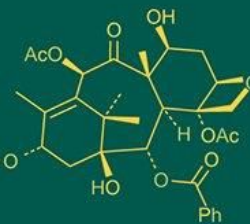
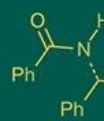
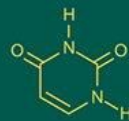
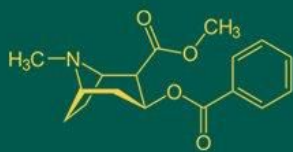


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Phytochemical evaluation of ethanol, acetone extract of leaves and seeds of medicinal plants

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Abstract

The current study focus on the phytochemical evaluation of seven medicinal aromatic plant species: *Azadirachta indica* (leaves), *Terminalia bellirica* (seeds), *Cinnamomum tamala* (leaves), *Mentha arvensis* (leaves), *Terminalia chebula* (seeds), *Eucalyptus globulus* (leaves), and *Cymbopogon citratus* (leaves), which were collected from their natural habitats in the Dehradun district of Uttarakhand. Ethanolic and acetone extracts of these plants tested positive for a various phytochemical constituent, including alkaloids, proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, and terpenoids. The acetone extract of *Mentha arvensis*, as well as the ethanolic extracts of *Cinnamomum tamala*, *Cymbopogon citratus*, and *Terminalia chebula*, showed positive results for all tested phytochemicals. Whereas, the acetone extract of *Cymbopogon citratus* showed negative results for all phytochemicals except tannins, steroids, flavonoids, and carbohydrates. The other plant extracts showedmixresult for different phytochemicals. The presence of these bioactive compounds may explain the therapeutic applications of the seven aromatic plant species studied.

Keywords: Phytochemicals, therapeutic, ethanolic, acetone, medicinal and aromatic plants

1. Introduction

Northern India is rich in biodiversity and possesses a vast wealth of medicinal plants, which have been integral to traditional Indian medicine systems such as Ayurveda, Unani, and Siddha. These medicinal plants have played a crucial role in health care by preventing, managing, and curing various diseases, as well as offering protection against coronary heart disease, cancer, and exhibiting anti-carcinogenic and anti-mutagenic properties. Used since ancient times, they continue to be an essential part of traditional medical practices worldwide. Many modern pharmaceutical drugs (such as aspirin, quinine, morphine) have been derived from plants. Phytochemicals present in these plants demonstrate a wide range of pharmacological and biological activities, including antioxidant properties, and include compounds such as hydrolysable tannins, phenolic acids, flavonoids, alkaloids, carbohydrates, terpenoids, and phytosterols [5, 8, 10]. According to the World Health Organization (WHO), medicinal plants are considered one of the most valuable sources for obtaining a wide range of therapeutic drugs. Around 80% of people in developing nations used traditional medicine, which includes compounds extracted from medicinal plants. Nevertheless, these plants need to be thoroughly studied to better comprehend their characteristics, safety, and effectiveness [3]. Medicinal plants contain natural compounds called phytochemicals that have specific physiological effects on the human body. These bioactive compounds are produced in nearly all parts of the plant, such as the bark, leaves, stems, roots, flowers, fruits, and seeds. Over the past several years, phytochemicals have been widely used in traditional herbal medicine across the world, as both the pharmaceutical industry and scientific researchers have placed increased focus on phytochemical research. These compounds, found in various plant parts, are also used by local communities to treat certain ailments [25]. These are also extensively utilized in the agricultural sector. Phytochemicals are generated by plants through both primary and secondary metabolic processes. Plants produce two types of metabolites: primary and secondary metabolites. Primary metabolites are essential for the growth and development of the plant. Secondary metabolites hold significant economic value and are used in the manufacture of pharmaceuticals, flavors and fragrances, dyes and pigments, pesticides, and food additives.

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A large number of drugs derived from secondary metabolites are either slight synthetic alterations or replicas of these naturally occurring compounds ^[10]. Understanding the chemical components of plants is important, as this knowledge can be valuable for the synthesis of complex chemical compounds ^[15, 16]. Keeping in view, the present study involved a qualitative analysis of phytochemicals in various plant parts, such as seeds and leaves, from seven different plant species using standard testing methods. These seven medicinally significant plant species from the northern region of India include: *Azadirachta indica* (Neem), *Terminalia bellirica* (Baheda), *Cinnamomum tamala* (Tejpatta), *Mentha arvensis* (Pudina), *Terminalia chebula* (Harad), *Eucalyptus globulus* (Eucalyptus), and *Cymbopogon citratus* (Lemongrass).

plants — *Azadirachta indica* (leaves), *Terminalia bellirica* (seeds), *Cinnamomum tamala* (leaves), *Mentha arvensis* (leaves), *Terminalia chebula* (seeds), *Eucalyptus globulus* (leaves), and *Cymbopogon citratus* (leaves) were collected from their natural habitats in the Dehradun district of Uttarakhand. The leaves were washed thoroughly under running water, and all plant materials, including seeds, were left to dry naturally in the shade. Mature seeds were also cleaned to eliminate any dirt or debris and then air-dried. Afterward, they were dried in an oven at below temperature 100 °C, to make them suitable for grinding. Once the drying process was complete, the plant materials were ground using a grinder, and the powder was sieved through a 20-mesh screen. The resulting powder was stored in a clean, dry, and cool place in a properly labelled plastic container for later use.

2. Materials and Methods

2.1 Plant sample collection: Fresh parts of seven medicinal



Fig 1: (A-B) *Eucalyptus globules* (leaves), (C) *Terminalia bellirica* (seeds), (D-F) *Azadirachta indica* (leaves), (G) *Cinnamomum tamala* (leaves), (H) *Mentha arvensis* (leaves), (I) *Terminalia chebula* (seeds), (J-K) *Cymbopogon citratus* (Lemongrass) and (L) Dry leaves of *Azadirachta indica*.

2.2 Preparation of plant extracts

2.2.1 Solvent extraction

The crude plant extract was obtained using the Soxhlet extraction technique. Approximately 100 grams of the powdered material was measured using a digital balance. The ground plant material was then evenly placed into a thimble and extracted separately with 500 ml of ethanol and acetone as solvents. The extraction process was carried out for 24 hours or until the solvent in the siphon tube of the extractor turned colorless ^[19]. The resulting crude extracts were concentrated using a rotary evaporator at 45 °C under reduced pressure, then filtered through Whatman No. 1 filter paper. The filtrate was subsequently used for phytochemical analysis ^[14].

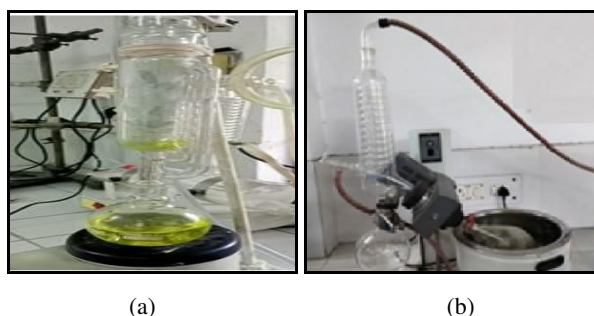


Fig 2: (a) Soxhlet Extraction of *Azadirachta indica* (leaves), (b) Distillation and purification of *Azadirachta indica* (leaves) by Rotary evaporators equipment.

2.3 Phytochemical Analysis

All these extracts were subjected to qualitative analysis to detect the presence of various phytochemical constituents, including steroids, alkaloids, flavonoids, phytosterols, and saponins, using the following standardized methods^[2, 7, 8, 11, 19, 24].

2.3.1 Test for flavonoids

Alkaline reagent test

The crude extract was mixed with 2 ml of a 2% sodium hydroxide solution. A bright yellow color appeared, which turned colorless upon the addition of a few drops of diluted acid, indicating the presence of flavonoids.

Shinoda test

The crude extract was combined with a few pieces of magnesium ribbon, and concentrated hydrochloric acid was added gradually. A pink to scarlet coloration appeared after a few minutes, indicating the presence of flavonoids.

2.3.2 Test for saponins

Foam test

A small quantity of the extract was mixed with 2 ml of sodium bicarbonate and combined with distilled water, then the mixture was shaken vigorously. The formation of froth in the test indicates the presence of saponins.

2.3.3 Test for glycosides

Liebermann's test

The crude extract was combined with 2 ml each of chloroform and acetic acid. The mixture was then cooled on ice. Concentrated sulfuric acid (H_2SO_4) was carefully added. A color transition from violet to blue to green indicated the presence of a steroidal nucleus, i.e., the glycone part of the glycoside.

Salkowski's test

The crude extract was mixed with 2 ml of chloroform, followed by the careful addition of 2 ml of concentrated sulfuric acid. The mixture was gently shaken. The appearance of a reddish-brown coloration confirmed the presence of a steroidal ring, i.e., the glycone portion of the glycoside.

2.3.4 Test for proteins

Millon's test

When the crude extract was treated with 2 ml of Millon's reagent, a white precipitate formed, which turned red upon mild heating, indicating the presence of proteins.

Ninhydrin test

When the crude extract was heated with 2 ml of a 0.2% Ninhydrin solution, a violet coloration developed, indicating the presence of amino acids and proteins.

2.3.5 Test for carbohydrates

Fehling's test

Equal volumes of Fehling's reagent A and B were combined, and 2 ml of the mixture was added to the crude extract, followed by gentle boiling. The formation of a brick-red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's Test

When the crude extract was mixed with 2 ml of Benedict's reagent and heated, a reddish-brown precipitate was formed, confirming the presence of carbohydrates.

Molisch's Test

The crude extract was treated with 2 ml of Molisch's reagent, and the mixture was shaken thoroughly. Subsequently, 2 ml of concentrated sulfuric acid was carefully added along the side of the test tube. The appearance of a violet ring at the junction of the two layers indicated the presence of carbohydrates.

2.3.6 Test for tannin

This method is based on the use of standard traditional procedures as described by ^[8]. A portion of 0.5 g of the sample was accurately weighed and stirred with 10 ml of distilled water, followed by filtration. To 2 ml of the resulting filtrate taken in a test tube, a few drops of 1% ferric chloride solution were added. The formation of a blue-green precipitate confirmed the presence of tannins.

2.3.7 Test for Alkaloids

Mayer's test

2 ml of the extract were treated with two drops of Mayer's reagent (a solution prepared by dissolving mercuric chloride and potassium iodide in distilled water). The appearance of a yellowish precipitate indicated a positive result for alkaloids.

Dragendorff's test

The procedure described ^[8] was followed for alkaloid detection. About 0.5 g of the sample was dissolved in hydrochloric acid and filtered. To 2 ml of the filtrate, Dragendorff's reagent (a solution of potassium bismuth iodide) was added. The formation of a reddish precipitate confirmed the presence of alkaloids. This method is known as Dragendorff's test.

Hager's reagent test

To 2 ml of the filtrate, Hager's reagent (prepared by dissolving 1 g of picric acid in 100 ml of water) was added. The formation of a yellow precipitate confirmed the presence of alkaloids.

2.3.8 Test for quinones

1 ml of the extract was mixed with 2 ml of diluted sodium hydroxide solution. The appearance of a blue-green or red color confirmed the presence of quinones.

2.3.9 Test for phenols

Two milliliters of each crude extract were transferred into separate test tubes, and 2 ml of 5% ferric chloride solution in distilled water was added. The formation of a blue color signified the presence of phenolic compounds in the sample.

2.3.10 Test for terpenoids

The crude extract was dissolved in 2 ml of chloroform and then evaporated until dry. Afterward, 2 ml of concentrated sulfuric acid (H_2SO_4) was added and the mixture was heated for approximately 2 minutes. The appearance of a greyish colour indicated the presence of terpenoids.

2.3.11 Iodine test

The crude extract was combined with 2 ml of iodine solution. The appearance of a dark blue or purple color indicated the presence of carbohydrates.

2.3.12 Test for phytosterols

Salkowski's Test

A few drops of concentrated sulfuric acid (H_2SO_4) were carefully added along the side of the test tube containing 2 ml of crude extract. The appearance of a red coloration indicates the presence of phytosterols.

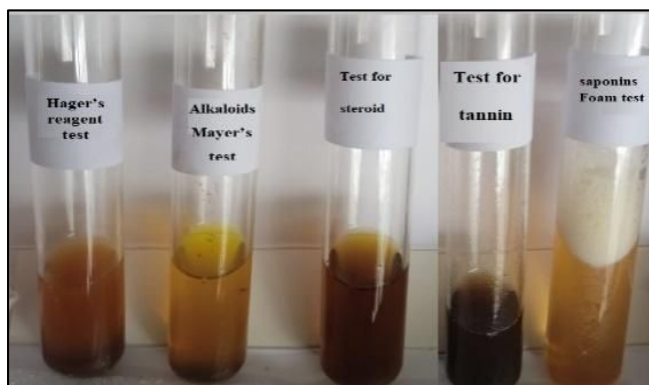


Fig 3: Preliminary qualitative screening for phytochemical

2.4 Determination of Ashcontent

Ash content refers to the inorganic residue left behind after water and organic substances have been eliminated through heating in the presence of oxidizing agents (usually at elevated temperatures). It reflects the total mineral content found in plant materials such as leaves, seeds, etc. [17, 21].

Dry the leaf and seed samples at a temperature of 60-70 °C until a constant weight is achieved. Then, grind the material into a fine powder using a mortar and pestle. Sieve the

powder (typically using a 1 mm mesh) to obtain uniform particle size. Accurately measure 3 gm of the dried leaf and seeds powder and transfer it into a clean, dry, pre-weighed silica crucible. Place the crucible in a muffle furnace and gradually raise the temperature to 500-600 °C. Maintain this temperature for about 6 hours, or until the sample turns white or grey, indicating the complete combustion of organic matter. Carefully remove the crucible and place it in a desiccator to cool to room temperature. Finally, weigh the crucible containing the ash residue and calculate the ash content using the following formula:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

Where:

Weight of ash = Final weight of crucible with ash – Weight of empty crucible

Weight of dry sample = Initial weight of leaf powder taken



Fig 4: Ash content of *Azadirachta indica* (leaf)

3. Results

Seven medicinally significant plant species — *Azadirachta indica* (Neem), *Terminalia bellirica* (Baheda), *Cinnamomum tamala* (Tejpatta), *Mentha arvensis* (Pudina), *Terminalia chebula* (Harad), *Eucalyptus globulus* (Eucalyptus), and *Cymbopogon citratus* (Lemongrass) — from northern India were analysed for their phytochemical constituents and percentage of ash content. Among the seven medicinal plants evaluated for total ash content, the leaves of *Azadirachta indica* exhibited the highest ash percentage, containing 8.23% (Table-1), which indicates a high concentration of minerals. Minerals function as coenzymes and cofactors in various biochemical reactions [9], making it essential to assess the mineral content in the extracts.

The phytochemical profiles of the seven medicinal plants examined are summarized in Table-2. It was observed that all plants contain a considerable proportion of important key phytochemicals, which were easily identified through qualitative analysis. As seen in Table-2, steroids, carbohydrates, phenols, tannins, terpenoids, alkaloids, proteins, and flavonoids were present in the ethanol extracts of all the plants. However, saponins were found to be absent in the leaves of *Azadirachta indica* and the seeds of *Terminalia bellirica*.

Table 1: Analytical analysis of ash content

Sr. No.	Plant sample	Local name	Part used	Ash content %
1	<i>Azadirachta indica</i>	Neem	Leaves	8.23%
2	<i>Terminalia bellirica</i>	Baheda	Seeds	6.1%
3	<i>Cinnamomum tamala</i>	Tejpatta	Leaves	5.42%
4	<i>Mentha arvensis</i>	Pudina	Leaves	2.08%
5	<i>Terminalia chebula</i>	Harad	Seeds	2.9%
6	<i>Eucalyptus globules</i>	Eucalyptus	Leaves	0.73%
7	<i>Cymbopogon citratus</i>	Lemongrass	Leaves	5.1%

Table 2: Preliminary phytochemical analysis of seven medicinal plants studied.

Plant Extract	Phytochemicals									
	Saponin	Tannins	Phenol	Protein	Glycoside	Terpenoids	Carbohydrate	Flavonoids	Alkaloids	Phytosterols
<i>Azadirachta indica</i> (Ethanol)	-	+	+	+	+	+	+	+	+	+
<i>Azadirachta indica</i> (Acetone)	+	+	+	+	+	+	+	-	+	-
<i>Terminalia bellirica</i> (Ethanol)	-	+	+	+	+	+	+	+	+	+
<i>Terminalia bellirica</i> (Acetone)	-	-	+	-	+	+	-	+	-	+
<i>Cinnamomum tamala</i> (Ethanol)	+	+	+	+	+	+	+	+	+	+
<i>Cinnamomum tamala</i> (Acetone)	-	-	+	-	-	+	-	+	+	+
<i>Mentha arvensis</i> (Ethanol)	+	+	+	-	+	+	+	+	+	+
<i>Mentha arvensis</i> (Acetone)	+	+	+	+	+	+	+	+	+	+
<i>Terminalia chebula</i> (Ethanol)	+	+	+	+	+	+	+	+	+	+
<i>Terminalia chebula</i> (Acetone)	-	+	+	-	-	-	-	+	+	+
<i>Eucalyptus globules</i> (Ethanol)	+	+	+	+	-	+	+	+	-	+
<i>Eucalyptus globules</i> (Acetone)	+	+	+	-	-	+	+	+	+	+
<i>Cymbopogon citratus</i> (Ethanol)	+	+	+	+	+	+	+	+	+	+
<i>Cymbopogon citratus</i> (Acetone)	-	+	-	-	-	-	+	+	-	+

Where; + Positive, -Negative

4. Discussion

The medicinal plants used in this study all contain phytochemical constituents, and a preliminary phytochemical screening confirmed that these compounds play numerous significant biological roles [22]. Analysis of the plant extracts revealed the presence of various phytochemicals, including phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. Phenolic compounds represent one of the largest and most widespread categories of plant metabolites. They exhibit a range of biological activities, such as anti-apoptotic, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic effects, cardiovascular protection, enhancement of endothelial function, and the suppression of angiogenesis and cellular proliferation [6].

Multiple studies have highlighted the antioxidant potential of medicinal plants rich in phenolic compounds [4, 12]. Natural antioxidants primarily originate from plants and are often present as phenolic substances like flavonoids, phenolic acids, and tocopherols [1]. Research shows that alkaloids possess several pharmacological properties, including antibacterial, antiarrhythmic, analgesic [20], and antihyperglycemic effects [18]. Tannins also contribute to the management of non-insulin-dependent diabetes mellitus by promoting glucose absorption and inhibiting fat cell formation [13].

Glycosides have emerged as a diverse group of naturally occurring compounds with broad therapeutic applications. They are characterized by a distinct glycone-aglycone structure. Their roles in addressing a variety of health conditions—such as antibacterial and anticancer effects, along with anti-inflammatory [27], cardiovascular, and neuroprotective benefits—demonstrate their value as important sources of therapeutic agents from medicinal plants.

Saponins exhibit a range of biological effects, including altering cell membrane permeability, lowering serum cholesterol, possessing abortifacient properties, exhibiting immunomodulatory effects, showing cytotoxicity toward cancerous cells, and enhancing the potency of immunotoxins through synergistic mechanisms [23]. Additionally, saponins also shows antidiabetic potential [26]. The findings from this study suggest that the identified phytochemical constituents may serve as the biologically active components, reinforcing the role of these plants as a valuable source of bioactive compounds with significant medicinal potential.

5. Conclusion

Plant-derived secondary metabolites are not directly involved in the essential life processes of plants but play a crucial role in their interaction with the surrounding environment. These bioactive compounds are extensively utilized in both traditional and contemporary medicinal systems. They possess numerous therapeutic and healing properties and are used in the formulation of pharmaceutical drugs. Many modern medications have originated from natural substances extracted from plants. Ongoing research continues to explore secondary metabolites from various plant species due to their potential to combat a wide range of diseases, offering eco-friendly alternatives in the pharmaceutical industry.

In the current study, the selected plants were analysed for the presence of these secondary metabolites, such as alkaloids, terpenoids, phenolic compounds, and flavonoids, which are widely applied in the treatment of numerous ailments. Plant-based metabolites are a vital source for sustainable development and innovation across diverse fields, including the food industry, agriculture, biotechnology, and overall human health and wellness.

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