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Preliminary phytochemical analysis of different extracts of *Ruellia patula*, *Luffa aegyptiaca* and *Thunbergia alata*

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ABSTRACT

Ruellia patula, Luffa aegyptiaca and Thunbergia alata are luxuriant and dominant growing herbs on the road sides and on waste lands. The present study deals with the comparative phytochemical analysis of the ethyl acetate, ethanol and ethanol water mixture extracts of arial parts of these plants. It was found that some phytochemicals were plant specific whereas some are extract specific. The absence of Amino acids, proteins, and flavonoids was detected in all extracts of all the three plants. Other phytochemicals like steroids, saponins, terpenoids, glycosides etc. were plant specific and extract specific.

Key words: *Ruellia patula, Luffa aegyptiaca, Thunbergia alata,* Ethyl Acetate, Saponins, Tannins, Sugars, Flavoniods

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INTRODUCTION

The present study aims at finding out the phytochemical constituents of arial parts of *Ruellia patula, Luffa aegyptiaca* and *Thunbergia alata,* which grow luxuriantly on the road sides and along railway tracts in Tamil Nadu, India. The plants were chosen for study because these plants have not been exploited for their potential as medicinal plants, since scanty reports on the medicinal values of these plants are available.

The use of medicinal plants for their curative properties is a time tested experience of humans. Some of the phytochemicals like tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids have therapeutic values. [1, 2, 3] The knowledge of these phytoconstituents is very helpful in drug discovery and new drug molecule formulation. [4, 5, 6] The discovery, development and use of modern medicines have a deep rooted connection with the age old practice of folk and traditional medicinal background of the natives. [7 Thus the ancient wisdom has been the basis of modern medicine and will remain as one of the important sources of future medicine and therapies. [8] The World health Organization also has recognized the importance of traditional medicines and has been active in creating strategies, guidelines and standards for botanical medicines. [9]

Ruellia patula is a Perennial or shrubby herb or subshrub; stems to 0.6(–1) m long, erect to trailing, when young subglabrous to densely puberulous, pubescent or pilose, sometimes with stalked capitate glands. Leaves with petiole 2–32 mm long; lamina ovate to elliptic or narrowly so (rarely lanceolate), largest 1–6≈0.5–3.7 cm, apex acute to rounded (rarely retuse), base cuneate to truncate, sparsely to densely puberulous to pubescent (rarely subglabrous), densest along veins, sometimes with scattered capitate glands. Flowers solitary or in 3(–5)-flowered axillary cymes; peduncles in cymes 1–6 (–12) mm long; pedicels 0–2(–5) mm long, glabrous to puberulous; bracteoles (bracts in cymes) ovate-elliptic or narrowly so, 7–18≈3–8 mm. Calyx 3–8(–9) mm long, divided to 1–2 mm from base, uniformly puberulous or with ciliate lobes and midribs or glabrous; lobes narrowly triangular. Corolla falling early in the morning, white to mauve or purple; tube (10–) 12–34 mm long of which the basal cylindric part 3–12(–20) mm and the throat (7–) 9–19 mm long; lobes 4–15≈3–15 mm, ovate-elliptic or broadly so with entire to crenate-dentate margin. Stamens included in throat (rarely slightly exserted), didynamous, anthers not or slightly

overlapping; filaments fused for 1–3 mm at base, free parts 2–5 and 3–7 mm long; anthers 1.5–2.5 mm long. Ovary glabrous; ventral stigma lobe 1–1.5≈± 0.5 mm. Capsule clavate, glabrous, 11–20(–22) mm long, (4–)6–14(–16)-seeded. Seed broadly ellipsoid to circular, dark brown, 3–4.5≈2.5–4.5 mm.

In vitro antimicrobial activity, DNA sequencing and phytochemical screening of *Ruellia patula* was performed by Saranya, 2014. [10] They have reported the presence of alkaloids, steroids, glycosides, flavonids and tannins in different extract of the plant. Identification of β-carotene and β-sitosterol in methanolic extract of *Dipteracanthus patulus* (Jacq) and their role in antimicrobial and antioxidant activity was reported by Bumrela and Naik, 2011. [11]

Luffa aegyptiaca is a monoecious, annual, climbing or trailing herb up to 15 m long, stem 5-angled, finely hairy; tendrils 2–6-fid. Leaves alternate, simple; stipules absent; petiole up to 15 cm long; blade ovate in outline, 6–25 cm × 6–27 cm, palmately 3–7-lobed with triangular or ovate lobes, cordate at the base, lobes acute or subacute and apiculate at the apex, margin sinuate-dentate, scabrous, dark green, palmately veined. Male inflorescence racemose, 5–20-flowered; peduncle 7–32 cm long, finely hairy; female flowers solitary. Flowers unisexual, regular, 5-merous, 5–10 cm in diameter; petals free, entire, broadly obovate, 2–4.5 cm long, deep yellow; male flowers on bracteate pedicels 3–13 mm long, receptacle tube obconic below, expanded above, 3–8 mm long, with triangular lobes 8–12 mm long, sepals ovate, 8–14 mm long, stamens 3 or 5, free, inserted on the receptacle tube, connectives broad; female flowers on pedicel 1.5–14.5 cm long, receptacle tube shortly cylindrical and 2.5–6 mm long, with ovate lobes c. 1 cm long, sepals ovate-lanceolate or lanceolate, 8–16 mm long, ovary inferior, stigmas 3, 2-lobed. Fruit an ellipsoid or cylindrical capsule up to 60(–90) cm × 10(–12) cm, beaked, not prominently ribbed, brownish, dehiscent by an apical operculum, glabrous, many-seeded. Seeds lenticular, broadly elliptical in outline, compressed, 10–15 mm × 6–11 mm × 2–3 mm, smooth, dull black, with a narrow, membranous wing-like border. Seedling with epigeal germination; cotyledons ovate, c. 5 cm long.

Antimicrobial Activity of whole Plant of *Luffa cylindrica* was reported by Indumathy *et al*, 2011. [12] Azeez *et al*, 2013 have reported that *Luffa cylindrica* contains chemical components that have effects on hypersensitivity reactions, serve as immunostimulant, as antiinflamatory agent, function in glycosidase activity, inhibit protein synthesis with structure-function relationship of type I RIPs suggesting potentials for antitumour and antiviral activities and also induce uterine contraction to hasten child birth (Oxytocics). [13]

Thunbergia alata

Thungergia alata is an herbaceous vine belonging to family Acanthaceae. This plant is creeping or climbing, twining, 2-3 m in length. Stems are cylindrical, slender, puberulous. Leaves opposite; blades 4.5-10.5 × 3.2-6 cm, ovate, lobed, chartaceous, the apex acute, the base subcordiform; upper surface dark green, dull, pubescent; lower surface pale green, dull, with prominent venation; petioles 4-8 cm long, winged, pubescent. Flowers axillary, solitary; pedicels pubescent, 4-5 cm long; bracts green, ovate, pubescent, 1.5 cm long, covering the calyx and the corolla tube. Calyx yellowish green, with 12 filiform lobes, approximately 4 mm long; corolla orange, pale yellow, or less frequently whitish, infundibuliform, with 5 lobes, the tube approximately 2.5 cm long, narrow at the base, dark violet inside, the lobes approximately 2.5 cm long with the apex truncate, the limb approximately 5 cm in diameter; stamens with glandular hairs on the basal portion. Capsules approximately 4 mm long, depressed-globose to 4-lobed at the base, the upper half in the form of a beak, dehiscent by two valves; seeds 2 or 4, 1.2-1.5 mm long, semicircular, reticulate. Several cultivars have been developed, including some with white, yellow, and even pinkish-coloured flowers.*T. alata* has been widely used as an ornamental for its attractive flowers. [14, 15 *T. alata* is also used in traditional medicine. In India, the fresh root extract is used as a health tonic and an aphrodisiac. [16, 17] Medicinally it is used for skin problems, back and joint pains, eye inflammation, piles and rectal cancer. Gall sickness and some ear problems in cattle are also treated with this plant. Okello *et al*, 2010 have reported the leaves of *T. alata* are used for curing boils. [18] The leaf powder of this plant is used to treat snake bite as reported by Maregesi *et al*, 2013; Tekle, 2014). [19, 20] Ng *et al*, 2011 have reported the anti HIV activities of Luffin1 present in this plant. [21] Du *et al*, 2006 have shown the antioxidant activities of fruit of this plant. [22] The anti-inflammatory effect of the plant parts was reported by Kao *et al*, 2012. [23]

The present study deals with the phytochemical analysis of the three plants to have an understanding of the phyto-constituents present in them.

EXPERIMENTAL SECTION

2.1. Collection of samples

The plants used for the experiment were whole plants of *Ruellia patula*, *Luffa aegyptiaca* and *Thunbergia alata* were collected from the nearby road side of Grand Southern Trunk Road near Chennai, India. The plants were identified and authenticated by qualified botanist.

2.2. Preparation of extracts

30 grams of dried powder of the plants was separately packed in separate round bottom flask for sample extraction using ethyl acetate, ethanol and mixture of water and ethanol (1:1) as solvents. The extraction was conducted by 500 ml of the solvent for a period of 96 hours. The extract was separated from the plant mass and filtered. The solvent was evaporated by gentle heating in a water bath at 50 degree Celsius. The resultant semi dried extract was collected and stored for further experiment in refrigerator.

2.3 Phytochemical analysis

The extracts prepared was dissolved in distilled water and homogenised by shaking analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, triterpinoids, cardiac glycosides, Amino acids and sugars based on the protocols available in the literature. [24, 25, 26]

Test for alkaloids

The extract of the crude dry leaf powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, one portion was treated with equal amount of Dragondorff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The appearance of creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids.

Test for saponins

About 0.5 g of the plant leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for tannins

About 0.5 g of plant leaf extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for steroids

2 ml of acetic anhydride was added to 2 ml of plant leaf extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange colour for flavones.

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of glycosides.

Test for Proteins

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for Triple Sugar

To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of con.H_2SO_4 was added on the side of the test tube. A reddish violet ring appeared at the junction of two layers immediately, indicated the presence of carbohydrates.

Test for Phenolic Compounds

To one ml of extract 1ml of Ferric Chloride was added and observed for characteristic violet precipitate, which indicated the presence of Phenolic compounds.

Test for amino acids
To 2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet colour indicated the presence of peptide linkage of the molecule.

Test for Tri-Terpenoids
5ml of each extract was added to 2ml of chloroform and 3ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

RESULTS AND DISCUSSION

The preliminary study of the various phytochemicals present in the ethyl acetate, ethanol and a mixture of ethanol and water (1:1) extraction of the arial parts of plants *Ruellia patula, Luffa aegyptiaca* and *Thunbergia alata are* indicated in Table 1, 2 and 3 respectively.

Table 1

Sl. No	Phytochemicals	Plant extracts of *R. patula*		
		Ethyle Acetate	Ethanol	Ethanol+ Water (1:1)
1.	Tannin	-	+	+
2.	Tri-terpenoids	-	+	-
3.	Saponins	-	+	+
4.	Flavonoids	-	-	-
5.	Glycosides	-	-	-
6.	Alkaloids	+	+	+
7.	Phenolic Componds	-	-	-
8.	Steroids	+	+	+
9.	Proteins	-	-	-
10.	Amino acids	-	-	-
11.	Sugars	-	-	-
12.	Anthroquinons	-	+	+

'+' = Present; '-'= Absent

Table 2

Sl. No	Phytochemicals	Plant extracts of *Luffa aegyptiaca*		
		Ethyle Acetate	Ethanol	Ethanol+ Water (1:1)
1.	Tannin	-	+	+
2.	Tri-terpenoids	-	-	+
3.	Saponins	-	-	+
4.	Flavonoids	-	-	-
5.	Glycosides	-	-	+
6.	Alkaloids	+	+	+
7.	Phenolic Componds	-	-	-
8.	Steroids	-	-	-
9.	Proteins	-	-	-
10.	Amino acids	-	-	-
11.	Sugars	+	+	+
12.	Anthroquinons	-	-	+

'+' = Present; '-'= Absent

Table 3

Sl. No	Phytochemicals	Plant extracts of *T. Alata*		
		Ethyle Acetate	Ethanol	Ethanol+ Water (1:1)
1.	Tannin	-	+	+
2.	Tri-terpenoids	-	-	+
3.	Saponins	+	+	+
4.	Flavonoids	-	-	+
5.	Glycosides	-	+	-
6.	Alkaloids	-	-	-
7.	Phenolic Componds	-	+	+
8.	Steroids	+	+	+
9.	Proteins	-	-	-
10.	Amino acids	-	-	-
11.	Sugars	-	-	-
12.	Anthroquinons	-	-	-

'+' = Present; '-'= Absent

From the results as shown in Table 1, 2 and 3 indicate the following facts.

1. Tannis, saponins, alkaloids, steroids and anthroquinones were present in ethanolic and ethanolic water extracts whereas they were absent in ethyl acetate extracts of *Ruellia patula*. The presence of these compounds clearly indicates the possibility of using this plant as antibacterial and antioxidant. Flavonoids, glycosides, phenolic compounds, proteins, amino acids and sugers were conspicuous by their absence in all the three extracts.

2. Phenolic compounds and anthroquinones were present in all the three extracts of *Luffa aegyptiaca* whereas Glycosides, steroids, proteins, amino acids and sugars were absent all the three extracts. Tannins were presnt in ethyl acetate and ethanoilc water extracts. Terpenoids, saponins, flavonoids and alkaloids were present in ethanolic water extracts. The presence of so many important phytochemicals augur well with the claim that *Luffa* is a good antioxidant, anti-inflammatory , immunostimulant, antitumor and antiviral agent.

3. Saponins and steroids were present in all the three extracts of *T. alata*. Tannins, phenolic compounds were present in ethanolic and ethanolic water extracts. Alkaloids, proteins, amin acids, sugars and anthroquionos were absent in all the extracts. The use of *T. alata* for skin problems, back and joint pains, eye inflammation, piles etc can be attributed to the saponins and steroids present in all the three extracts.

The present day medicines owe their origin to plant products and plant extracts. In all the three Indian systems of medicine, Ayurveda, Sidha and Unani, most of the medicines are produces by herbal plants or their derivatives. Traditional systems of medicine all over the world hinge on herbal medicine. The range of treatment by the herbals spans from common cold to cancer. The present day medicine is crippled due to the massive side effects they produce and poor affordability. It is high time to search for medicines which are natural, with less or no side effects and are available at cheap cost [27] (Nair *et al*, 2005). It is known that alkaloids, saponins and tannins are known to have antimicrobial activity. [28, 29] Saponins are useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergents. [30] Tannins have astringent properties that hasten the healing of wounds and prevention of decay. [31] Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effects. Flavonoids are known anti-oxidant agent. [32, 33, 34]

CONCLUSION

The phytochemical analysis of the three plants namely *Ruellia patula, Luffa aegyptiaca* and *Thunbergia alata* revealed the presence or absence of various phytochemical as shown in the results. Although the three plants have similar habit and habitats they vary significantly in their phytochemical constituents and also in some medicinal properties. Further work is required to quantify and understand the medicinal values of these plants.

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