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Phytochemical screening of selected medicinal plants from the Dakshina Kannada and Udupi Districts in the Western Ghats of Karnataka

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Abstract
Background: Herbal drugs are a potential source of therapeutic agents for the treatment and prevention of various ailments. The plant kingdom is a gold mine of potential drugs, and in recent years there has been increasing awareness about the importance of medicinal plants. Drugs derived from plants are readily available, inexpensive, safe, and effective, and they rarely have side effects. Understanding the phytochemical profile of different parts of a plant is essential for determining which part to explore for any specific activity, as well as for evaluating possible synergistic effects.
Results: The qualitative phytochemical analysis examined several important phytochemicals such as alkaloids, steroids, terpenoids, flavonoids, carbohydrates, phenolics, tannins, saponins, and anthraquinones. The tests revealed that some phytochemicals are present in certain medicinal plants while others are absent. The plants studied include *Rubia cardifolia, Pongamia pinnata, Alstonia scholaris, Andrographis paniculata, Glycyrrhiza glabra, Cassia fistula, Hedyotis corymbosa, Murraya koenigii, Eugenia jambolana,* and *Picrorhiza kurroa*.
Conclusions: The ten selected medicinal plants are rich sources of secondary metabolites such as alkaloids, flavonoids, terpenoids, and reducing sugars. These secondary metabolites play a vital role in the plants' anti-inflammatory, anticancer, antiviral, antibacterial, and antifungal activities, thereby contributing to their ability to prevent various diseases.

Keywords: Medicinal plants, phytochemical analysis, alkaloids, steroids, terpenoids, flavonoids, carbohydrates, phenolics, tannins, saponins, anthraquinones

Introduction
Western Ghats of India is a great repository of plant wealth and one among the 35 biodiversity hot spots of the world which harbours more than 4500 plant species of medicinal importance. Western Ghats region of Dakshina Kannada and Udupi have a greater ethnic diversity [1]. Herbal drugs are a potential source of therapeutic agents for the treatment and prevention of a wide range of ailments. Numerous studies have emphasized the promise of herbal medicines as a pipeline for novel bioactive molecules. The rich biodiversity of plants makes them a treasure trove for obtaining new compounds that can serve directly as drugs or as lead molecules for developing drugs with different mechanisms of action [2]. The demand for plant-derived raw materials is increasing at a rate of 15% to 25% annually and is expected to exceed US$5 trillion by 2050. In India, the total trade in medicinal plants is estimated at approximately US$1 billion annually [3].
The importance of plants is well recognized, and there has been growing awareness of the value of medicinal plants in recent years. Drugs derived from plants are readily available, relatively inexpensive, safe, effective, and typically have few side effects. The plants selected for medicinal use over thousands of years represent the most promising candidates for discovering new therapeutically effective drugs [4].
Phytochemicals are categorized into primary and secondary constituents based on their roles in plant metabolism. Primary constituents include common sugars, amino acids, proteins, and chlorophyll, while secondary constituents comprise alkaloids, terpenoids, phenolic compounds, and others such as flavonoids and tannins [5]. The concentration of these phytochemicals varies among different parts of the same plant as well as among different plants. The therapeutic efficacy of plants is largely attributed to these compounds, which include alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, and tannins. Each of these secondary metabolites is known to help treat various diseases.

For example, alkaloids exhibit antispasmodic, antimalarial, analgesic, and diuretic activities, while terpenoids possess antiviral, anthelmintic, antibacterial, anticancer, antimalarial, and anti-inflammatory properties. Terpenoids are also recognized for their ability to inhibit cholesterol synthesis and their insecticidal properties, which are useful in the storage of agricultural products. Saponins are known for their anti-inflammatory, antiviral, and cholesterol-reducing properties, as well as their role in plant defence. Phlobatannins possess astringent properties, glycosides have antifungal and antibacterial effects, and phenols and flavonoids are known for their antioxidant, anti-allergic, and antibacterial activities [6]. Researchers have increasingly focused on herbal remedies, and various plants are currently under investigation for their potential therapeutic benefits [7]. Collaborative work in ethnobotany, ethnomedicine, ethnopharmacology, and phytochemical analysis is crucial for advancing research in the field of medicinal plants [8]. Most studies have concentrated on the phytochemical screening of medicinal plants using one or two solvents for extraction [9]. Understanding the phytochemical profile of different plant parts is essential for determining which parts to explore for specific activities and for selecting candidates for synergistic evaluations. Establishing the phytochemical profile at the beginning of any experiment is far more advantageous than randomly selecting plants.

Medicinal plants used for phytochemical analysis

1. **Rubia cordifolia** (Family: Rubiaceae) *Rubia cordifolia*, known as Indian madder, is a flowering plant cultivated for the red pigment derived from its roots.
   Part used for extraction: Root
   Major phytoconstituents: Munjistin, alizarin, mollugin, and rubilactone

2. **Pongamia pinnata** (Family: Fabaceae) *Pongamia pinnata* is a tree native to eastern and tropical Asia, Australia, and the Pacific islands, and it is the sole species in the genus *Pongamia*.
   Part used for extraction: Seeds
   Major phytoconstituents: Karanjin, pongaflavanol, isopongachromene, and glabrachalcone

3. **Alstonia scholaris** (Family: Apocynaceae) *Alstonia scholaris* is an evergreen tropical tree native to southern China, tropical Asia (primarily the Indian subcontinent and Southeast Asia), and Australasia, where it is also used as an ornamental plant.
   Part used for extraction: Bark
   Major phytoconstituents: Isogentialutine, strictosamine, leuconoxine, and dicentrine

4. **Andrographis paniculata** (Family: Acanthaceae) Commonly known as green chiretta, *Andrographis paniculata* is an annual herb native to India and Sri Lanka.
   Part used for extraction: Whole plant
   Major phytoconstituents: Andrograpanin, andrographiside, apigenin and andrographolide

5. **Glycyrrhiza glabra** (Family: Fabaceae) *Glycyrrhiza glabra* is a flowering plant known for its sweet, aromatic root extract, which is widely used for flavouring. It is an herbaceous perennial legume native to Western Asia, North Africa, and Southern Europe.
   Part used for extraction: Bark
   Major phytoconstituents: Isoliquiritin, glabridin, glycyrrhetic acid, and liquiritin

6. **Cassia fistula** (Family: Fabaceae) *Cassia fistula*, commonly known as the golden shower, is a flowering plant in the subfamily Caesalpinioideae of the legume family. In India, it is typically found in the sub-

Himalayan region, the outer Himalayas (from the Indus eastwards to Assam), the Gangetic Valley, central India, the Deccan, and South India.
Part used for extraction: Seed/fruit
Major phytoconstituents: Anthraquinone derivatives such as sennoside-A and sennoside-B, flavanols, and flavanol derivatives

7. **Hedyotis corymbosa** (Family: Rubiaceae) *Hedyotis corymbosa* is a weedy herb widely distributed throughout India.
Part used for extraction: Whole plant
Major phytoconstituents: Oleanolic acid, sitosterol, asperuloside, and asperulosidic acid

8. **Murraya koenigii** (Family: Rutaceae) *Murraya koenigii*, commonly known as curry leaf, is a small tropical to subtropical tree or shrub that typically grows to 6-15 feet tall. It is renowned for its pungent, aromatic leaves, which are an important flavouring in Indian and Asian cuisine. This plant is native to moist forests in India and Sri Lanka.
Part used for extraction: Leaves
Major phytoconstituents: Mahanimbin, girinimbine, mahanine, and nerolidol

9. **Eugenia jambolana** (Family: Myrtaceae) *Eugenia jambolana* is a native Indian tree widely distributed in the Asian subcontinent and other tropical regions. It is a rapidly growing plant that is considered invasive in many parts of the world. Its fruits are consumed by various native birds and small mammals.
Part used for extraction: Seeds
Major phytoconstituents: Quercetin, gallic acid, ellagic acid, and ferulic acid

10. **Picrorhiza kurroa** (Family: Plantaginaceae) *Picrorhiza kurroa* is a plant that grows in the Himalayan mountains and is used in Ayurvedic medicine. Due to overharvesting, it has been driven nearly to extinction.
Part used for extraction: Root
Major phytoconstituents: Cinnamic acid, ferulic acid, vanillic acid, apocyanin, and androcyn

Materials and Methods

Collection of Plant Materials

Fresh parts of ten medicinal plants were collected from various regions in the Dakshina Kannada and Udupi districts of Karnataka

Table 1: Ethnobotanical information of selected medicinal plant species for phytochemical analysis.

Name	Part used	Local name
Rubia cardifolia (RCR)	Root	Manjishta
Pongamia pinnata (PPS)	Seed	Karanja
Alstonia scholaris (ASB)	Bark	Saptaparna
Andrgraphis panniculata (APWP)	Whole plant	Kalmegh
Glycyrrhiza glabra (GGB)	Bark	Licorice
Cassia fistula (CFB)	Bark	Indian Laburnum
Hedyotis corymbosa (HCWP)	Whole plant	Flattop Nille Grass
Murraya koenigii (MKL)	Leaf	Curry leaf tree
Eugenia jambolana (EJS)	Seed	Java plum
Picrorhiza kurroa (PKR)	Root	Picrorhiza

Preparation and Extraction Process

The collected plant materials were sprayed with 70% ethanol and then shade-dried until completely devoid of moisture. Once dried, the plant materials were ground into a fine powder using a mechanical blender. Approximately 100 g of each powdered sample was subjected to extraction using a Soxhlet apparatus. Both non-polar solvents (e.g., hexane) and polar solvents (e.g., methanol) were employed for extraction. Extraction was performed using a Soxhlet hot successive extraction method:

- **Hexane Extraction:** 1 L of hexane was used for 8-12 hours at a temperature of 40-45°C.
- **Methanol Extraction:** 1 L of methanol was used for 44-48 hours at a temperature of 45-50°C.

After extraction, the solution was filtered and concentrated to remove the solvents. The resulting paste was further dried in a vacuum oven at 50°C to eliminate any trace amounts of solvent and was then stored in an airtight container under refrigerated conditions.

Phytochemical testing

Chemical tests

Alkaloids [10]

Test	Observation	Inference
Extract+ mayer's reagent	Cream precipitate	Positive
Extract + Dragendroff's reagent	Red precipitate	Positive
Extract+Hager's reagent	Yellow precipitate	Positive
Extract + Wagner's reagent	Brownish yellow precipitate	Positive

Steroids [11]

Test	Observation	Inference
Extract in CHCl3+ H2SO4 + acetic anhydride	Reddish brown layer at interface	Positive
Liebermann buchard test: Extract + few drops of acetic anhydride, boil, cool. Add H2SO4 along the sides of the test tube.	Green colour	Positive
Salkowski test: Extract + 2ml chloroform+ few drops of H2SO4. Shake well and allow to stand.	Reddish brown layer is formed at the interface.	Positive

Terpenoids [12]

Test	Observation	Inference
Extract+H2SO4+ Add 2, 4 DNP.	Reddish brown colour at interface.	Positive
Extract + chloroform + H2SO4	Reddish brown colour at interface.	Positive

Carbohydrates [11]

Test	Observation	Inference
Extract+ Molisch reagent. Shake well. Add conc.H2SO4 along the sides of the test tube allow it to stand for two minutes.	Violet ring is formed at the junction.	Positive

Flavonoids [13]

Test	Observation	Inference
Extract + few drops of NaOH gives yellow colour. Add few drops of dil. HCl	Yellow colour disappears on addition of HCl	Positive
Extract +few drops of lead acetate solution. Shake well.	Yellow colour	Positive

Phenolics and tannins [14]

Test	Observation	Inference
Extract + FeCl3 solution.	Olive green colour	Positive

Saponins [15]

Test	Observation	Inference
Extract+water. shake	Formation of foam	Positive

Proteins and amino acids [11]

Test	Observation	Inference
Extract + ninhydrin. Shake.	Blue or purple colour	Positive
Extract + biuret reagent, shake	Purple colour	Positive

Glycosides [16]

Test	Observation	Inference
Extract + conc. H2SO4. Allow to stand for 2min	Red colour solution or red colour precipitate.	Positive

Anthraquinones [17]

Test	Observation	Inference
Extract + 5ml of 5% Fecl3 solution + 5ml of dil. Hcl. Heat on water bath for 5 mins. Cool. Shake gently with benzene. Separate the benzene layer and add equal volume of ammonia.	Cherry red or red colour	Positive

SL. No.	Secondary Metabolites	Tests
1	Alkaloids	• Extract+Mayer's Reagent, shake well. • Extract+Dragendroff's Reagent, shake well. • Extract+Hager'sReagent, shake well. • Extract+Wagner's Reagent, shake well.
2	Steroids	• Extract in $CHCl_3$ +H_2so_4+Acetic anhydride • Liebermann-Buchard: Extract+Few drops of acetic anhydride, boil and cool. Add conc. H_2so_4 along the sides of test tube. • SalkowskiTest: Extract+2ml of chloroform few drops of H_2so_4 shake well and allow to stand.
3	Terpenoids	• Extract+ H_2so_4 heat, add 2,4 DNP • Extract +chloroform+ H_2so_4
4	Carbohydrates	Extract + Molish's reagent shake well, add conc. H_2so_4 along the sides of the test tube, allow it to stand for 2 minutes.
5	Flavonoids	• Extract+aq.NaOH, to it add Mg-HCl. • Extract+Few drops of NaOH gives yellow colour. Add few drops of dilute HCl. • Extract+Few drops of lead acetate solution, shake well.
6	Phenolics and tannins	Extract+$FeCl_3$ solution.
7	Saponin	Extract +water, shake well.
8	Proteins and Amino acid	• Extract+Ninhydrinsolution, shake well. • Extract+BiuretReagent, shake well.
9	Glycosides	Extract+con.H_2so_4 allow the contents to stand for five minute.
10	Anthraquinones	Extract+5ml of 5% $FeCl_3$ solution+5ml of dilute HCl and heat on water bath for 5 minutes, cool the solution and shake gently with Benzene. Seperate the benzene layer and add equal volume of ammonia.

Phytochemical test results of hexane and methanol extract of ten medicinal plants

Table 2: Phytochemicals result of hexane extract of ten medicinal plants

Plant name	Alkaloid	Steroids	Terpenoids	Carbohydrates	Phenolics and tannins	Saponins	Protein and AA	Glycosides	Anthraquinones	Flavonoids
RCR	--	++	++	--	--	--	--	++	++	++
PPS	--	--	++	--	++	++	--	--	--	++
ASB	--	--	++	--	--	--	--	--	--	++
APWP	--	++	++	--	++	--	--	--	++	++
GGB	--	++	--	--	++	--	--	--	++	++
CFB	--	++	++	--	++	--	--	++	++	++
HCWP	--	--	++	--	++	++	--	--	++	++
MKL	--	++	++	--	++	--	--	--	--	++
EJS	--	--	--	++	++	++	--	++	++	++
PKR	--	--	++	++	++	--	--	--	++	++

++ present, --absent

Table 3: Phytochemicals result of methanol extract of ten medicinal plants

Plant name	Alkaloid	Steroids	Terpenoids	Carbohydrates	Phenolics and tannins	Saponins	Protein and AA	Glycosides	Anthraquinones	Flavonoids
RCR	++	++	++	++	--	++	++	++	++	++
PPS	++	++	++	--	++	++	--	++	--	++
ASB	++	--	++	++	++	++	--	--	++	++
APWP	++	++	++	++	++	--	--	--	++	++
GGB	++	++	--	++	++	--	--	++	++	++
CFB	++	++	++	--	++	++	--	++	++	++
HCWP	++	--	++	++	++	++	--	--	++	++
MKL	++	++	++	--	++	--	--	--	--	++
EJS	++	--	--	++	++	++	--	++	++	++
PKR	++	++	++	++	++	--	--	--	++	++

++ present, --absent

Results

The phytochemical constituents of the hexane and methanol extracts of the ten medicinal plants are summarized in Tables 2 and 3. Briefly, the results are as follows:

- ***Rubia cordifolia* (root):** Positive for alkaloids, steroids, terpenoids, carbohydrates, saponins, glycosides, anthraquinones, and flavonoids.
- ***Pongamia pinnata* (seed):** Positive for alkaloids, steroids, terpenoids, phenolics, tannins, saponins, glycosides, and flavonoids.
- ***Alstonia scholaris* (bark):** Positive for alkaloids, terpenoids, phenolics, carbohydrates tannins, saponins, anthraquinones, and flavonoids.

- ***Andrographis paniculata* (whole plant):** Contains alkaloids, steroids, terpenoids, carbohydrates, phenolics, tannins, anthraquinones, and flavonoids.
- ***Glycyrrhiza glabra* (bark):** Contains alkaloids, steroids, carbohydrates, phenolics, tannins, glycosides, anthraquinones, and flavonoids.
- ***Cassia fistula* (bark):** Contains alkaloids, steroids, terpenoids, phenolics, tannins, saponins, glycosides, anthraquinones, and flavonoids.
- ***Hedyotis corymbosa* (whole plant):** Positive for alkaloids, terpenoids, carbohydrates, phenolics, tannins, saponins, anthraquinones, and flavonoids.
- ***Murraya koenigii* (leaf):** Contains alkaloids, steroids, terpenoids, phenolics, tannins, and flavonoids.
- ***Eugenia jambolana* (seed):** Positive for alkaloids, carbohydrates, phenolics, tannins, saponins, glycosides, anthraquinones, and flavonoids.
- ***Picrorhiza kurroa* (root):** Contains alkaloids, steroids, terpenoids, carbohydrates, phenolics, tannins, anthraquinones, and flavonoids.

Discussion

Plants produce a wide variety of bioactive metabolites that have been only partially studied. Understanding the physiology, biochemistry, and ecology of secondary metabolism offers an opportunity to breed plants with enhanced protection against microbes and herbivores. In plants, three major pathways are responsible for most secondary metabolites: the shikimate pathway, the isoprenoid pathway and the polyketide pathway. After the formation of the basic skeletons, further modifications result in species-specific compounds.

- **Shikimate Pathway:** This pathway is the major source of aromatic compounds. It occurs in both microorganisms and plants (but not in mammals), making it an attractive target for herbicides and antibiotics because these compounds are unlikely to affect mammalian systems. The phenylpropanoid pathway, a branch of the shikimate pathway, plays a critical role in carbon flux and leads to the formation of lignin, lignans, flavonoids, and anthocyanins. A key enzyme in this pathway is phenylalanine ammonia lyase (PAL), which converts phenylalanine into trans-cinnamic acid via non-oxidative deamination. While some plants have a single PAL enzyme, others possess several isoenzymes.
- **Isoprenoid (Terpenoid) Pathway:** Terpenoids constitute more than one-third of all known secondary metabolites. The C_5 isoprene unit not only forms the basis of terpenoids but is also incorporated into other classes of compounds such as anthraquinones, naphthoquinones, and cannabinoids, furanocoumarins, and terpenoid indole alkaloids.
- **Natural Antioxidants and Tannins:** Natural antioxidants, mainly in the form of phenolic compounds such as flavonoids, phenolic acids, and tocopherols, are predominantly derived from plants [18] (Ali *et al*., Food Res. Int., 2008). Tannins, which bind to proline-rich proteins, interfere with protein synthesis.
- **Other Bioactive Compounds:** The plant extracts were found to contain saponins, which are known to inhibit inflammation [19]. Steroids have been reported to possess antibacterial properties [20], while alkaloids have been associated with medicinal uses for centuries, including cytotoxic effects [21].

Conclusion

The selected ten medicinal plants are rich sources of secondary metabolites, including alkaloids, flavonoids, terpenoids, and reducing sugars. These compounds contribute to the anti-inflammatory, anticancer, antiviral, antibacterial, and antifungal activities observed in these plants. The phytochemical analysis not only confirms the traditional use of these medicinal plants but also highlights their potential in the discovery and development of new drugs. The identification of important phytochemical properties in this study will aid in the design of novel therapeutic agents for various diseases and has significant commercial interest for both research institutions and pharmaceutical companies.

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