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SCREENING AND QUANTIFICATION OF PHYTOCHEMICALS IN LEAF EXTRACTS OF *SCHREBERA SWIETENIOIDES* AND *HOMALIUM ZEYLANICUM*

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ABSTRACT

Phytochemical screening and quantification in leaf extracts of *Schrebera swietenioides* and *Homalium zeylanicum* plants were carried out with an intention of finding the type and diversity of phytochemicals present in them. The phytoconstituents in the plant were extracted using ethyl acetate, methanol and water as solvents. The extracts showed positive results for biologically active compounds like alkaloids, flavanoids and phenolic compounds. The quantitative studies indicated that the more components were extracted in highly polar solvent water with high amount of alkaloids (31.00mg/g) in *Schrebera swietenioides*, and phenolic compounds (20.28mg/g) in *Homalium zeylanicum*.

KEYWORDS: ***Schrebera swietenioides*, *Homalium zeylanicum*, Leaf extract, Phytochemicals, Screening and Estimation.**

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PLANT INTRODUCTION

Schrebera swietenioides

Schrebera swietenioides is belongs to the Oleaceae family and found in Peru, Tropical and Southern Africa, India and Southeast Asia[1, 2]. Commonly known as Weaver's Beam Tree, Banpalas (Hindi), Bullakaya, Magalings, and Tondamukkudi (Telugu). It is a moderate sized deciduous tree, growing up to 20 m tall, with thick grey bark. The roots, bark and leaves are bitter, acrid, appetizing, digestive, thermogenic, stomachic, depurative, constipating urinary astringent and anthelmintic. The fruits are reported to be useful in curing hydrocele. Leaves are pinnate, with 3-4 pairs of opposite leaflets, and a terminal one. Various studies have been conducted on medicinal properties of the plant like antioxidant, anti-Inflammatory and antipyretic activity studied in root [3], healing potential in the dexamethasone-suppressed wound healing in rodents [4], antidiabetic and antioxidant effect in fruit [5].

Figure I
Schrebera swietenioides Plant

Figure 2
Homalium zeylanicum Plant

Classification

Classification	*Schrebera swietenioides*	*Homalium zeylanicum*
Kingdom	Plantae	Plantae
Order	Lamiales	Violales
Family	Oleaceae	Flacourtiaceae
Tribe	Oleaceae	----
Genus	*Schrebera*	*Homalium*

Homalium zeylanicum

Homalium zeylanicum Benth plant belongs to Flacourtiaceae family and is distributed in evergreen and semi-evergreen forests, native to South India and Srilanka. It is also found in Bangladesh, Laos, Myanmar, Nepal, Thailand and Vietnam [6]. Common name of the plant includes kalladamba, liyan, mukki. The various parts of plant including, bark and leaf having many traditional medicinal uses, mainly in diabetes, rheumatism and wound healing. It has been traditionally used for treating several ailments including rheumatism, anti-inflammatory, hepatoprotective and anti-diabetic agent in Rayalaseema region of Andhra Pradesh. In Nigeria, it is used as traditional medicine for the treatment of malaria, ulcer, and inflammatory diseases and as an aphrodisiac. Various studies have been conducted to evaluate the medicinal properties of plant [7-12]. Though the traditional medicinal uses of both plants are known, but the phytochemical bases for their uses are not known. Hence in the present paper, we are reporting the screening and quantification of phytochemicals present in both plants.

MATERIALS AND METHODS

Chemicals and reagents

The solvents used for extracting the phytochemicals are Methanol and Ethyl Acetate. The reagents used for phytochemical screening and estimation were of laboratory reagent grade and were purchased for Merck chemicals private limited, Mumbai, Fisher scientific, Mumbai and SD fine chemicals Mumbai.

Plant Collection and Identification

Both plants were collected from in and around regions of Tirumala hills and were identified with the help of botanists of SV University, Tirupati.

Extraction of phytochemicals

The phytochemicals present the leaves of the collected plants were isolated using different solvents like ethyl acetate, methanol and water in a series of extraction method from low polarity to high polarity using soxhlet extraction method.

Preparation of reagents

Bromocresol green solution

Solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.

Phosphate buffer solution (pH 4.7)

Buffer solution was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Folin- Ciocalteu's (FC) reagent

10ml of of Folin- Ciocalteu's solution was dissolved in 90ml of double distilled water.

Iron (III) chloride solution

500mg of ferric chloride was weighed and dissolved in 100ml of distill water.

Potassium hexacyanoferrate (III) solution

500mg of potassium hexacyanoferrate was weighed and dissolved in 100ml of distill water.

Preliminary phytochemical screening

1. Test for steroids

Salkowski Test

Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour.

Liebermann Burchard's Test

To the extract, few drops of acetic anhydride was added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.

2. Tests for triterpenoids

Salkowski Test

Few drops of concentrated sulphuric acid are added to the extract, shaken and on standing, lower part turns golden yellow colour.

Lieberman Burchard's Test
To the extract, few drops of acetic anhydride was added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a red ring indicates triterpenes.

Ischugajiu Test
Excess of acetyl chloride and pinch of zinc chloride are added to the extract solution, kept aside for reaction to subside and warmed on water bath, cosin red colour is produced.

Brickorn and Brinar Test
To the extract, few drops of chlorosulfonic acid in glacial acetic acid (7:3) are added, red colour is produced.

3. Test for Saponins
Foam Test
Small amount of extract is shaken with little quantity of water; the foam produced persists for 10 minutes. It confirms the presence of saponins.

Haemolysis Test
To 2ml of 1.8% Sodium chloride solution in two test tubes, 2ml distilled water is added to one and 2ml of 1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract indicates the presence of saponins

4. Test for Steroidal Saponin
The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

5. Tests for Triterpenoidal Saponin
The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

6. Tests for Alkaloids
Mayer's Test
The acid layer when mixed with Mayer's reagent (Potassium mercuric iodide solution) gives creamy white precipitate.

Dragendroff's Test
The acid layer with a few drops of Dragendroff's reagent (Potassium bismuth iodide) gives reddish brown precipitate.

Wagner's Test
The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) gives brown to red precipitate.

Hager's Test
The acid layer when mixed with few drops of Hager's reagent (Saturated solution of pricric acid) gives yellow coloured precipitate.

7 Tests for Carbohydrates
Fehlings's Test
The extract when heated with Fehling's A and B solutions gives an orange red precipitate showing the presence of reducing sugar.

Molischs's Test
The extract is treated with Molisch's reagent and concentrated sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.

Benedict's test
The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.

Barfoed's Test
Barfoed's reagent is added and boiled on water bath for few minutes, reddish precipitate is observed for the presence of carbohydrate.

8. Test for Flavonoids
Shinoda Test
The extract solution with a few fragments of magnesium ribbon and concentrated hydrochloric acid produced magenta colour after few minutes.

Ferric chloride test
Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and given blackfish green color.

Lead Acetate Test
Alcoholic solution of extract reacts with 10% lead acetate solution and given yellow precipitate.

9. Test for Glycosides
Anthraquinone test
Drug is powdered and extracted with either ammonia or caustic soda. The aqueous layer shows pink color

Keller-killiani test
This is for cardiac glycosides. The extract and 0.4 glacial acetic acid are mixed with ferrous chloride and 0.5 mi of concentrated sulphuric acid. The acetic acid layer shows blue color.

10. Test for Phenolic Compounds
Ferric chloride test
Treat the extract with ferric chloride solution then blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Gelatin test
To the test solution add 1% gelatin solution containing 10% NaCl, and then precipitate is formed.

Test for chlorogenic acid
Treat the test solution with aqueous ammonia and expose to air gradually, green color is developed.

Estimation of phytochemicals
Quantitative estimation of Alkaloids
To 1ml of extract, 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shaked a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Quantitative estimation of Steroids
1ml of an extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2^0C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Determination of total Flavonoid content
Total flavonoid content was determined using Aluminium chloride ($AlCl_3$), using quercetin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% $NaNO_2$ (0.03ml). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm.

Quantitative Estimation of Phenolic Compounds
The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, 1ml of extract was mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min, 4 ml of sodium carbonate solution was added. The final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

RESULTS AND DISCUSSION

The solvent extraction and the preliminary screening of phytochemicals (secondary metabolites) is an important step in identification and evaluation of bioactive compounds present in plants. This may lead to medicinal plant drug discovery and development of phytomedicine. The biological activity of medicinal plants such as hypoglycemic, antidiabetic, antioxidant,

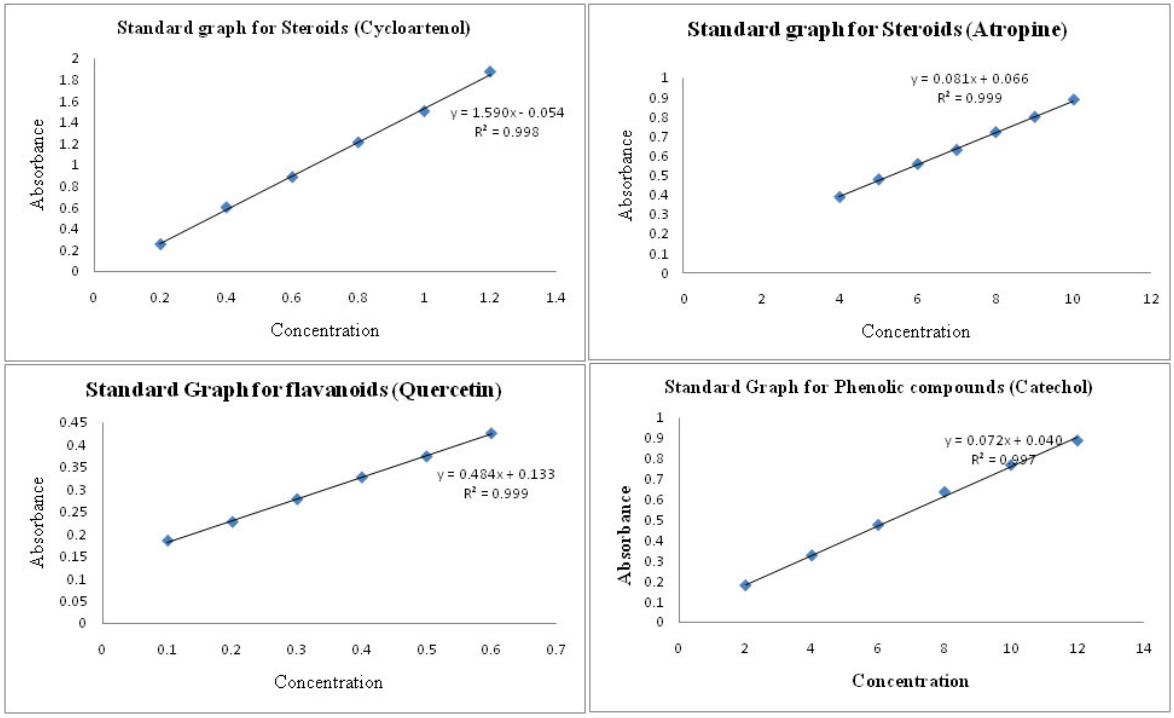
antimicrobial, antiinflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc., was due to the presence of theses secondary metabolites in plants (12). In the present study, chief phytoconstituents in the leaves of selected medicinal plants, *Homalium zeylanicum* and *Schrebera swietenioides* were identified in order to relate their presence with bioactivities of the plants. The solvents like Ethyl Acetate, Methanol and Water were used for extracting the secondary metabolites form selected medicinal plants. The results of the preliminary studies indicated that the leaf extract of *S. swietenioides* and *H. zeylanicum* contains more number of phytochemicals. Table 1 shows the screening results for the medicinal plants in the study. Results indicated that the plant, S*. swietenioides* contains Steroids, Triterpenoids, Saponins, Steroidal saponin, Triterpenoid saponin, Alkaloids, Carbohydrates, Flavanoids and Phenols. Aqueous extract of leaves showed positive results for all the phytochemicals. The leaf extract of *H. zeylanicum* showed positive results for Steroids, Triterpenoids, Carbohydrates, Alkaloids, Flavanoids and Phenolic compounds. In *H. zeylanicum* methanolic and aqueous extract showed positive results for number of phytochemicals.

Table 1
Qualitative phytochemical analysis of S. swietenioides and H. zeylanicum leaf extracts

S. No	Screening Tests	*Schrebera swietenioides*			*Homalium zeylanicum*		
		Ethyl Acetate	Methanol	Water	Ethyl Acetate	Methanol	Water
1	Steroids	+ve	+ve	+ve	-ve	-ve	+ve
2	Triterpenoids	+v e	+v e	+ve	+ve	+ve	-ve
3	Saponins	_ve	+ve	+ve	_ve	_ve	_ve
4	Steroidal saponin	_ve	_ve	+ve	_ve	_ve	_ve
5	Triterpenoid saponin	_ve	_ve	+ve	_ve	_ve	_ve
6	Alkaloids	_ve	+ve	+ve	_ve	+ve	+ve
7	Carbohydrates	+ve	+ve	+ve	+ve	+ve	+ve
8	Flavanoids	+ve	+ve	+ve	+ve	+ve	+ve
9	Phenols	+ve	+ve	+ve	+ve	+ve	+ve

Visible spectrophotometric methods were followed for the quantitative estimation of the identified phytochemicals in selected plants. Extractive spectrophotometric method with Bromocresol green was followed for estimation of Alkaloids and Atropine was used as standard compound and results were expressed in Atropine equivalent units. Aluminium chloride method with quercetin standard for flavonoid, Folin- Ciocalteu's method with catechol standard for Phenoilc Compounds and Potassium hexacyanoferrate in presence of Iron (III) chloride method was followed for estimation of Steroids with Cycloartenol standard. The standard calibration curves obtained for estimation of phytochemicals was given in Figure 1.

Figure 3
Standard calibration curve for estimation of phytochemicals in plant extract



The quantitative estimation of phytochemicals present in different solvent extracts of *Schrebera swietenioides* and *Homalium zeylanicum* leaves indicated high amount of phytochemicals in the leaves. In *S. swietenioides* plant, alkaloids, flavanoids and phenolic compounds were found to be very high in water extract i.e 31.00mg/g, 11.404mg/g and 16.57mg/g respectively. Ethyl Acetate extract shows more amount of Steroids (5.930mg/g). Among the three solvents used for isolating of phytochemicals in leaves of *S. swietenioides*, water was the best solvent for isolating the compounds. Water extract shows positive results for more number of compounds and also the compounds present in very high amount. Ethyl acetate was used as best solvent for isolating steroids and it was found to be unsuitable for remaining phyto-constituents. The polarity of the solvent plays an important role for isolation. The leaf extract of *S. swietenioides* proves that more polar active components present in the plant. The medicinal activity of the plant and the ayurvedic use of plant may be due to the presence of high polar active compounds in high amount. The quantitative study results of leaf extract of *S. swietenioides* were given in table 2. The aqueous extract of *Homalium zeylanicum* shows positive results for maximum number of phytochemicals. The quantitative studies confirm that Alkaloids (17.375 mg/g) and Phenolic compounds (20.28mg/g) were found to be very high in aqueous extract. Ethyl acetate extract shows high amount of Flavanoids (12.21mg/g). Table 3 shows the quantitative analysis results of leaf extract of *H.zeylanicum*.

Table 2
Quantitative estimation results of leaf extract of Schrebera swietenioides

S. No	Name of Phytochemical compound	*Schrebera swietenioides* extract					
		Ethyl Acetate		Methanol		Water	
		Absorbance	Amount*	Absorbance	Amount*	Absorbance	Amount*
1	Steroids	0.889	5.930	0.214	1.685	0.485	3.389
2	Alkaloids	…	…	0.198	16.500	0.314	31.00
3	Flavanoids	0.586	9.359	0.203	1.446	0.685	11.404
4	Phenolics	0.06	11.42	0.056	10.285	0.078	16.57

Amount is give as mg of the phytochemical compound present in one gram of the plant extract

Table 3
Quantitative estimation results of leaf extract of Homalium zeylanicum

S. No	Name of Phytochemical compound	*Homalium zeylanicum* extract					
		Ethyl Acetate		Methanol		Water	
		Absorbance	Amount*	Absorbance	Amount*	Absorbance	Amount*
1	Steroids	…	…	…	…	0.663	4.509
2	Alkaloids	…	…	0.105	4.875	0.205	17.375
3	Flavanoids	0.724	12.21	0.531	8.223	0.851	14.83
4	Phenolics	0.035	4.28	0.087	19.143	0.091	20.28

Amount is give as mg of the phytochemical compound present in one gram of the plant extract

CONCLUSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. In this study, by using ethyl acetate, methanol and water as solvent, phytochemicals were isolated from leaves of medicinal plants *Schrebera swietenioides* and *Homalium zeylanicum*. Preliminary and quantitative studies confirm that the plants are the rich source of phytochemicals. Ethyl acetate extract shows less quantity of components and aqueous extract of both the plants shows high amount of compounds. Further studies need to be carried out for medicinal or biological activity based identification of molecules and their pharmaceutical evaluation to conform in vivo or in vitro activity of phytochemicals.

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