typically grows in tropical and subtropical climates and is a typical fresh vegetable or spice in human diets. Its potent and distinct scent makes it stand out in meat, vegetables, salad, sauce, herbal tea, and other culinary applications. However, medicinal properties of this herb increased its value and has expanded beyond its original just culinary uses. Italian basil leaves contain a unique and recognizable fragrance, thanks to unusual oil glands making it a highly beneficial herb. Hence, practically all nations and cultures utilize it in both fresh and dry conditions. Both soil and soilless cultures are appropriate for basil production. Cultivation of Basil through Hydroponic system is well-established and in use in most of the countries as per their requirements. Basil grows well in hydroponic systems because of its excellent growth potential while hydroponics and Aquaponic grown Basil have a very high demand because of its quality and is free from pesticides used in soil cultivation. Consequently, hydroponic farming of basil is believed to be more ecologically friendly and efficient than traditional soil system farming. Due to its great value along with its consumption, it is a cash crop, which make more concerns for farmers worldwide. Till date soil system is one of the most complex and abundant methods of growing basil (*O. basilicum*). Hydroponically produced basil, requires less water and no soil and contains a high-quality crop output. This method is not dependent on the environment due to its controlled system and hence sudden changes in the climate and other environmental changes do not affect the crops production yield as well as its quality grown in hydroponic system. As per research data published previously revealed that basil is one of the most often utilized plants in the kitchen and various other business sectors due to its many medicinal qualities and attributes. This study is conducted to check the performance of *Ocimum basilicum* in both the hydroponic as well as in traditional soil systems[1-23].

Material and Methods

Experiment Location

The experiment was carried out at Himalaya Wellness Company, Faridabad Unit, India.

Seed Collection and Germination

The certified seeds of it were obtained from agriculture material supplier in Faridabad, India. Seeds of it were sown in the nursery in foam plates under slight media layer and kept in the greenhouse conditions. Water spray was performed one time a day. Plants were germinated after 5–7 days. Normal soil cultivation practices were followed for soil-based plants in the using soil.

Plant Growth Conditions and Parameters Analyzed

Basil was harvested 40 and 70 days after sowing of seeds. Basil characteristics of each harvest was evaluated by measuring the root length, total weight, average leaf number per plant. The total number of fully developed leaves were counted and expressed as number of leaves per plant. The total leaf area of fully developed leaves was measured and expressed in cm per plant. Fresh weight was determined by using an electronic balance and the values were expressed in grams. The plants were dried for 48 hrs at 60 °C in a hot air oven just after taking its weight. After drying, the weight was measured and the values were expressed in grams[24-25].

Ascorbic Acid Determination- total Ascorbic acid is determined according to the method described by Omaye et al. (1979). Water extract (100 µL) of sample was mixed with 900 µL of 5% TCA, 1 ml of 10% TCA and 100 µL of DTC reagent. The DTC reagent was prepared using 0.04 g thiourea, 0.05 g copper sulphate, 0.3 g 2, 4-DNPH and 10 ml of 9 N Sulphuric acid. The mixture was then incubated at 37˚C for 3 hours for the formation of orange red osazone crystals. The osazone crystals were dissolved in 750 µL of 85% sulphuric acid and incubated at room temperature for 30 minutes. The absorbance was measured at 540 nm against 5% TCA as blank. Total ascorbic acid was expressed in mg per g of weight sample.

Result and Discussion

Morphological Parameters- The plants in hydroponic and soil system exhibited an increase in height with days, as they started growing since the first day. However, when compared to the hydroponic system at 40 days 49.42 cm height, the soil-grown plants exhibited 45.55 cm, a decreased height. A similar trend was noticed in the case of 70 days, when hydroponic-grown plants measured 72.05 cm, while the soil-grown plants measured only 68.21 cm in height. The root length increased with age in both systems. The root length increased in the hydroponic system when compared to soil-grown plants. The number of leaves increased with age and found more in number about 111.00 and 185.00 with hydroponic system as compared to traditional soil system which reduced to 101.00 and 156.00 evaluated on 40 days and 70 days respectively. The total leaf area showed a similar trend, representing 3.88 cm and 3.92 cm in the hydroponic system while in traditional soil system is 3.18 cm and 3.75 cm at 40 and 70 days respectively.

The fresh weight was higher in case of hydroponic system grown plant when compared to soil grown plants. This is due to the higher shoot and root growth of hydroponic plants as compared to soil grown plants and hence contributed to the increased fresh and dry weights of the plants in the hydroponic system.

Ascorbic Acid- The hydroponic system significantly increased the ascorbic acid content of the plants as compared to the traditional soil system plants; it represented 3.67 mg/g in hydroponic system when compared to 3.2 mg/g in the traditional soil system evaluated on 70 days.

Conclusion`

The main aim of this research is to standardize the hydroponic production of Italian basil plants and to compare with the plants grown in soil system, regarding the growth parameters like size, weight of plant, leaves numbers and their size and content of vitamin C at different interval mainly after 40 and 70 days. Based on the obtained results, the growth parameters like plants weight, leaves number and their size along with vitamin C content is better in hydroponic system on both the interval of time that is after 40 days and after 70 days, as compared to soil system and hence we can say that production of herbal and aromatic plants in hydroponic system is better option as compared to that of traditional soil system.

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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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Preparation and Assessment of a Herbal Multipurpose cream for Cosmetic Application

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Abstract- Herbal medicine, often known as herbalism or botanical medicine, involves using herbs for therapeutic or medical purposes. An herb is a plant or plant component appreciated for its medicinal and fragrant properties. Herb plants generate and contain several chemical compounds that affect the body. Herbal cosmetics are beauty products with beneficial physiological effects, including skin healing, smoothing, attractiveness enhancement, and conditioning, due to their herbal constituents. The herbal multi-functional cream was studied with the goal of creating a new recipe. Formulations were tested for physicochemical properties such as appearance, emulsion type, color and odor stability, extrubility, pH, texture, application feel, particle contamination, and spreadability. Formulation C out performed other formulations in terms of product stability and effectiveness. Its usage may be responsible for such a high-performing stable formulation.

Keywords: Multipurpose cream, Herbal Formulation, Medicinal Herb.

Introduction

Herbal cosmetics are sometimes known as botanical origin products. Plant-based personal care products are becoming increasingly popular in both local and international markets (Kaliya, 1998). In the 1990s, cosmetics manufacturers began using the term "cosmeceuticals" to describe over-the-counter skin care products with therapeutic benefits. These products use plant-based active ingredients like Alfa-hydroxyl acids, retinoic acid, ascorbic acid, and coenzymes Q-10 (Ubiquinone) to increase skin elasticity, reduce wrinkles, and protect against UV radiation.The herbal cosmetics can be grouped in to following major categories (Pawar and Gaud, 2001). A Herbal cosmetics are beauty products with beneficial effects such as skin healing, smoothing, and enhancement, as well as conditioning, due to their herbal constituents.

a. For enhancing the appearance of the facial skin.
b. For hair growth and care.
c. For skin care, especially in teenager (acne, pimples, sustaining).
d. Shampoos, soap, powder and perfumery etc.
e. Miscellaneous products.

In the future years, skin care products—particularly those used for professional procedures like chemical peels and facial implants that improve appearance—will account for the majority of cosmetic demand within the aforementioned categories (Kokate CK et al., 2003). The primary market need was for anti-wrinkle products. Therapy, sunscreens,

analgesics, hair growth promotion, and micro-circulation enhancement. Creams are semi-solid emulsions that are used topically or mucosal membrane. Cream has relatively low yield value and pseudoplastic flow characteristics.

Material and Methods

Formulation of Herbal Multi-purpose Cream

Preparation of Cream Base- Every component was precisely weighed. After melting beeswax in a porcelain plate, liquid paraffin was poured. Almond oil was homogenized, and then applied to the melted foundation above. After dissolving borax in enough water, the mixture was heated. Drop by drop, the water was vigorously stirred into the greasy area. The necessary consistency was then achieved by allowing the molten bulk to cool (Ahmad et al., 2005).

Preparation of Herbal Multipurpose Cream- The following ingredients were accurately measured and homo-genized separately: aloe, papaya, amla, neem, tulsi, and turmeric. Papaya pulp was crushed and combined with aloe and powdered amla. Neem and tulsi extracts were added to the combination above. The resulting mass was continuously stirred as it was added to the base. After that, perfume and turmeric powder were added.

Evaluation tests for cream

Evaluation of *In-Vitro* Skin Permeation

Determination of Amount of Drug Deposited in Skin- This approach uses a diffusion cell set at 32 ±1 °C and conducts the in vitro drug release research in two phases. 10 ml of PBS (pH 6.5) is utilized as the receptor in the first step medium for ten hours, and in-vitro skin penetration is done. After 10 hours, the donor compartment is cleaned five times using warm receptor fluids (45°C). The second stage operates without a donor phase and employs 50% v/v ethanol as the receptor solution for a further 12 hours.

The carrier system that may have penetrated and deposited in the tissue will be disrupted by the ethanolic receptor's diffusion into the skin at this step, releasing both carrier-bound and free drug for collection in the receptor.

Evaluation of Skin Sensitivities Open Epicutaneaous Test- Six to eight guinea pigs' shaved flanks are covered with 0.025mm of various concentration across a 2 cm sq. area to assess the irritancy profile. Test locations are aesthetically. Assessed 24 hrs after the test solutions were applied on the erythema. The dosage at which 25% of animals do not respond (minimum irritating concentration) is identified. According to Previously, topical dose forms were made to be used topically to address local conditions. It may or may not be advantageous for there to be some penetration under the stratum corneum. Topical products, as opposed to trans-dermal formulations, are not meant to cause significant systemic circulation absorption. A topical medication that

1. reaches a concentration in the target tissue high enough to produce required pharmacological response;
2. exhibits a systemic toxicity level that is acceptable; and
3. leaves the skin in an inactive state.

Draize Test- The first predicted sensitization test approved by regulatory bodies was the Draize Sensitization Test (DT). Twenty guinea pigs had one flank shaved, and 0.05 on day 0, the anterior flank receives an injection of

milliliters of a 0.1% test material solution in saline, paraffin oil, or polyethylene glycol. Through day 20, each animal receives a fresh injection of 0.1 ml of the solution every other day (challenge). Twenty untreated flanks are shaved, and 24 and 48 hours after injection, 0.05 of the test solution is visually assessed. According to Tortora et al. (1993), a favorable reaction is one that is more or more strongly erythematous than that of controls.

Results and Discussion

In order to create a novel recipe for herbal multipurpose cream, the herbal multi-purpose cream was made and assessed. Before the herbs were chosen and gathered from various sources in order to formulate the herbal cream. Four formulas in all were created for this investigation. The comprehensive breakdown is displayed in Table No. 5. Several physicochemical criteria, such as appearance, emulsion type, color and odor stability, extensibility, pH, texture, feel upon application, particle contamination, spredability, etc., were assessed for the formulations. An ideal formulation was assessed in light of the intended physicochemical characteristics.

The following test settings were applied to the chosen formulation:

1. Appearance
2. Type of emulsion
3. Stability of color and odor
4. Extrubility
5. Ph
6. Texture
7. Feel upon application
8. Particulate contamination
9. Spredabilit

Table-1 Medicinal Herbs Used for the Beuty Therapy (Abdolhossein et al., 2003)

Sr. no	Action	Medicinal herbs
01	Antiseptic	Amba haldi, neem, tulsi, Liquorice, Bavachi, citrus peel
02	Anti-inflammatory	Chandan, Khus, rose, Lodhra, Aloe, Raktchandan, Anantmul
03	Antiwrinkle	Manjishtha, Papaya, Aloe, Nagarmotha, Ginseng
04	Astringent	Arjun, Harada, Triphala, Manjishtha, Neem
05	Bleaching	Amba haldi, Kachur sugandhi, Aloe, Rakta chandan, Anantmul.
06	Cleansing	Aloe, Papaya, citruspeel, amla, Lemon.
07	Enzyme action	Aloe, Papaya, citruspeel, amla, Lemon.
08	Moisturizers	Aloe, Rose, Khus, Neem.
09	Nutritive	Liquorices, Ashvagandha, Ginseng, nagarmotha, tulsi.
10	To increase circulation	Kachur sugandhi, Nagarmotha, bavachi, Amba haldi.
11	Rejuvenating	Ginseng, papaya, Liquorices, Aloe, Raktachandan, Anantmul

Table-2 Materials used in the preparation of Herbal Multipurpose Cream (Jerajani et al., 2004)

Sr. no	Name of Ingredients	Role of Ingredients
01	Beeswax	Flavouring agent
02	Liquid paraffin	Preservative
03	Almond oil	Vehicle
04	Rose oil	Flavouring agent
05	Borax/methyl paraben	Preservative
06	Aloe	Antiwrinkle, Cleansing and Moisturizers
07	Papaya	Antiwrinkle, Cleansing and Enzyme action
08	Amla	Antioxidant property and cleansing
09	Neem	Antiseptic, astringent, and moisturizers
10	Tulsi	Antiseptic and nutritive
11	Turmeric	Colouring agent and antiseptic
12	Water	Vehicle

Table-3 Formula for preparation Cream Base

Sr. no	Name of ingredient	Quantity
01	Beeswax	10gm
02	Liquid paraffin	12gm
03	Almond oil	30ml
04	Rose oil	2ml
05	Borax/methyl paraben	01gm
06	Water	q.s upto 100gm

Table-4 Overall formulation design for the Herbal Multipurpose Cream

Sr. no	Ingredients	A	B	C
01	Aloe	25	30	30
02	Papaya	15	25	15
03	Amla	15	10	20
04	Neem	1.5	2.5	4.0
05	Tulsi	2.5	2.5	4.0
06	Turmeric	2.5	4.0	3.0
07	Acacia	5.0	3.0	3.0

Table-5 Evaluation Parameters of all formulations

Sr. no	Evaluation Parameters	A	B	C
01	Appearance	Pale Yellow	Pale Yellow	Orange Colour
02	Type of emulsion	O/W	O/W	O/W
03	Stability of color and odor (After 2 week)	Unstable	Unstable	Unstable
04	Extrubility	Good	Good	Good
05	PH	6.3	**6.5**	**6.4**
06	Texture	Gritty	Smooth	Smooth
07	Feel upon application	Cooling	Cooling	Cooling
08	Particulate contamination	No	No	No
09	Spreadability	Not good	good	good

The goal of the study was to create a herbal multipurpose cream, and the literature review revealed that demand for multipurpose creams is growing daily. Today, amla exhibits antioxidant properties, neem and tulsi have antiseptic, astringent, moisturizing, and nutritive properties, and turmeric has antiseptic and coloring properties. In the current work, an attempt has been made to create a herbal multipurpose cream that contains aloe, papaya, amla, neem, tulsi, and turmeric. A total of four formulations (A, B, and C) were made by varying the proportion of all ingredients of herbal origin, and all four formulations were O/W type of emulsion. possess high extensibility and a cooling feeling following application. There was no particle contamination in any of the formulations. The color of Formulation A was light yellow and after two weeks, it became unsteady. Its texture was grainy, its spreadability was poor, and its PH of A was determined to be 6.3. Formulation B was unstable after two weeks and had a pale yellow color. Its texture was smooth, its spreadability was adequate, and its PH of B was determined to be 6.5. After two weeks, formulation C, which had an orange hue, remained steady. The pH of C was determined to be 6.4, and it had a smooth texture with excellent spreadability.

Conclusion

The successful development of a stable herbal multifunctional cream was determined. It was discovered that Formulation C worked best with in terms of its use and exhibiting improved product stability. Its usage may be the reason for this formulation's exceptional performance and stability.

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 Potential of *Ocimum gratissimum* (Clove Basil)

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Abstract- The aim of this study was to evaluate the antimicrobial activities of the aqueous and alcoholic (ethanol and acetone) extracts of *Ocimum gratissimum* leaves against different bacterial and fungal strains. Agar well diffusion method has been used to determine the antimicrobial activities of different plant extracts against Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), and fungi (*Candida albi-cans, Penicillium crysogenum, Asper-gillus niger and Aspergillus flavus)*. The extracts exhibited both antibacterial and antifungal activities against tested microorganisms. The plant leaves were tested positive for alkaloids, saponins, flavonoids, tannins and other tested phytochemicals in a preliminary phytochemical screening analysis. This study showed that the phytochemicals of leaf extract of *Ocimum gratissimum* have antimicrobial properties.

Key words: *Ocimum gratissimum,* antimicrobial, phytochemical screening

Introduction

Medicinal plants play a vital role in the treatment and prevention of various diseases and their promotion and there is growing interest in the search for new drugs from natural resources (Ullah et al., 2018). Medicinal plants offer a substantial opportunity as they contain various bioactive chemical constituents (phytochemicals) that can act as antimicrobial agents.

Ocimum gratissimum (clove basil) as the medicinal herb. Basil is the main ingredient of pesto sauce but is also used to flavour other sauces and soups. Different parts including the leaves, stems, flowers, roots, seeds, and even the whole plant are useful. The seeds are edible, and when soaked in water become mucilaginous. The leaves can be eaten as a salad. Basil is widely used in traditional medicine. It is used in Ayurveda and in traditional Chinese medicine for treating digestive system disorders, such as stomach ache and diarrhoea, kidney complaints, and infections. *Ocimum gratissimum* belongs to the group of plants known as spices. *Ocimum gratissimum* commonly known as clove basil

belonging to family Lamiaceae is an important aromatic and medicinal plant existing wild or cultivated in various tropical and subtropical parts of the globe. The plant possesses two unique features firstly it contains essential oil with diversity in chemical composition and water stress tolerance capacity. Plant oil is considered the Mother Nature's chemical factory (Tanko et al., 2008; Akara et al., 2021) shows a unique composition of alcohols, aldehydes, ketones, ethers, esters, lactones, oxides, peroxides (Venuprasad et al., 2014) tannins (Irondi et al., 2016) and flavonoids (Benitez et al., 2009; Melo et al., 2019). It is also known as African basil, exhibits significant antimicrobial activity, particularly against bacteria and fungi.

Material and Methods

Collection of plant material

The leaves and flowers of. *Ocimum gratissimum* were collected from the Botanical Garden, Patanjali yoga peeth, Haridwar, Uttarakhand. The leaves were washed with distilled water and subjected to crude extract preparation at room temperature for further studies.

Preparation of crude extract

The leaves of the plant *Ocimum gratissimum* were used for crude extract preparations separately for phytochemical and antimicrobial analysis. 25 mg and 50 mg (fresh weight) of leaves of *Ocimum gratissimum* were homogenized in 3-5 folds of aqueous and organic solvent (50% ethanol and acetone) separately in pestle and mortar at room temperature. The extracts were filtered through sterilised whattmaan filter paper and filterate was centrifuged at 10000 rpm at 4^0C for 5 minutes. The clear supernatant was used as crude extract for phytochemical analysis and antimicrobial testing. Extracts were kept at 4°C for the further analysis.

Isolation, purification and identify-cation of microbial strains from soil and spoiled fruits

The microbial strains were isolated from soil by serial dilution method. (Cappuccino and Sherman, 2005; Aneja, 2009) and by some spoiled fruits. The isolated colonies of *Penicillium crysogenum, Aspergillus niger and Aspergillus flavus, Candida albicans* were streaked onto the fresh SDA plates while *E. coli*, *Bacillus* sp., *Staphylococcus aureus* and *Pseudomonas s*p. were streaked onto the fresh NAM plates.

Screening of antimicrobial activity: Antimicrobial activity of plant extract was screened by agar disc diffusion method on SDA plate for fungal strains and NAM plates for bacterial strains and measure the zone of inhibition in mm. (Khokra *et al*, 2008). Sterile agar plates were prepared, and the fresh standardized broth culture of each test organism was inoculated. Subsequently, a sterile cork borer of 5 mm diameter was used to punch 4 wells on each of the plates. sixty micro-litres (60 µl) of each of the varying concentrations (25mg/L, 50 mg/mL) of the test extracts was dropped into three of the wells, while the remaining one well was filled with sterile distilled water to serve as the control. The plates were then left for one hour to allow the contents in the well to diffuse into the agar, followed by incubation at 37^0C for 24 hours for NAM plate while 28^0C for SDA plate. The diameter zones of inhibition were then measured in millimetres (mm).

Phytochemical screening of different parts of *Ocimum gratissi-mum*

Phytochemical tests were carried out in the aqueous and organic extract of *Ocimum gratissimum* using standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), Omoya and Akharaiyi (2012), Jyothiprabha and Venkatachalam (2016), Harborne and Williams (2000).

Screening for Tannins

5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One ml ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

Screening for Terpenoids

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for Flavonoids

A pinch of zinc dust was added to 2 ml of extract followed by the addition of 1 ml concentrated HCl. Appearance of pink colour indicate the presence of flavonoids

Screening for Saponins

5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence for the presence of the saponins.

Screening for Anthraquinone

One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in isopropyl alcohol. After two minutes, formation of red colour indicated the presence of anthraquinone.

Screening for Glycosides

5 ml extract was mixed thoroughly with 1 ml of glacial acetic acid and 1 ml of 5% $FeCl_3$ solution in a test tube. 1ml of concentrated sulphuric acid was added to the above reaction mixture carefully along the side of test tube. Development of green-blue colouration shows the presence of glycosides. (Kellar- Kiliani test).

Screening for Phytosterols

(Salkowski Test) 5 mL of extract mixed with 2 ml of chloroform then 2 mL of concentrated H_2SO_4 were added into it. Red colour was observed in lower layer of chloroform indicates the presence of phytosterols.

Screening for Alkaloids

Mayer's test- 1 ml of every extract mix with a drop of Mayer's chemical agent is additional by the aspect of the test tube. A creamy or white precipitate indicates the presence of alkaloids.

Screening of Phenols- Few drops of 10% lead acetate solution were added to 5ml of test solution. Formation of white precipitates indicates the presence of phenol in the test solution.

Results and Discussion

Various extracts, including ethanolic extracts, have shown effectiveness against a range of pathogens. This study was to evaluate the antibacterial activities of the aqueous and the alcoholic extracts of *O. gratissimum* leaves against different bacterial strains. (Table:1) The antibacterial activity against gram positive and gram-negative strains was performed using the agar diffusion technique. The inhibition zone was determined through the microtiter broth

dilution method. Studies have shown *O. gratissimum* effectiveness against *Bacillus, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Aqueous and ethanolic extracts of the leaves of *O. gratissimum* were active against *E. coli*, *P. aeruginosa*, and *S. aureus*, and the aqueous extract of the leaf was active against *P. aeruginosa* at the investigated concentrations. The extract of *O. gratissimum* was more effective in inhibiting *E.coli than P. aeruginosa* bacteria. All fractionated extracts showed the highest inhibition zone diameter against gram positive bacterial strains.

Table-1 Study of antibacterial activity of different leaves extracts of *O. gratissimum* samples on selected bacterial isolates

S.No.	Test Organism (Bacterial Strains)	Inhibition zone in diameter (mm± SD)					
		25 mg/L			50mg/L		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	***E. coli***	15±0.22	20±0.28	19±0.25	17±0.29	25±0.34	20±0.28
2	***P. aeruginosa***	12±0.21	21±0.23	18±0.27	19±0.26	28±0.28	19±0.26
3	***S. aureus***	18±0.36	31±0.26	27±0.32	22±0.24	27±0.38	28±0.23
4	***Bacillus subtilis***	19±0.32	28±0.29	26±0.30	21±0.25	29±0.29	25±0.33

The antimicrobial activity of the leaves extracts of *O. gratissimum* were studied in different concentrations (25 and 50 mg/ml) on fungal strains (*Penicillium crysogenum, Aspergillus niger, Aspergillus flavus, Candida albicans*). The extracts of *O. gratissimum* as shown antifungal activity against *Candida albicans* and other fungal pathogens (Table-2). The anti-fungal activities of the extracts increased linearly with increase in concentration of extracts (mg/ml). The growth inhibition zone measured ranged from 23 to 38 mm for all the sensitive fungal strains.

Table-2 Study of antifungal activity of different leaves extracts of *O. gratissimum* samples on selected fungal isolates.

S.No.	Test Organism (Fungal Strains)	Inhibition zone in diameter (mm)					
		25 mg/L			50mg/L		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	***Candida albicans***	25±0.32	23±0.28	25±0.25	27±0.29	28±0.34	23±0.28
2	***Penicillium crysogenum***	22±0.25	27±0.23	28±0.27	29±0.26	29±0.28	29±0.26
3	***Aspergillus niger***	28±0.36	34±0.26	29±0.32	32±0.24	32±0.38	19±0.23
4	***Aspergillus flavus***	29±0.32	38±0.49	31±0.30	30±0.25	29±0.29	22±0.33

The existence of phytochemicals with therapeutic potential was checked in the leaves of *O. gratissimum*. Preliminary phytochemical screening was done using standard techniques. The phytochemical components discovered in the leaves of *O. gratissimum* included alkaloids, flavonoids, saponins, and tannins. Among these, the tannin and flavonoids content

were remarkably high. The current results could provide a rational support for the traditional use of *O. gratissimum* to treat infections. Cytotoxic qualities, anti-bacterial, anti-viral properties, are credited to the presence of saponin (Bailly & Vergoten, 2020). The Flavonoids and phenols are major compounds that act as antioxidants or free radical scavengers (Bhandary *et al.,* 2012). Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth while the phenolic compound, tannin, terpenoid, flavonoids possess an ant-helmintic property so the plant Zanthoxylum, Acorus could be used to treat stomach problems (Nath & Yadav, 2016).

Table-3 Screening of phytochemicals in different leaves extracts of *O. gratissimum*.

S.No.	Phytochemicals	Preliminary qualitative Identification					
		25 mg/L			50mg/L		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	Tannin	++	+++	++	++	+++	+++
2	Alkaloids	+	+++	++	+	+++	+++
3	Glycosides	+	+++	++	+	+++	+++
4	Anthraquinone	+	+++	++	+	+++	++
5	Phytosterols	+	++	+	+	++	++
6	Terpenoids	+	++	++	+	+++	++
7	Flavonoids	++	+++	++	++	+++	+++
8	Saponin	-	+	+	+	+++	++
9	Phenols	+	++	++	+	+++	+++

Conclusions

This study suggests the exploration of *O. gratissimum* as sources of natural products for future use in the management of bacterial infections. The findings could also be of commercial interest to both pharmaceutical companies and research institutes. Furthermore, further studies are required to be conducted concerning the botanical preparation of the traditional sources of medicinal plants in various fields, including pharmacology, phytochemistry, ethnobotany and other biological activities associated with drug recovery.

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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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Decoding the Pharmacognostical Blueprint of *Abutilon indicum* Linn.: A Study of Roots and Leaves

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Abstract - Herbal medications are natural products, and their phyto-constituents change with time, geography, processing, and storage. Variations in a herb's gathering, processing, or storage may have an influence on its efficacy profile. Tradition may be utilized as a guide to quality standards since it contains past knowledge about the right gathering and application of most medicinal herbs. This study evaluates the pharmacognostic properties of root and leaf extracts of *Abutilon indicum*. Research indicates a lack of focus on establishing standardized limits for plant roots and leaves. Our goal is to determine WHO standard limits for macroscopic and microscopic studies, including LOD, ash values, and extractive values, to control the quality of crude drugs.

Keywords: *Abutilon indicum Linn.*, Taxonomic classification, Microscopic study, Physicochemical Investigation.

Introduction

Our pharmaceutical field's best buddy is nature. Natural medications work well and don't cause any negative side effects. Commonly known as *Abutilon indicum* (Linn.) sweet (Malvaceae), The perennial plant known as "Country Mallow" can grow up to three meters in height. *A. indicum* is a common weed in the hotter regions of India and the sub-Himalayan region. According to reports, *A. indicum* possesses antibacterial, hepato-protective, hypoglycemic, male contraceptive, and antidiarrheal properties[1]. *Abutilon indicum*'s leaves are cordate, ovate, acuminate, tothed, and occasionally subtrilobate. They may reach a maximum length of 12 cm, with petioles of 3.8 cm stipules with linear, sharp, deflexed pedicles that are 9 mm long and frequently 2.5–5 long auxiliary single jointed very close to the top 12.8 mm long calyx with oval, apisculate middle lobes.

With a diameter of 2.5 cm, the corolla opens in the evening. Long carpals, typically 15-20 with a distinguishing little sharp tip hairy and a final shiny dark brown seed that is thickly minutely scrobiculate, are characteristically hairy of the base filaments of the staminal tube. It's a somewhat common roadside plant that grows in hotter regions of India[2] with the capacity to produce a wide range of chemical components, including flavonoids, proteins, alkaloids, and steroids, glycosides, phytosterols, and phenolic

compounds. Plants are a crucial and vital part of the prescription drug industry. These plants yield carbohydrates, amino acids, saponins, and glycosides[3] which are used to treat a variety of illness including bronchitis, jaundice, toothaches, piles, diabetes, fever, leprosy, cystitis, ulcers, gonorrhea, diarrhea, cough, urine production, and lung disease. They are also used to treat cough, pulmonary TB, mumps, high fever, deafness, and ringing in the ears. In Ayurvedic formulations, the whole herb is used to cure menorrhea, diabetes and hemorrhoids. In rats, A. indicum leaf extracts have hypo-glycemic effects.

Taxonomic Classification[4]

Kingdom	: Plantae
Class	: Magnoliopsida
Order	: malvales
Family	: malvaceae
Genus	: *Abutilon*
Species	: *indicum*

Ayurvedic properties[5]

Rasa	: Madhura
Guna	: Snigdha
Veerya	: Sita
Vipaka	: Madhura
Karma	:Balya,Vatahara,Vrsya

Figure- 1 Abutilon indicum

Pharmacological Potential and Medicinal Use of *A. indicum*

The majority of medications or compounds with minor to considerable pharmacological activity against enormous creatures and illnesses come from plants. Table-1 shows the phytoconstituents and pharmacological action.

Traditionally, the plant is used in inflammation, piles, gonorrhea treatment and as an immune stimulant. Root and bark are used as aphrodisiac, anti-diabetic, nervine tonic, and diuretic. Seeds are used as aphrodisiac and in urinary disorders[6] along with other therapeutic applications. Ayurvedic Pharmacopoeia of India indicates the use of the root in gout, polyuria and haemorrhagic diseases.

Traditional Claims

Nearly every portion of Atibala has therapeutic value and has long been used to treat a range of illnesses. The plant's roots are used to treat urethritis, chest infections, and diuretics. The root infusion is recommended as a cooling remedy for fevers and is thought to be helpful in leprosy, strangury, and haematuria. The leaves have been shown to help with ulcers and to soothe sore areas of the body. The leaves' decoction is used internally to treat bladder irritation and to treat toothaches and sore gums. The bark has diuretic, astringent, alexeteric, febrifuge, and anthelmintic properties. The seeds are used to treat chronic cystitis, gleets, gonorrhea, piles, laxatives, and expectorants[7]. The herb has historically been used to treat inflammation, piles, gonorrhea, and as an immune booster. Bark and root are used as a diuretic, nervine tonic, anti-diabetic, and aphrodisiac. Seeds are used to treat urinary issues and as an aphrodisiac. The Ayurvedic

Pharmacopoeia of India lists the root's usage in treating hemorrhagic disorders, polyuria, and gout in addition to other medicinal uses.

Material and Methods

Collection and Authentication of Plant material

For Root and Leaf of *Abutilon indicum* Linn. (FamilyMalvaceae), plants were collected in the month of September from village Shankerpur, Distt. Dehradun (Uttarakhand). It was authenticated by Botanical survey of India, Allahabad U.P., India.

Drying of Plant material

After being detached from the *Abutilon indicum* plant, the root and leaf sections were given a water wash. After 30 minutes in the sun, it was allowed to dry for 15 days at room temperature in the shade, coarsely ground and then sieved through a 60 screen to ensure uniform particle size.

Macroscopic study

Macroscopic features of the leaf and root were observed using both the unaided eye and a magnifying glass. We detected the root part's shape, size, colour, odour, taste, surface features, fractures, etc[8,9].

Microscopical study

a. **T.S. of the Leaf-** To completely remove the chlorophyll, the leaf fragments were cooked in a test tube with chloral hydrate for a number of minutes. Both the dorsal and ventral sides of the leaf could be inspected.

   A sharp blade was used to cut the leaf portion, including the midrib, to create a transverse slice. Safranin was then used to stain the lignified tissue red.

b. **T.S. of the Root-** The hard drug sample was softened by boiling the root portion, which had a diameter of 3 to 5 mm and a length of around 2.5 cm in water for a few minutes. At this point, fine sections were taken using the softened samples. A cylindrical section of the root was cut perpendicular to the long axis and along its radial plane to create transverse sections[10, 11].

Physicochemical Investigation

Loss on drying

The mass loss as a percentage of weight is known as loss on drying. Water and volatile materials in the crude medication are determined by drying loss. Crude drugs will inevitably include moisture, which needs to be removed as much as feasible. A tarred glass petridish with around 2g of precisely weighed powdered medication was taken. The powder was dispersed uniformly. The sample was dried for two hours at a temperature between 100 and 105^0C while the petridish remained open in a vacuum oven, until a consistent weight was noted. After cooling to room temperature in a desiccator, it was weighed and noted in %. The following formula was used to determine the drying loss.

% Alcohol soluble extractive value = 80 X (Wt. of residue)

Water soluble extractive value

The procedure as above was followed using chloroform water I.P. instead of alcohol. **% Loss on drying = Loss in weight of the sample/ Weight of the sample X 100**

Determination of Ash values

Ash values are useful for assessing a crude drug's quality and purity, particularly when it is powdered. The goal of ashing vegetable medications is to eliminate any organic matter residue that may otherwise obstruct an analytical result.

i. **Total Ash value-** It is the entire quantity of material that is left over after ignition. This comprises "physiological ash," which comes from the actual plant tissue, and "nonphysiological ash," which is the leftover material. After placing the drug (about 2 grams) in a crucible and heating it to 60^0 Celsius for two hours, the total ash value was determined. The percentage yield of the ash value was then computed using the air-dried medication as a reference.

ii. **Acid-Insoluble Ash**- The residue left over after the remaining insoluble material is ignited and the complete ash is boiled with diluted hydrochloric acid. 25 milliliters of diluted hydrochloric acid were added to the ash and boiled for five to ten minutes. The insoluble material was then collected in a crucible on ash-free filter paper, burned, and weighed. The % yield of acid-insoluble ash was then computed using the medication that had been air-dried.

% Acid insoluble ash value = Wt. of acid insoluble ash/ Wt. of crude drug taken X 100

iii. **Water soluble Ash value**- Boiled the total ash for five minutes with 25 ml of water; collected the soluble matter in a crucible, ignited, and weighed. Calculated the percentage or water soluble ash with reference to air dried drug.

% water insoluble ash = wt. of total ash – wt. of water insoluble ash / wt. of crude drug taken X 100

Determination of extractive values

Determination of extractive values is useful for evaluation of crude drug. It gives idea about the nature of the chemical constituents present in a crude drug.

Alcohol soluble extractive value

In a stoppered flask, macerate 5 grams of precisely weighed coarse powdered medication with 100 millilitres of 90% v/v alcohol for 24 hours, stirring the flask often for the first 6 hours quickly passed through filter paper while being cautious not to lose too much alcohol. 25 millilitres of alcoholic extract were dried out in a tray and then weighed. The following formula was used to get the percentage w/w of alcohol soluble extractive in relation to the air-dried medication.

Extractive value = weigt of residue /weight of crude drug taken *100

Results and Discussion

Macroscopic characteristics of root of *Abutilon indicum*

1. **Shape -** Cylindrical or slightly straight.
2. **Surface characteristic –** Surface was fissured, longitudinal corru-gations were present on it's surface.
3. **Colour** – Yellowish Brown
4. **Odour** – odourless
5. **Taste** – characteristic

Macroscopic characteristics of Leaf of *Abutilon indicum*

1. **Shape -** oval
2. **Surface characteristic -** Surface was Rough
3. **Colour** – Light Green
4. **Odour** – Characteristic & persistent
5. **Taste** – Slightly Sweet

Histological study of Root

A thin cork of four to seven or more tangentially elongated rectangular cells, cork cambium and at the lenticel regions are followed by two or three layers of secondary cortex layers of thin- walled, nearly cubical or

rectangular cells, with small clusters of calcium oxalate in the majority of cells, and phellogen, which is followed by three to four layers of thin-walled cells of cortex.

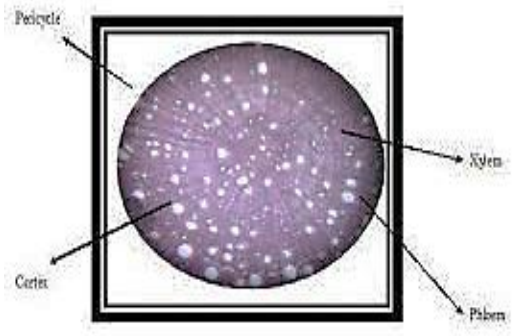


Figure-2 T.S. of *Abutilon indicum* Root

a. Study of Root powder

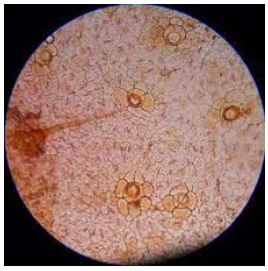
The root powder had a distinct smell, a slightly bitter flavor, and a brilliant yellow to brownish yellow colour. The following reagents were used to observe calcium oxalate crystals, tannins, and starch grains in the root powder.

Figure-3 T.S. of *Abutilon indicum* Leaf Study of Powder

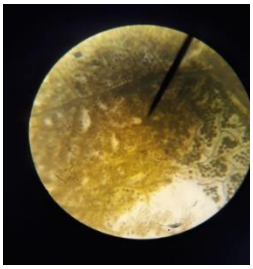
Table-1 Characteristics of Powder microscopy of *Abutilon indicum* Root

S. NO.	Reagents	Observation	Inference
1.	Iodine solution	Blue color	Starch grains present
2.	FeCl3 Solution	Blue black color	Tannins were present
3.	Lectochloral	Observed calcium oxalate crystals	Calcium oxalate crystals Present

[A]- Starch present



[B]- Tannins present



[C]- Calcium oxalate crystals Present

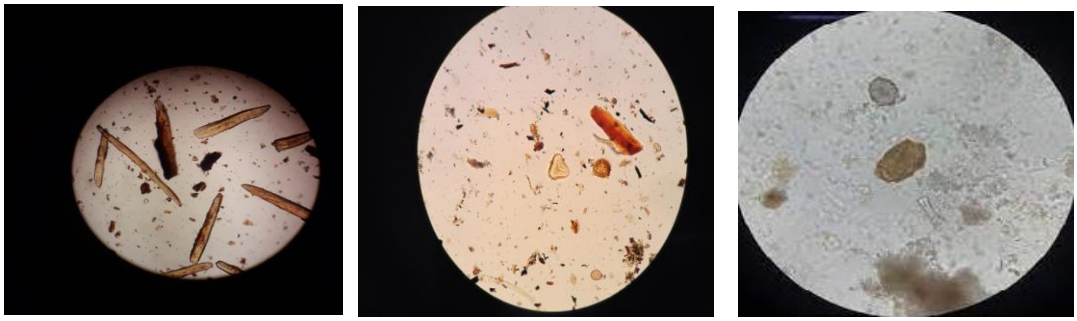
Figure-4 Powder Microscopy of Root

Study of leave's powder

The powdered leaves had a distinctive smell, a slightly sweet flavor, and a brilliant yellow to greenish color. The following reagents were used to observe calcium oxalate crystals, tannins, and starch grains in the leaf powder.

Table-2 Characteristics of Powder microscopy of *Abutilon indicum* Leaf

S. NO.	Reagents	Observation	Inference
1.	Iodine solution	Blue colour	Starch grains present
2.	FeCl3 Solution	Blue black colour	Tannins were present
3.	Lectochloral	calcium oxalate crystals	Calcium oxalate crystals Present



[A]- starch present **[B]- Tannins present** **[C]- Calcium oxalate crystals present**

Figure-5 Powder Microscopy of Leaves

Physicochemical Evaluation for Root

According to WHO guidelines, root powder was examined for physicochemical findings characterisation in this study. The following were discovered: drying loss, ash levels, and extractive values.

Table-3 Physicochemical Parameters of *Abutilon indicum* Root

S. No.	Physical Constants	Results
1.	Loss on drying	5.25%
2.	Total ash value	7.67%
3.	Acid-insoluble ash value	1.%
4.	Water soluble ash value	4.33%
5.	Alcohol soluble extractive value	4.6%
6.	Water soluble extractive value	9.89%

Physicochemical Evaluation for Leaves

According to WHO guidelines, leaf powder was examined for physicochemical characterisation in this study. The following findings were discovered.

Table 4 Physicochemical Parameters of *Abutilon indicum* Leaves

S. No.	Physical Constants	Results
1.	Loss on drying	6.47%
2.	Total ash value	7.53%
3.	Acid-insoluble ash value	1.%
4.	Water soluble ash value	4.31%
5.	Alcohol soluble extractive value	3.81%
6.	Water soluble extractive value	9.74%

Conclusion

The roots and leaves of *Abutilon indicum* were the focus of a pharmacognostic investigation in this study. Several standardized characteristics, including macro-scopic and microscopic analysis, drying loss, ash values, extractive values, etc., were established for the *Abutilon indicum* root and leaf in pharmacognostic research. It was discovered that the crude drug's water-soluble extractive value was greater than its alcohol-soluble extractive value.

Acknowledgement

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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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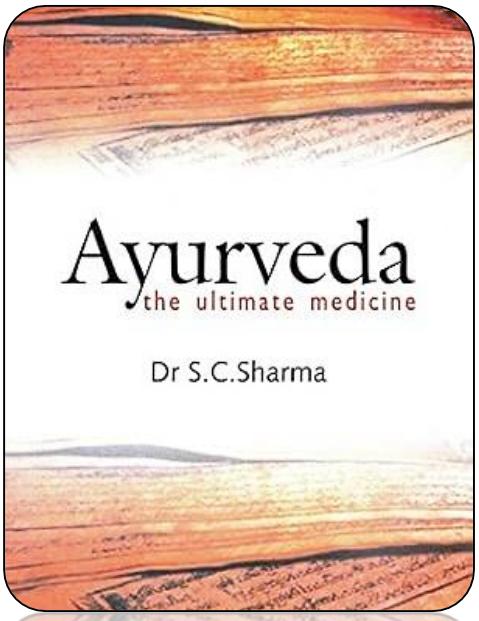
BOOK REVIEW



This review is on the book on Ayurveda, the ultimate medicine authored by Dr. S. C. Sharma, Deputy Advisor, Ministry of Health and Family Welfare and a CSIR member. This book was published for the first time in 2005 and reprinted in 2007, 2009, 2014 and 2017. It contains twenty four chapters.

It introduces the subject on Ayurveda, the ultimate medicine. It is comprised of twenty three chapters, prominent amongst them are chapters on Prakriti and Constitution, Genetics and Ayurveda, Psychiatry, Toxicology, Aphrodisiacs and spiritualism and Ayurveda.

The chapter on Spiritualism and Ayurveda is Captivating. The treatment of Ayurveda takes theories from both the materialistic and spiritual world.

Ayurveda not only deals with the physical body but also describes a complete human being. All diets, herbs, life styles or exercises described in Ayurveda refer to complete *purusa* which was used in the Vedas to mean Ultimate reality Epistemologically, the evolved *purusa* from unmanifested entity was considered to be composed of six elements, five primordial evolved entities and consciousness. The newly evolved *purusa* or *bhutani*, however are made of 24 elements and one conscious entity.

Subsequently, the existence of mind was proved. The mind is responsible for thinking, considerations, ambitions, hypothesis, attention, determination, consideration etc. Control of sensory organs and self-restraint and so on are also action of mind. Beyond this flourishes the domain of intellect. The intrinsic qualities of Ayurvedic aphorism is not disturbed in any way when modern methods are added.

Dr. I. P. Saxena
Editor

About Flowers on the cover page

Antioxidant and Antimicrobial Herbs

Eclipta alba (Bhringraj/ Bhangra)

Eclipta alba, commonly known as Bhrringaraja or Bhangra, belongs to the *Asteraceae* (Compositae) family. Here's its taxonomic classification:

Eclipta alba, commonly known as Bhringraj, is a medicinal plant with a wide range of traditional uses.

i. **Promotes hair growth:** Eclipta alba is considered a potent hair tonic, helping to stimulate hair growth and improve hair quality.
ii. **Prevents hair loss:** It is used to combat hair fall and reduce premature graying.
iii. **Strengthens hair:** Eclipta alba oil is often used to nourish and strengthen hair follicles, making hair less prone to breakage.
iv. Liver:
v. **Hepatoprotective:** Eclipta alba is known to protect the liver from damage and promote healthy liver function.
vi. **Treats liver disorders:** It is used to manage conditions like hepatitis and cirrhosis.
vii. **Improves bile flow:** Eclipta alba is believed to help improve bile flow, which is crucial for digestion and liver detoxification.
viii. **Treats skin disorders:** It is used to treat various skin conditions, including eczema, psoriasis, and urticaria.
ix. **Anti-inflammatory:** Eclipta alba has anti-inflammatory properties, which can help reduce skin irritation and inflammation.
x. **Treats wounds:** Traditionally, Eclipta alba has been used to promote wound healing.

Hemidesmus indicus (Anantamool)

Hemidesmus indicus, **Indian sarsaparilla**, is a species of plant found in South Asia.
Hemidesmus indicus, commonly known as Anantamool or Indian Sarsaparilla, has a long history of medicinal use in Ayurvedic and traditional Indian medicine.
It can be used as:

a. **Blood Purifier**
   It's used to purify the blood and reduce itching, potentially beneficial for skin conditions and sexually transmitted diseases.
b. **Anti-inflammatory**
   It's known for its anti-inflammatory properties, which can be helpful in treating conditions like rheumatism and arthritis.
c. **Diuretic**
   The plant is believed to increase urine production, aiding in fluid retention and potentially relieving symptoms like swelling and bloating.
d. **Urinary Disorders**
   It's used to treat various urinary problems, including painful urination, scanty urine, and kidney infections.
e. **Skin Diseases**
   Traditionally used for skin conditions like leprosy and dry skin with rashes.
f. **Fever**
   It's considered a fever-reducing agent in traditional systems of medicine.

Ocimum basilicum (Sweet Basil)

Ocimum basilicum (basil) has been used in traditional medicine for a variety of ailments, including digestive issues, respiratory problems, and infections. It's known for its antispasmodic, anti-diabetic, and anti-inflammatory properties. Additionally, basil essential oil possesses antimicrobial and antioxidant activities.

Medicinal Uses

i. **Digestive Issues**

Basil is traditionally used to treat stomach pain, nausea, abdominal pain, and diarrhea.

ii. **Respiratory Problems**

It's believed to help with coughs, colds, and bronchitis.

iii. **Infections**

Basil's antimicrobial properties have led to its use for treating infections and even insect bites.

Wedelia chinensis (Pilabhangra)

Wedelia chinensis, a plant widely used in traditional Indian medicine, is known for its various medicinal properties. It is traditionally used to treat viral hepatitis, and its leaves, stems, and fruits have been used in childbirth, bites, stings, fevers, and infections.

Medicinal Properties and Uses

i. **Viral Hepatitis:** Research indicates that Wedelia chinensis is specific in treating viral hepatitis.
ii. **Childbirth:** The plant is used during childbirth, and its leaves and fruits are traditionally used.
iii. **Infections:** Wedelia chinensis is used in the treatment of various infections, including bites, stings, fevers, and infections.
iv. **Kidney Dysfunction:** The leaves are used to address kidney dysfunction.
v. **Dermatological Disorders:** The leaves are used to treat skin problems, including eczema and acne.
vi. **Hair Growth and Dying:** Leaves are used to promote hair growth and dye gray hair.
vii. **Cough and Headache:** The leaves are used to treat coughs and headaches.
viii. **Anti-inflammatory:** The plant's leaves extract is considered a natural alternative to anti-inflammatory drugs.
ix. **Wound Healing:** Wedelia chinensis is used for wound healing.
x. **Antimicrobial:** The plant has been shown to possess antimicrobial properties, which may contribute to its therapeutic effects.
xi. **Antioxidant:** Wedelia chinensis extracts have been shown to possess antioxidant properties.
xii. **Anticancer:** The plant has shown potential anticancer properties.
xiii. **CNS Depression:** The plant's extract has been shown to have CNS depressant activity.
xiv. **Deobstruent:** The decoction of the plant is used as a deobstruent.
xv. **Uterine Hemorrhage:** A decoction of the plant is used in uterine hemorrhage.
xvi. **Mental Stress and Sleep:** Wedelia chinensis is used to treat mental stress and sleep disturbances.

Forthcoming Events

1. International Conference on Agricultural Biotechnology Research (ICABR) - Lagos, Nigeria
August 07, 2025

2. International Conference on Applications of Alternative Medicine and Acupuncture (ICAAMA) - Nicosia, Cyprus
August 21, 2025

3. 73RD International Congress And Annual Meeting Of The Society For Medicinal Plant and Natural Product Research (Ga), Naples (Italy)
August 31-September 3, 2025

4. 11th International Conference & Exhibition on Herbal & Traditional Medicine, Dubai, UAE
September 22-23, 2025

5. International Conference on Applications of Alternative Medicine and Acupuncture (ICAAMA) - Paris, France
September 25, 2025

6. 12th International Conference on Natural, Traditional & Alternative Medicine, Zurich, Switzerland
October 06-07, 2025

7. International Conference on Alternative Medicine and Medical Sciences (ICAMMS) - New York, United States
October 09, 2025

8. Global Congress on Advances in Chemical, Biological & Environmental Sciences - Istanbul, Turkey
November 13, 2025

9. 3rd Traditional Medicine and Plant Science, Bali, Indonesia
November 13-14, 2025

10. International Conference on Molecular Biology, Biochemistry and Biotechnology (ICMBBB) - Honolulu, United States
December 25, 2025

Instruction to Contributors

The Universities' Journal of Phyto-chemistry and Ayurvedic Heights is a *bi-annual* Journal publishing *free of charge* the research work on *herbs, natural products, phytochemicals and indigenous traditional system* on Indian medicines for Human Health Protection, Nutrition and Plant Defence. It is open to research workers in India and abroad. Original articles, Research articles, short communications and Letters to the Editor on relevant topics are published in English. The copyright lies with the publisher. Publication of paper will be in clear understanding that they have not been submitted for publication in any other journal.

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6. It is emphasized that the manuscripts should be neatly typed, double spaced throughout including tables on pages of uniform size with at least 2.5 cms margin on all sides. Author(s) should not break or hyphenate the words. Authors are required to include:
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   ii. The w/w yield of prepared extracts in terms of starting crude material.
   iii. Complete formulation details of all crude drug mixtures.
   iv. The herbarium specimen number of plant(s) studied in case of less well known plants be cited using the collector and collection number (eg. Doe 123) as well as indicating the names of the herbarium institution where it has been deposited.
7. **Keywords:** Authors are requested to assign 3-6 keywords to the manuscript preferably taken from index Medicus of Excerpt Medica Index for abstracting and indexing purposes. They should be

mentioned after abstract and before Introduction of the Manuscript.

8. References to literature are to be arranged alphabetically or with superscript number as given in the manuscript wise and to be placed at the end of the article and referred to by name and year chronologically.
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Uttarakhand State Council for Science & Technology

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Prof. Durgesh Pant
Director General
UCOST

Uttarakhand State Council for Science and Technology (UCOST), Government of Uttarakhand, is an autonomous apex body established with the primary aim of taking science and technology to the common people. It also works to implement the Government of India's science and technology policies at the state level and promote scientific awareness.

Given the importance of science and technology in the overall development of a Himalayan state like Uttarakhand, UCOST plays a pivotal role. Taking into account the unique ecological conditions and economic challenges of the region, UCOST continues to engage in efforts to connect remote areas with mainstream development through science and technology.

The council has contributed significantly to placing Uttarakhand on the national map in the field of science and technology. In collaboration with prominent academic institutions, universities, and national research and technical organizations, it has undertaken and supported numerous multidimensional programs that promote scientific research and innovation. This has enabled the creation of a conducive environment for researchers and scientists within the state.

As part of its mission to foster inclusive development through science and technology, UCOST strives to identify need-based innovations, carry out high-level research, and promote public engagement and awareness for the social and economic benefit of both the state and the nation. It provides policy recommendations to the government and helps identify thrust areas where science and technology can benefit people at the grassroots level.

Currently, UCOST is managing several impactful projects across the state to facilitate science and technology outreach in remote and under served areas. These include initiatives like the establishment of Science City, Regional Science and Innovation Centers, Patent Information Centers, Labs on wheel, Seemant janpad baal vigyaan mahoutsav and R&D initiatives.

The Universities' *Journal of Phytochemistry and Ayurvedic Heights (UJPAH)* is will provide a scholarly platform for researchers and scientists to share and discuss their work on herbs and to conduct scientific assessments of traditional Indian herbal medicines. The goal is to create innovative, evidence-based models grounded in indigenous knowledge systems.

I Extend my best wishes to the *Journal of Phytochemistry and Ayurvedic Heights* for their endeavor in herbal research.

(Prof. Durgesh Pant)
Director General