

Physicochemical Evaluation, Preliminary Phytochemical Investigation, Fluorescence and TLC Analysis of Leaves of *Schleichera oleosa* (Lour.) Oken

S. MUTHUKRISHNAN* AND T. SIVAKKUMAR

Department of Pharmacy, Annamalai University, Chidambaram-608 002, India

Muthukrishnan and Sivakkumar: Pharmacognostic and Phytochemical Evaluation of Leaves of *Schleichera oleosa*

Schleichera oleosa (Lour) Oken, family: Sapindaceae, is medium to large sized tree widely distributed in the tropics and subtropics of India. In the present study, physicochemical parameters, fluorescence, phytochemical constituents and thin-layer chromatogram of the leaves of the plant were investigated. The total, acid-insoluble, water-soluble and sulphated ash values and the moisture content of the leaf powder of *Schleichera oleosa* were found to be 9.06, 7.16, 5.73, 13.5 and 5.21 %, respectively. The fluorescent characteristics of the leaf powder with various chemical reagents were examined under visible and UV light. Percent yields of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous leaf extracts were 3.26, 1.35, 1.70, 7.73 and 4.21 %, respectively. A significant amount of phenolic and flavonoid content was detected. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins and steroids in ethanol and aqueous extracts. The ethyl acetate, ethanol and aqueous extracts were subjected to thin-layer chromatography for identification of bioconstituents, which revealed various colored phytochemical compounds with different Rf values in different solvent systems. The results indicated that the leaves contain an appreciable number of bioactive compounds and presence of these phytochemicals especially the phenols and flavonoids could be the reason behind the use of *Schleichera oleosa* in ethnomedicine for the management of various ailments. These results could help in standardization, identification and in carrying out further research on *Schleichera oleosa* leaf-based herbal drugs.

Key words: Physicochemical standards, fluorescence, extractive values, phytochemical studies, flavonoids, phenolic compounds, TLC, Rf values

Plant-based drugs have been used worldwide in traditional medicines for treatment of various diseases. World plant biodiversity is the largest source of herbal medicine and still about 60-80 % world population relies on plant-based medicines, which are being used since the ancient ages as traditional medicine. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world. It is now clear that the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on the human body. These natural compounds also formed the base of modern drugs as we are using today^[1-3].

Schleichera oleosa (Lour.) Oken, commonly known as *Lac* tree or *Kusum*, is a forest species of tropical and subtropical region. The tree is utilized for multifarious purposes and production of natural, biodegradable

and commercially important *lac* resins that serve as a livelihood support to millions of poor farmers^[4]. It also has many medicinal uses and is used in traditional medicine for several indications. The powdered seeds are applied to wounds and ulcers of cattle to remove the maggots. The bark is used as an astringent and against skin inflammations, ulcers, itching, acne and other skin infections^[5]. It is generally used as an analgesic, antibiotic and against dysentery^[6]. Recently, it was reported that the bark along with water is used to treat menorrhoea^[7].

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*Address for correspondence

E-mail: smuthukrishnan.smk@gmail.com

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Hence the present investigation was taken up to study the physicochemical characteristics, phytochemical constituents, fluorescence and thin-layer chromatography (TLC) analysis of leaves of *S. oleosa* (Lour.) Oken extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water and also to perform qualitative and quantitative analysis of some secondary metabolites and minerals present in this plant^[8].

MATERIALS AND METHODS

Fresh leaves of *S. oleosa* were collected during May 2015 from the forest regions of Kanyakumari District, Tamil Nadu. The plant material was identified and authenticated in the Botany Laboratory, Central Council for Research in Ayurveda and Siddha (Government of India), Tirunelveli, Tamil Nadu. Voucher specimens were preserved in the department herbarium library (PS-08/14/1/5/15).

Taxonomy and nomenclature:

Synonym(s): *Schleichera Trijuga* Willd & Klein, family: *Sapindaceae*. Vernacular/common names: lac tree (English), *kusum* (India), *pongro* (French, Khmer of Cambodia), *gum-lac tree* (Filipino), *kasambi* (Indonesian), *kusambi* (Malay), *takhro* (Thai)^[9]. Scientific synonyms are *Cussambium oleosum* O. Kuntze, *Pistacia oleosa* Lour. *Schleichera trijuga* Wild.^[4]. The vernacular names are *Kusum*, *Kusam* (Hindi); *Sagada* (Kannada); *Pava*, *puvan* (Tamil), *Pusuku* (Telugu)^[4].

Processing of the sample:

Fresh leaves of plants were washed well using tap water followed by washing twice using distilled water and were dried in the shade for a period of 10-15 d, at an ambient temperature of 33°. After drying, leaf materials were crushed into small pieces. The dried samples were ground properly to obtain a coarse powdered, which was stored at room temperature till used in the investigation^[10,11].

Macroscopic evaluation:

The leaves were collected from the surrounding environment. The fresh leaves were deep green in colour. The leaves were separated from the stem and shoots, cleaned manually and kept over a dry plastic sheet and macroscopic characters of the fresh leaves were noted. A magnifying glass and scale were used to measure the parameters like morphology, length, width,

shape, surfaces, venation, presence and absence of petiole, the apex, margin, base, lamina and texture^[12-14].

Fluorescence analysis:

The fluorescence analysis of the plant was done by placing dry, powdered leaves on a slide and observing the colour changes under visible and UV lights after treating with several drops of specific reagents such as ammonia, acetic acid, 1 N hydrochloric acid, 1 N nitric acid, 1 N sulphuric acid, 10 % ferric chloride, I₂ solution and 1 N sodium hydroxide. Similarly, different solvent crude extracts were observed without adding any reagents under visible and UV light. The development of colour was noted within 1-2 min in order to avoid drying and resultant colour change^[12,15,16].

Preparation of extracts:

The dried, powdered leaf material (500 g) was extracted successively with petroleum ether, chloroform, ethyl acetate, ethanol and water in the increasing order of polarity by hot percolation method using a Soxhlet apparatus. After distillation, the resultant extracts were collected in a clean dry glass beaker. The extracts were evaporated to dryness in a Buchi rotary vacuum evaporator (Rotavapor R-210, Switzerland) at 40-50° and the final extracts were kept at low temperature for further investigations^[17,18].

Physicochemical standardization:

Standardization of the extracts of *S. oleosa* leaves was carried out as per guidelines of WHO and different procedures listed in the pharmacopeia. The standardization studies on different physicochemical parameters including extractive values in different solvents, total ash, water-soluble ash, acid-insoluble ash, moisture content and loss on drying (LOD) were carried out^[19,20]. Cold maceration estimated water and alcohol-soluble extracts according to the method prescribed by World Health Organization (WHO). All estimations were performed six times and the result, which was presented as mean±standard error of the mean (SEM).

Total ash value:

The total ash value gave an estimate of the total material remained after ignition. About 2 g of the dried crude drug was accurately weighed in a silica crucible and incinerated gently at first and gradually increased the temperature to 675±25°, until free from carbon. Then the ash was cooled in a desiccator and weighed. The percent of ash was calculated with reference to the air

dried powder. The procedure was repeated to get the constant weight^[21].

The total ash was boiled with 25 ml of water for 5 min and filtered through ash less filter paper (Whatman No. 41). The insoluble matter was collected in a filter paper followed by washing with hot water. The filter paper was ignited in silica crucible for 15 min at a temperature not exceeding 450°. The remaining ash was cooled in a desiccator and weighed. Subtracting the weight of the water-insoluble matter from the weight of the total ash yielded the weight of water-soluble ash. Percent water-soluble ash in the dried powder was calculated^[21].

Sometimes, variable inorganic constituents like calcium oxalate, silica and carbonate content of the crude drug affected the total ash value. Such variables were removed by treating the total ash powder with hydrochloric acid. The acid-insoluble ash measures the amount of silica, especially as sand and siliceous earth present in the plant material. The acid-insoluble ash was determined from the total ash obtained. It was boiled for five minutes with 25 ml of 2 M hydrochloric acid and filtered through an ash less filter paper (Whatmann No. 41). The collected insoluble matter was washed with hot water, ignited in a silica crucible and cooled in desiccator. The residue was weighed and the acid-insoluble ash of the plant material reference to the air-dried powder was determined^[21].

Sulphated ash value:

A silica crucible was heated to redness for 10 min, allowed to cool in a desiccator and weighed. About 1 g of the dried crude powder was accurately weighed in a silica crucible and ignited gently at first until the substance is thoroughly charred. Then cooled and the residue was moistened with 1 ml sulphuric acid, heated gently until the white fumes no longer evolved and ignited at 800°±25° until all black particles have disappeared. Then the crucible was allowed to cool, few drops of sulphuric acid were added and heated. The process was repeated and weighed^[21].

Determination of LOD:

The test for LOD determines both water and volatile matter. About 5 g of the powdered plant material was accurately weighed in a flat tarred weighing bottle. The bottle was dried in an oven at 105° for 1 h. It was cooled in a desiccator and again weighed. The LOD was calculated with reference to the amount of air-dried powder taken^[21].

Determination of crude fibre content:

Estimation of crude fibre content represented the measurement of the content of cellulose, lignin and cork cells in the plant tissue. Here the plant material was defatted and boiled with dilute acid and alkali to eliminate the soluble material. The excess of crude fibre might indicate adulteration with woody tissues like kernels. The crude fibre content was determined by the method recommended in USP XX. About 2 g of the coarse powder of the plant material was accurately weighed and extracted with ether. The marc was boiled with 200 ml of 1.25 % sulphuric acid for 30 min under reflux in a 500 ml flask. The mixture was then filtered through tarred filter and the residue was washed with boiled water until free from acid. The residue was again transferred into the flask and boiled with 200 ml 1.25 % sodium hydroxide solution under reflux for 30 min. Then the liquid was filtered quickly through a tarred filter. The residue was washed with boiling water until it became neutral. Then the residue was dried in a hot air oven at 110° and weighed. After weighing the residue, it was incinerated and weighed again. The difference in the weight of the residue before and after incineration provided the crude fibre content of the plant material^[22].

Determination of swelling index:

The swelling index is the swelling of 1 g of plant material under specified conditions. Plant materials may have specific therapeutic or pharmaceutical utility because of their swelling properties, especially the gums and those containing mucilage, pectin or hemicellulose. About 1 g of coarse powder of the plant material was transferred into a 25 ml glass stoppered measuring cylinder containing 25 ml of water. The mixture was shaken thoroughly every 10 min for 1 h. Then it was allowed to stand for 3 h at room temperature. After that, the volume occupied by the plant material, including any sticky was measured. This method was used to determine the amount of active constituent present in the plant material, which are soluble in particular solvent^[23].

Extractive value:

This method was used to determine the amount of active constituents present in the plant material, which are soluble in given solvent. Extraction of any crude drug with a particular solvent yielded a solution containing different phytoconstituents soluble in that solvent^[24]. About 5 g of powder was accurately weighed and

placed in a round bottom flask containing 100 ml of ether, which was subjected to continuous extraction in a Soxhlet apparatus for 20 h. Then the extract was transferred into a porcelain dish, allowed to evaporate spontaneously followed by drying it over phosphorous pentoxide for 18 h and weighed. Finally, the percent ether-soluble extractive was calculated^[24].

Five grams of the powder was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 h shaking frequently during 6 h and allowed to stand for 18 h. It was filtered rapidly taking precautions against loss of alcohol; 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105° and weighed. The percent of alcohol-soluble extractive was calculated with reference to the air-dried drug^[24].

About 5 g of the powder was added to 50 ml of water at 80° in a stopper flask. It was shaken well and allowed to stand for 10 min, cooled to 15° and to it 2 g of kieselghur was added and filtered. About 5 ml of the filtrate was transferred to a tarred evaporating basin (7.5 cm in diameter), the solvent was evaporated on a water bath, drying was continued for half an hour, finally it was dried in a hot air oven for 2 h and weighed. The percent of water-soluble extractive was calculated with reference to the dried drug^[24].

Phytochemical screening of the plant material:

Phytochemical screening was performed to detect the presence of secondary metabolites that could be responsible for curing ailments. The phytochemical screening of the plant extract was carried out for all the extracts, as per reported standard methods^[25-27].

Quantitative phytochemical test:

Alkaloids were estimated by weighing 5 g of the sample into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one fourth of the total volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid matter, which was dried and weighed^[28].

Twenty grams of sample powder was put into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples heated over a water bath for 4 h

with continuous stirring at about 55°. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The extract was reduced to 40 ml over water bath at about 90°. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered. The purification process was repeated by extracting with 60 ml n-butanol. The combined n-butanol extracts and washed twice with 10 ml of 5 % aqueous sodium chloride. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated^[29].

Total flavonoids were estimated by extracting 10 g of the plant sample repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight^[30].

Total phenolic content was analysed spectrophotometrically using a modified Folin-Ciocalteu colorimetric method^[31]. The crude extracts were diluted suitable solvent to attain interpretations within the standard curve ranges of 0.0-100.0 µg of gallic acid/ml. Then the extract was mixed with 1 ml of distilled water in a test tube and 250 µl of Folin-Ciocalteu reagent was added. Then 2.5 ml of 7 % aqueous solution of sodium carbonate (Na₂CO₃) was added. This mixture was gently shaken and allowed to stand for 6 min. The solution was then made up to 6 ml by adding sufficient amount of distilled water. Samples were allowed to stand for 90 min at room temperature. Then absorbance was measured against the blank at 760 nm using a UV/Vis spectrophotometer. This reagent blank, which was composed of the same reagents but instead of sample using distilled water. The standard values prepared similarly with known concentrations of gallic acid and plot a standard curve. The interpretations of sample absorbance value in standard curve to find out the concentration of phenolic content of the extracts. All values were expressed as mean±SEM for six replications^[32,33]. The total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg of GAE/g DW) were calculated.

TLC analysis of various extracts of *S. oleosa*:

Based upon the preliminary phytochemical tests and quantitative estimation, the ethyl acetate, ethanol

and aqueous extracts were subjected to TLC. The semi-solid extract was chromatographed by taking a small quantity of the extract diluted with a drop of the solvent. These extracts were subjected to TLC as per conventional one dimensional ascending method by using standard protocol as per pharmacopoeia was followed. In this method silica gel 60F₂₅₄, 20×20 cm, with thickness 0.2-0.3 mm (Merck) pre-coated plate was used. Plate markings were made with soft pencil. Glass capillaries were used to apply the spot on the TLC plate, 1 µl of sample volume applied by using capillary at distance of 1 cm and the 3 tracks and developed in air tight chamber already saturated with same solvent system.

In the twin trough chamber with different solvent systems: chloroform:ethyl acetate:formic acid (5:4:1) solvent system I. In solvent system II, toluene:acetone:formic acid (4.5:4.5:1). In solvent system III, ethyl acetate:methanol:water (100:13.5:10), In solvent system IV, ethyl acetate:formic acid:acetic acid:water (100:11:11:26) used. After pre-saturation with mobile phase, 20 min for development was used. After the run, plates were dried and allowed for visualization^[34-36]. After development, initially three spots were visualized in UV chamber (254, 365 nm). In the present study, different visualizing reagents were used such as NH₃ vapour, vanillin HCl, 10 % alcoholic KOH, Folin-Ciocalteu. Always freshly prepared reagents were used to detect the better bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples^[34-36]. After visualized in UV chamber and spray reagents, the spots were shown in different in colour. The Rf values were measured correctly and carefully and the chromatogram were photographed and presented in the tabulated form.

TABLE 2: FLUORESCENCE CHARACTERISTICS OF POWDERED LEAVES OF *S. OLEOSA* TREATED WITH DIFFERENT CHEMICAL REAGENTS

Chemical Treatment	Day Light	UV	
		254 nm	366 nm
Powder	Green	Brown	Greenish-brown
Powder+H ₂ O	Green	Brown	Greenish-brown
Powder+1N HCl	Greenish-brown	Brown	Dark brown
Powder+5 % NaOH	Green	Brown	Greenish-brown
Powder+1N NaOH (alcoholic)	Greenish-yellow	Brownish-red	Greenish-brown
Powder+50 % HNO ₃	Brown	Dark brown	Brownish
Powder+50 % H ₂ SO ₄	Brownish-red	Dark brown	Dark brown
Powder+ammonia	Light green	Light green	Fluorescent Green
Powder+acetic acid	Light green	Light green	Fluorescent Green
Powder+I ₂ Solution	Brown	Yellowish-green	Dark green
Powder+FeCl ₃	Yellowish-green	Fluorescent green	Dark green

RESULTS AND DISCUSSION

Leaves are paripinnate, w/(2-)3(-4) pairs of pinnae, petiole cross section circular somewhat flattened or slightly grooved above, 2-6(-8) cm long, swollen at base; rachis cross section round to triangular; petiolule swollen, slightly grooved above, 1-3 mm long. Leaflets are elliptical to elliptic-oblong, coriaceous, 4.5-18.5 (-25)×2.5-9 cm, dark brown or greyish-green above, lighter-brown to greenish beneath, deep purple when young, veins in 12-15 pairs, looped and joined near the margin^[9].

Physicochemical standards such as total ash value (9.06±0.20), acid-insoluble ash value (7.16±0.53), water-soluble ash value (5.73±0.13), sulphated ash value (13.5±1.24), LOD (5.21±0.12), swelling index (19.33±0.23) and crude fibre content (4.13±0.24) were observed as per Indian Pharmacopoeia and WHO guidelines for the Quality control of herbal drugs.

The coarse powder of aerial parts of *S. oleosa* was subjected to the various systemic physicochemical evolutions as per standard procedure. Extractive values

TABLE 1: PHYSICOCHEMICAL STANDARD VALUES OF POWDERED LEAVES OF *S. OLEOSA*

Experiment	Mean (% w/w)±SEM
Total ash value	9.06±0.20
Water-soluble ash value	5.73±0.13
Acid-insoluble ash value	7.16±0.53
Sulphated ash value	13.5±1.24
Loss on drying	5.21±0.12
Crude fibre content	4.13±0.24
Swelling index	19.33±0.23
Ether-soluble extractive value	1.2±0.38
Alcohol-soluble extractive value	11.15±0.35
Water-soluble extractive value	8.76±0.38

Each value is presented as mean±SEM (n=6)

such as ether-soluble extractive (1.2 ± 0.38), alcohol-soluble extractive value (11.15 ± 0.35) and water-soluble extractive (8.76 ± 0.38) were observed. The volatile oil content of the aerial part of the plant was determined by hydro distillation method using Clevenger apparatus. No oil was collected in the graduated tube. All the physicochemical standards values were observed from the mean of six findings (Table 1).

Comparative fluorescence analysis was performed for powder and the extracts. The powder was suspended with various chemical reagents, whereas the extracts were dissolved in same solvent. The fluorescence nature was observed and recorded (Tables 2 and 3) by comparing the color development in day light and UV (254 and 366 nm) light.

The aerial parts of the plant were shade dried and made into coarse powder. The coarse powder was extracted by continuous hot percolation method in a Soxhlet apparatus using various solvents in increasing polarity such as petroleum ether, chloroform, ethyl acetate, ethanol and water. Extracts were concentrated by removal of solvent under reduced pressure and finally residue was dried in vacuum. The percent yields were such as petroleum extract 3.26 % w/w, chloroform extract 1.35 % w/w, ethyl acetate extract 1.70 % w/w, ethanol extract 7.73 % w/w and aqueous extract 4.21 % w/w as shown in Table 4.

All extracts were subjected to preliminary phytochemical screening to identify the phytoconstituents using qualitative chemical reagents. The phytochemical tests revealed the presence of phytosterols in all the extracts, alkaloids (ethanol, aqueous extract), glycosides (ethanol, aqueous extract), saponins (ethanol, aqueous extract), phenolic compounds (ethyl acetate, ethanol, aqueous extract) and flavonoids (ethyl acetate, ethanol, aqueous extract). Compared with petroleum ether and chloroform extracts, most of the phytoconstituents were present

TABLE 3: FLUORESCENCE CHARACTERISTICS OF VARIOUS CRUDE EXTRACTS OF LEAVES OF *S. OLEOSA*

Extracts	Day light	UV observation	
		254 nm	366 nm
Petroleum ether	Yellowish-green	Light brown	Dark brown
Chloroform	Green	Blackish-green	Black
Ethyl acetate	Greenish-brown	Dark brown	Reddish-brown
Ethanol	Brown	Dark brown	Reddish-brown
Aqueous	Reddish-brown	Reddish-brown	Brownish

TABLE 4: EXTRACTIVE VALUES OF POWDERED LEAVES OF *S. OLEOSA*

Extracts	Colour	Consistency	Percent yield
Petroleum ether	Yellowish-green	Solidify	3.26 %
Chloroform	Green	Solidify	1.35 %
Ethyl acetate	Greenish-brown	Sticky	1.70 %
Ethanol	Brown	Thick viscous	7.73 %
Aqueous	Reddish brown	Solidify	4.21 %

TABLE 5: QUALITATIVE PHYTOCHEMICAL SCREENING OF SUCCESSIVE EXTRACTS OF POWDERED LEAVES OF *S. OLEOSA*

Compounds	Observation				
	PET	CF	EA	ET	AQ
Carbohydrates	-	-	-	+	+
Glycosides	-	-	-	+	+
Proteins and aminoacids	-	-	-	-	-
Alkaloids	-	-	-	+	+
Saponins	-	-	-	+	+
Phenolic compounds and tannins	-	-	+	+	+
Flavonoids	-	+	+	+	+
Phytosterols	+	+	+	+	+
Fixed oils and fats	-	-	-	-	-
Gums and mucilage	-	-	-	-	+
Volatile oil	-	-	-	-	-
Resin	-	-	-	-	-

PET: petroleum ether extract, CF: chloroform extract, EA: ethyl acetate extract ET: ethanol extract, AQ: aqueous extract

in the ethyl acetate, ethanol and aqueous extracts. The results of preliminary phytochemical were given in Table 5.

Based upon the preliminary phytochemical test, quantitative determination of phytoconstituents were carried out for the powdered plant material of leaves of *S. oleosa* by various standard methods and found the alkaloid 1.32 ± 0.56 % w/w, total phenol 71.92 ± 2.92 % w/w, flavonoids 9.69 ± 1.38 % w/w and saponins 1.15 ± 0.21 % w/w were present. The values were recorded from the mean of six repeated observations (Table 6).

Based upon the preliminary phytochemical studies, ethyl acetate, ethanol and aqueous extracts were chosen for TLC studies. The chromatogram was developed for these three extracts with four different solvent systems. The extracts showed distinct spots with different intensity, spots were identified with the

TABLE 6: QUANTITATIVE PHYTOCHEMICAL ESTIMATION OF POWDERED LEAVES OF *S. OLEOSA*

Experiment	Mean±SEM
Estimation of total phenols	71.92±2.92
Estimation of alkaloids	1.32±0.56
Estimation of total flavonoids	9.69±1.38
Estimation of saponins	1.15±0.21

Each value is presented as mean±SEM (n=6)

TABLE 7: THIN LAYER CHROMATOGRAPHY ANALYSIS OF POWDERED LEAVES OF *S. OLEOSA*

Extracts	Mobile phase	No of spots	Rf values	Intensity
Ethyl acetate	SS I	3	0.85, 0.87, 0.91,	#, *, #
	SS II	2	0.51, 0.56	*, +
	SS III	2	0.84, 0.92	*, *
	SS IV	1	0.92	*
Ethanol	SS I	3	0.84, 0.86, 0.90	+, +, +
	SS II	2	0.51, 0.56	+, +
	SS III	2	0.84, 0.92	*, +
	SS IV	3	0.21, 0.83, 0.91	#, *, *
Aqueous	SS I	3	0.85, 0.86, 0.90	*, +, +
	SS II	2	0.51, 0.56	*, +
	SS III	1	0.84	#
	SS IV	1	0.92	+

SS I-IV= solvent system I-IV, '#' most intense, '**' moderate intense, '+' less intense

help of various visualizing agent and UV chamber (fig. 1). The resultant spots Rf values were calculated and summarized in the Table 7. The results indicated that the leaves contained an appreciable amount of bioactive compounds. The presence of these phytochemicals especially the phenols and flavonoids might explain the use of *S. oleosa* in ethnomedicine for the management of various ailments.

The present pharmacognostic data accentuated the awareness of quality and identity of the plant *S. oleosa*. The qualitative and quantitative parameters showed the important information of the plant. These studies revealed the presence of various important bioactive compounds and proved that the plant leaves can also be of medicinal importance. The plant being a morphologically variable species, these information will also be helpful to differentiate *S. oleosa* from the closely related other species. These results may help in standardization, identification and in carrying out further research in *S. oleosa* leaf-based drugs, which are used in traditional and modern pharmacopoeia.

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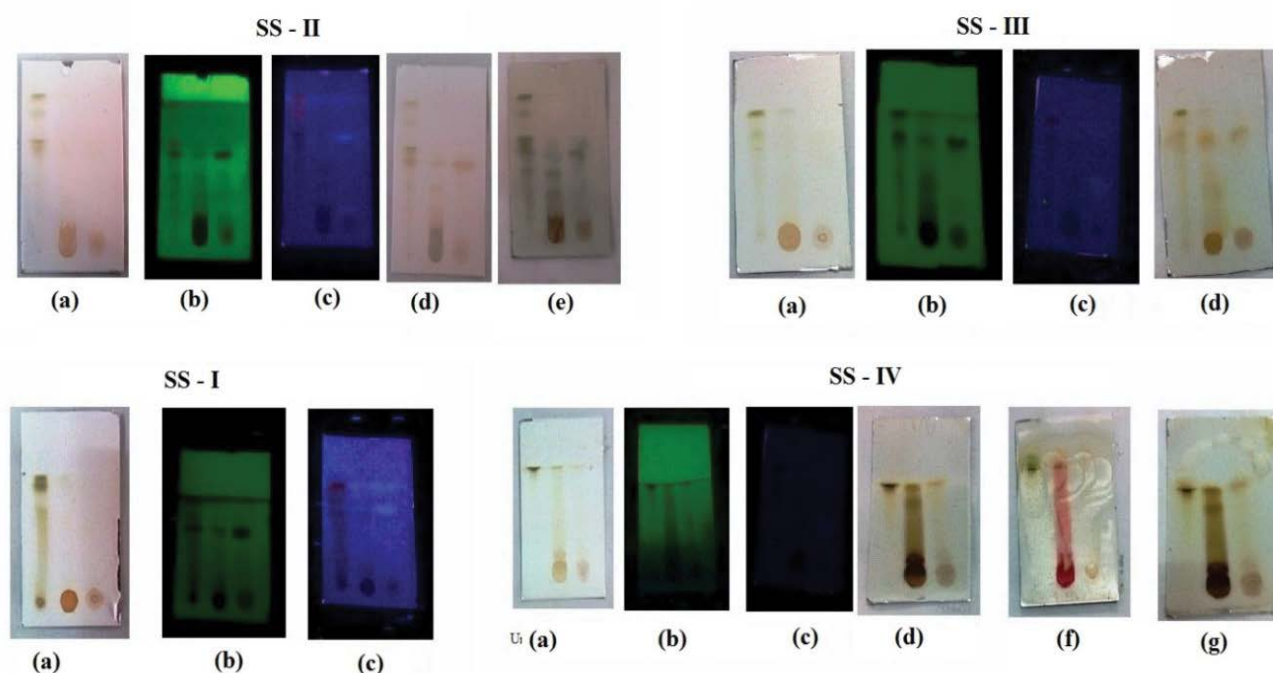


Fig. 1: TLC analysis of leaf extracts of *S. oleosa* in various solvent system

SS- Solvent system; a- under visible light; b- under UV short wave length; c- under UV long wave length; d- under NH_3 spray; e- under Folin-Ciocalteu spray, f- under vanillin HCl spray; g- under potassium hydroxide in ethanol spray

Conflicts of interest:

There are no conflicts of interest.

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REFERENCES

- Edeoga HO, Ok Wu DE, Mbaebie BO. Phytochemical constituents of some Nigerian Medicinal Plants. *Afr J Biotech* 2005;4(7):685-88.
- Akinmo-laudn AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituents and antioxidant activity of extracts from leaves of *O. gratissimum*. *Sci Res Essay* 2007;2:163-66.
- Rout SP, Choudhary KA, Kar DM, Das L, Jain A. Plants in traditional medicinal system-future source of new drugs. *Int J Pharm Pharm Sci* 2009;1(1):1-23.
- Saha D, Ramani R, Baboo B. Kusum - Multipurpose Tree, Yet Not Popular. *Science Reporter* 2010;47(02):20-2.
- Iwasa S. *Schleichera oleosa* (Lour.) Oken. In: Faridah Hanum I, van der Maesen LJG, editors. *Plant Resources of Southeast Asia No.11. Auxiliary Plants*. Bogor, Indonesia: Prosea Foundation: 1997. p. 27-229.
- Rout SD, Panda T, Mishra N. Ethno-medical plants used to cure different diseases by tribal of Mayurbhanj district of North Orissa. *Stud Ethno-Med* 2009;3:27-36.
- Mahatma SP, Sahoo HP. An ethano medico botanical study of Bolangi, Orissa, India: native plant remedies against gynaecological diseases. *Ethanobot Leaf* 2008;12:846-54.
- Varghese S, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Phytochemical screening and HPTLC finger printing analysis of *Citrullus lanatus* (Thunb.) seed. *J Acute Dis* 2013;122-26.
- https://forskning.ku.dk/find-en-forsker/?pure=files%2F34317454%2FSchleichera_oleosa_.pdf.
- Khandekar U, Bobade A, Ghongade R. Evaluation of antioxidant activity, *in vitro* antimicrobial activity and phytoconstituents of *Schleichera oleosa* (lour.) Oken. *IJBPR* 2015;6(2):137-43.
- Ghongade R. Phytochemical analysis of citrus fruit. *Int J Pharma Bio Sci* 2013;4(2B):1162-67.
- Kumar M, Mondal P, Borah S, Mahato K. Physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant *Lasia spinosa* (lour) Thwaites. *Int J Pharm Sci* 2013;5(2):306-10.
- Mohan GK, Sachin YS, Manohar VP, Dipak LR, Sanjay JS. Pharmacognostical investigation and physicochemical analysis of *Celastrus paniculatus* willd. Leaves. *Asian Pac J Trop Biomed* 2012;2(3):S1232-S36.
- Wallis TE. *Text book of Pharmacognosy*. London: J. & A. Churchill Ltd.; 1985. p. 572-75.
- Udayan D, Nair SN, Padinchareveetil SK, Palayullaparambil AKT, Juliet S, Ravindran R, *et al*. Evaluation of Phytochemical constituents, Proximate and Fluorescence analysis of ethanolic extract and its fractions of *Clerodendrum philippinum* 1 Schauer found in Wayanad region of Kerala, India. *Res J Chem Sci* 2014;4(9):1-6.
- Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J Am Pharm Assoc* 1949;38(6):324-31.
- Ahmad A, Husain A, Mujeeb M, Khan SA, Alhadrami HAA, Bhandari A. Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of *Piper nigrum* Linn fruits. *Asian Pac J Trop Biomed* 2015;5(2):101-07.
- Sharma D, Bhatia V, Patil S, Sharma PC. Antimicrobial activity of selected cryptogams from solan region. *IJBPR* 2013;4(6):448-54.
- Association of Official Analytical Chemists. Wet digestion for nonvolatile metals. In: AOAC official methods of analysis. 16th ed. Virginia: Association of Official Analytical Chemists; 1998.
- Khandelwal KR. *Practical pharmacognosy, techniques and experiments*. 17th ed. Pune: Nirali Prakashan Publishers; 2007. p. 149-56.
- Anonymous. *Indian Pharmacopoeia*. Vol. I. Controller of Publications, 7th ed. New Delhi: Government of India; 2014. p. 98-9.
- Mukherjee PK. *Quality control of herbal drugs*. New Delhi; Business Horizons; 2002. p. 160-219.
- Okerere O. Who guidelines for the assessment of Herbal Medicines. *Fitoterapia* 1992;63(2):99-110.
- Indian Herbal Pharmacopoeia*. Controller of Publications. 7th ed. New Delhi: Government of India; 2004. p. 493-97.
- Trease GE, Evans WC. *Pharmacognosy*. 11th ed. London: Bailliere Tindall Ltd.; 1978. p. 60-75.
- Harborne JB. *Phytochemical methods— A guide to modern techniques of plant analysis*. 2nd ed. London: Cassell and Collier Macmillan Publishers Ltd.; 1984. p. 9-15.
- Raman N. *Phytochemical Techniques*. New Delhi: New India Publishing Agency; 2006. p. 19-24.
- Harborne JB. *Phytochemical methods*. 1st ed. London: Chapman and Hall Ltd.; 1973. p. 49-188.
- Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global J Pure Appl Sci* 2001;8b:203-08.
- Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pac Sci* 1974;48:458-63.
- Dewanto V, Xianzhong Wu, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric Food Chem* 2002;50:3010-14.
- Medini F, Fellah H, Souri RK, Abdelly C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. *J Taibah Univ Sci* 2014;8:216-24.
- Kumari A, Sharma RV. Estimation of total phenol, falconoid contents and DPPH free radical scavenging activity of *Oxalis corniculata* linn. *IJBPR* 2015;6(3):178-81.
- Sandhya M, Nidhi R, Sudhanshu, Menghani E. Determination of natural compounds in dasmool extracts by thin layer chromatography and high pressure liquid chromatography. *Int J Res Ayu Pharm* 2012;3(6):814-17.
- Harborne JB. *Phytochemical methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall Ltd.; 1998. p. 40-96.
- Bladt S. *Plant drug analysis: A Thin Layer Chromatography Atlas*. Heidelberg, Germany: Springer-Verlag Berlin Heidelberg; 1996. p. 350-64.