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Preliminary phytochemical screening and TLC profile of selected four plants of Tirupati hills in Chittoor district, Andhra Pradesh

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Abstract

India being a rich and varied flora of medicinal plants since the Vedic period. The present study deals with the phytochemical screening and thin layer chromatographic studies of *Polygonum glabrum*, *Canthium dicoccum*, *Ochna obtusata*, *Argyreia nervosa* plants areal parts. Phytochemical screening determination by some chemical tests and thin layer chromatographic study was carried out by using various solvent system of varying polarity of hexane, chloroform, ethyl acetate, acetone and methanol extracts. Phytochemical screening reflects presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts. Thin layer chromatographic studies of the *Polygonum glabrum*, *Canthium dicoccum*, *Ochna obtusata*, *Argyreia nervosa* plants areal parts constituted different colored phytochemical compounds with different R_f values. The chloroform and methanol extracts in the drug is carried out to establish the biomarker compound. The result obtained in present study indicated *Polygonum glabrum*, *Canthium dicoccum*, *Ochna obtusata*, *Argyreia nervosa* plants areal parts are a rich source of natural antioxidants, and provides evidence that solvent extract of *Aerva lanata* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Keywords: *Polygonum glabrum*, *Canthium dicoccum*, *Ochna obtusata*, *Argyreia nervosa*, Phytochemicals, TLC Profile

Introduction

Plant based drugs are one of the most important cultural and traditional parts of the people. Today, most of the world population depends upon plant based drugs for their primary health care needs. World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicines plant. The Ethno-pharmacological approach provides the way for developing new drugs from plants sources, which have historical background. Although modern medicines may be available, due to socio-economical, cultural and historical reasons, these drugs have maintained their importance^[21-32].

Polygonum glabrum Willd. (Polygonaceae) commonly known as 'Attalareel' in Tamil & 'Neeru kanagilul' in Mangalore. *Polygonum glabrum* have been used as folk medicine and as ingredient in various Ayurvedic preparations. Traditionally it is used as Plant juice and rootstock—used in pneumonia, consumption, jaundice, fevers. Leaf—antispasmodic. Used for colic and pungent young shoots are cooked with other vegetables. The leaves are astringent, diuretic, rubefacient and vermifuge. An. An infusion has been used as a treatment for gravel and stomach pains. A decoction of the plant has been used as a foot and leg soak in the treatment of rheumatism^[1-8].

Canthium dicoccum also known as nalla balusu (Telugu), Nallamandharam (Tamil) in India belongs to the family Rubiaceae. The plant is found in Deccan peninsula, Maharashtra southwards, and extending from Bihar eastwards to Assam and Meghalaya. It is an unarmed shrub, grows upto 3m tall. In India the bark is used for fever and is also applied as plasters, decoction of the root is used in diarrhea. Bark powder with sesame oil is used in rheumatic pains. Used in inflammation, during night boiled leaf extract is taken for 2 months^[9].

Ochna obtusata DC. (Family- Ochnaceae). Habit: Small trees up to 8 m tall. Trunk & Bark: Bark greyish, smooth; blaze pinkish. Branches and branchlets: Branchlets terete, lenticellate, glabrous. Leaves: Leaves simple, alternate, distichous; stipules caducous and leaving scar;

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petioles ca. 0.4 cm long, planoconvex, glabrous; lamina 16 x 5 cm, elliptic or elliptic-oblong to obovate, apex acute to rounded, base acute to rounded, margin serrate, shining above, chartaceous, glabrous beneath; midrib raised above; secondary nerves ca. 12 pairs, ascending towards apex; tertiary nerves slender, reticulo-percurrent. Inflorescence / Flower: Inflorescence axillary or lateral racemes; flowers yellow; pedicels up to 2.5 cm long. Fruit and Seed: Drupe, 3-5 distinct drupes seated on the enlarged disk; seeds 1 drupe. Distribution: South Asia; in the Western Ghats, South, Central and Maharashtra Sahyadris. The leaves and roots of *Ochna obtusata* is used for ulcer, asthma and bronchitis^[10]. And also whole plants are used for the treatment of ulcers and sores. In spite of the use of *Ochna obtusata* in traditional medicine and its potential for toxicity, systematic evaluation of its toxic effects is lacking.

Argyrea speciosa (Linn.f.) sweet, invites attention of the researchers worldwide for its pharmacological activities ranging from aphrodisiac to nematocidal activities.^[11-20] *Argyrea speciosa* (Linn.f.) sweet belongs to family Convolvulaceae is a climbing shrub with woody tomentose stem, found mainly in Deccan, Karnataka and East slopes of the West Ghats at an altitude of 900m. It is commonly known as Elephant creeper and in Samudra-sok Hindi. Traditionally, leaves are used by Rajasthani tribes to prevent conception. Seeds of *Argyrea nervosa* found to possess hypotension, spasmolytic, and antiinflammatory activity. Chemical analysis revealed the presence of triterpenoids, flavanoids, steroids and lipids. Roots of *Argyrea nervosa* proved the immunomodulatory activity against the myelosuppressive effects induced by Cyclophosphamide.

Collection of the plant

Polygonum glabrum (Polygonaceae), *Canthium dicoccum* (Rubiaceae), *Ochna obtusata* (Ochnaceae) *Argyrea nervosa* (Convolvulaceae) plants areal parts were collected near Tirupathi hills, Chittoor district of Andhra Pradesh in India. The collected plant material was properly identified by the Botanist Prof. P. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai. He confirmed and authenticated its identity. A voucher specimen has been reserved in the Department of Pharmacognosy, Narasaraopet Institute of Pharmaceutical science, Narasaraopeta.

Preparation of the plant extracts

Polygonum glabrum, *Canthium dicoccum*, *Ochna obtusata*, *Argyrea nervosa* plants areal parts were collected and shade dried. Dried plant material was blended to make homogenous powder. The powdered material was mixed in equal proposal and Extraction carried out by maceration with the Hexane, Chloroform, Ethyl acetate, Ethanol and Methanol. The extract was concentrated in vacuum using rotary flash evaporator and the extracts were concentrated, percentage yield calculated and then subjected to phytochemical screening and TLC profiling studies. The dried extract was properly stored in the desiccators for further experiment and analysis.

Qualitative Phytochemical Screening.^[24-26]

Detection of Carbohydrates

About 100mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch's Test

To 2 ml of filtrate, two drops of alcoholic solution of α -naphthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction indicates the presence of carbohydrates.

Fehling's Test

To 1ml of filtrate was boiled on a water bath with 1 ml each of Fehling's solution A and B. Formation of red precipitate indicates the presence of sugar.

Barfoed's Test

To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugar.

Benedict's Test

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

Detection of Glycosides

For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the hydrolysate was subjected to the following tests.

Borntrager's Test

To 2 ml of filtrate hydrolysate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink color indicates the presence of anthraquinone glycosides.

Legal's Test

About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink color.

Detection of Saponins

Foam or Froth Test

A small quantity of extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

Detection of Alkaloids

About 50 mg of solvent – free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows.

Mayer's Test

To a few ml of filtrate, two drops of Mayer's reagent was added along the sides of the test tube. If the test is positive, it gives white or creamy precipitate.

Wagner's Test

To a few ml of the filtrate, few drops of Wagner's reagent was added along the sides of the test tube. Formation of reddish brown precipitate confirms the test as positive.

Hager's Test

To a few ml of filtrate 1 or 2 ml of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

Dragendroff's Test

To a few ml of filtrate, 1 or 2 ml of Dragendroff's reagent was added. A prominent reddish brown precipitate indicates positive test.

Detection of Proteins and Amino Acids. [33-37]

About 100 mg of extract was dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests for proteins and amino acids.

Millon's Test

To 2 ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins.

Biurette Test

An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicates the presence of proteins.

Ninhydrin Test

About 2 drops of ninhydrin solution was added to 2ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of Phytosterols and Triterpenoids. [28-33]**Liebermann – Burchard's test**

The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the side of test tube. Red, pink or Violet color at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

Salkowski test

Few drops of concentrated sulphuric acid are added to the chloroform extract, shaken on standing, red color to the lower layer indicates the presence of steroids and golden yellow color indicates the presence of triterpenoids.

Detection of Phenolic Compounds and Tannins. [33-36]**Ferric chloride test**

About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

Gelatin test

A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

Lead acetate test

A small quantity of extract was dissolved in distilled water and to this, 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

Alkaline reagents

An aqueous solution of extract was treated with 10% ammonium hydroxide solution – yellow fluorescence indicates the presence of flavonoids.

Shinoda test or Magnesium – Hydrochloric acid reduction

A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. hydrochloric acid (drop wise) were added. If any pink or crimson – red color develops, presence of flavanol glycoside is inferred.

Thin Layer Chromatography. [24-37]

Thin Layer Chromatography of extracts was done by using standard procedures and is mainly used for the detection of the nature of phytoconstituents present.

Thin Layer Chromatography is a very effective technique for the separation of chemical constituents of an extract and for their identification. The history of TLC has been reviewed by various authors. A major breakthrough in this field was the commercial availability of convenient precoated plates in the early 70's Pharmacopoeias are increasingly employing this technique for assessing the quality and purity of compounds of both synthetic and natural origin. TLC profiles developed for an extract from a defined solvent system and other parameters could be used as a fingerprint in comparative qualitative evaluation of herbal drugs. The trend of evaluation by this method is becoming popular in view of its simplicity and reproducibility.

TLC is an important analytical tool in the separation, identification and estimation of different classes of natural products. In this technique, the different components are separated by the differential migration of solute between two phases – a stationary phase and a mobile phase. Here, the principle of separation is adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phase, its preparation and use with different solvents, separation can be achieved on the basis of partition or a combination of partition and adsorption

Preparation of Plates. [27]

100 g of Silica gel-G was weighed and made into a homogenous suspension with 200 ml of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10 x 2, 10 x 5, 20 x 5, 20 x 10 cm etc.). The coated plates were allowed to dry in air, followed by heating at 100 – 105° C for 1 hour, cooled and stored in a dry atmosphere to protect from moisture. Before using, the plates were activated by heating at 100° C for 10 minutes.

Solvent systems used in TLC

Different solvent system [Hexane: Acetic acid (9:1)] solvent system I, In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)], In solvent system V [Hexane: Ethyl acetate: Acetic acid (2:7:1)] used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front of TLC}}$$

Results and Discussion

Table 1: Percentage of Herbal powder mixture using different extracts

S.no	Solvent	Colour of the extract	Yield of the extract (in grams)	Percentage yield
1	Hexane	White	5.020	2.51%
2	Chloroform	Light brown	4.080	2.04%
3	Ethyl acetate	Light brown	2.750	1.37%
4	Ethanol	Light brown	1.720	0.86%
5	Methanol	Dark brown	3.750	1.85%

Table 2: Qualitative Chemical Evaluation of Selected Herbal Extracts

S. no	Chemical Test	Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol
1	Carbohydrates	+	+	+	+	+
2	Glycosides	+	+	+	+	+
3	Saponins	+	+	+	+	+
4	Alkaloids	+	+	+	+	+
5	Proteins And Amino Acids	+	+	+	+	+
6	Phytosterols And Triterpenoids	+	+	+	+	+
7	Phenolic Compounds And Tannins	+	+	+	+	+

Thin layer chromatographic studies

A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used. Thin layer chromatographic studies of the hexane extract of Selected mixed herbal powder Solvent system I [Hexane: Acetic acid (9:1)], 3 spots were visible R_f values 0.20, 0.36 and 0.52. In solvent system II [Hexane: Ethyl acetate: Acetic acid (5:4:1)], 1 spot detected R_f value 0.90. In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], 1 spot detected R_f value 0.90. In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)], 2 spots were visible R_f values 0.07 and 0.81. In solvent system V [Hexane: Ethyl acetate: Acetic acid (2:7:1)], 3 spots were obtained having R_f of 0.09, 0.81 and 0.94.

TLC studies of the Chloroform extract of Selected mixed herbal powder Solvent system I [Hexane: Acetic acid (9:1)], 2 spots were visible R_f values 0.14 and 0.40. In solvent system II [Hexane: Ethyl acetate: Acetic acid (5:4:1)], 3 spots were detected R_f values 0.10, 0.82 and 0.90. In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], 2 spots were detected R_f values 0.05 and 0.90. In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)], 2 spots were visible R_f values 0.09 and 0.78. In solvent system V [Hexane: Ethyl acetate: Acetic acid (2:7:1)], 2 spots were obtained having R_f of 0.18 and 0.94.

TLC studies of the Ethyl acetate extract of Selected mixed herbal powder Solvent system I [Hexane: Acetic acid (9:1)], 2 spots were visible R_f values 0.10 and 0.40. In solvent system II

[Hexane: Ethyl acetate: Acetic acid (5:4:1)], 2 spots were detected R_f values 0.82 and 0.90. In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], 1 spot detected R_f value 0.85. In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)], 2 spots were visible R_f values 0.07 and 0.81. In solvent system V [Hexane: Ethyl acetate: Acetic acid (2:7:1)], 2 spots were obtained having R_f of 0.03 and 0.94.

TLC studies of the Acetone extract of Selected mixed herbal powder Solvent system I [Hexane: Acetic acid (9:1)], 2 spots were visible R_f values 0.16 and 0.44. In solvent system II [Hexane: Ethyl acetate: Acetic acid (5:4:1)], 1 spot detected R_f value 0.82. In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], 2 spots were detected R_f values 0.85. In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)], 2 spots were visible R_f values 0.05 and 0.90. In solvent system V [Hexane: Ethyl acetate: Acetic acid (2:7:1)], 2 spots were obtained having R_f of 0.09 and 0.80.

TLC studies of the Methanol extract of Selected mixed herbal powder Solvent system I [Hexane: Acetic acid (9:1)], 1 spot detected R_f value 0.10. In solvent system II [Hexane: Ethyl acetate: Acetic acid (5:4:1)], 1 spot detected R_f value 0.92. In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], 4 spots were detected R_f values 0.05, 0.25, 0.80 and 0.90. In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)], 2 spots were visible R_f values 0.10 and 0.81. In solvent system V [Hexane: Ethyl acetate: Acetic acid (2:7:1)], 2 spots were obtained having R_f of 0.09 and 0.81 (Table.no. 3).

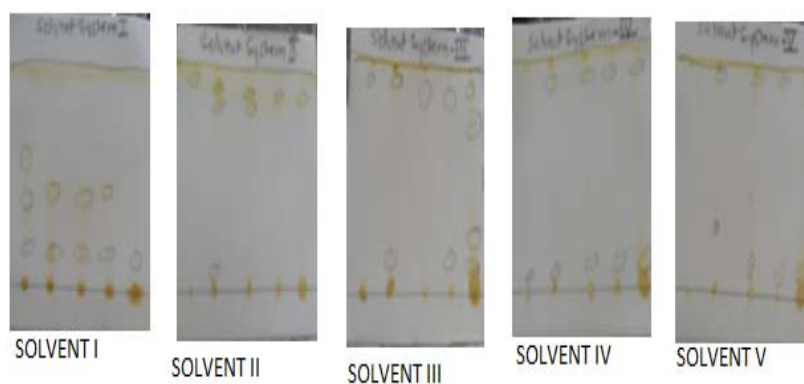


Fig 1: Photo graphic pictures of TLC showing with different solvent systems

Table 3: R_f values of TLC with respect to Different extracts of Selected mixed Herbal extract using different solvent systems

S. no	Extract	Solvent I		Solvent II		Solvent III		Solvent IV		Solvent V	
		No of spots	R _f values	No of spots	R _f values	No of spots	R _f values	No of spots	R _f values	No of spots	R _f values
1	Hexane	3	0.20 0.36 0.52	1	0.90	1	0.90	2	0.07 0.81	3	0.09 0.81 0.94
2	Chloroform	2	0.14 0.40	3	0.10 0.82 0.90	2	0.05 0.90	2	0.09 0.78	2	0.18 0.94
3	Ethyl acetate	2	0.16	2	0.82 0.90	1	0.85	2	0.07 0.81	2	0.03 0.94
4	Ethanol	2	0.44	1	0.82	2	0.05 0.90	2	0.03 0.81	2	0.09 0.80
5	Methanol	1	0.10	1	0.92	4	0.05 0.25 0.80 0.90	2	0.10 0.81	2	0.09 0.81

Conclusion

Results revealed the presence of saponins, tannins, flavonoids, alkaloids, carbohydrates, phenolic compounds by phytochemical investigation with respect to chemical tests and chromatographic techniques. The herbal powder mixture of *Polygonum glabrum*, *Canthium dicoccum*, *Ochna obtusata*, *Argyrea nervosa* mixture contains various primary and secondary metabolite which are pharmaceutically important. The present study useful for preparation of poly herbal preparations and further investigation required to isolate the individual bioactive compounds which are present in the mixture and their activities.

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