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ETHNOBOTANICAL AND PHYTOCHEMICAL ANALYSIS OF *SCHLEICHERA OLEOSA* (LOUR.) OKEN

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ABSTRACT

1.*Schleichera oleosa* (Lour.) Oken is a medicinal plant belongs to family Sapindaceae, commonly found in tropical and subtropical regions of the world. The leaves, bark, seeds and other parts are used against various diseases in the Indian traditional medicinal system. The oil extracted from the seeds is used against skin diseases, acne, burns, rheumatism and for promoting hair growth. The bark of plant is used as an astringent, antipyretic agent and in treatment of ulcers and boils. The leaves are being the most potential and supportive organs of the plant used against various bacteria and helminthes. The leaves of *S. oleosa* were extracted in water, ethanol, methanol and chloroform for the analysis of phytochemicals. The percentage yield obtained through Soxhlet extraction in ethanol, methanol, chloroform for leaves were 16.3%, 15.93% and 11.45% respectively, while the percentage yield for aqueous extract was 6.3%. The phytochemical analysis revealed the presence of saponins, tannins, steroids, terpenoids, carbohydrates, cardiac glycosides and various other phytochemicals in varying solvents. The present study is undertaken to ascertain the possibilities of various phytochemical constituents responsible for medicinal activities of leaves of *S.oleosa*. The potential of phytochemical analysis in leaves and its ethnomedicinal uses suggests that *S. oleosa* could serve as a potential natural source of traditional medicine.

KEYWORDS: Ethnobotany, Phytochemicals, *Schleichera oleosa*.

2. INTRODUCTION

The *S. oleosa*, popularly known as Kusum is a well reputed deciduous to evergreen tree up to 40 metres tall having compound and paripinnate leaves. Inflorescence is axillary panicles, flowers are polgamodioecious and subsessile. Fruits are 1-2 seeded, ellipsoidal to sub globular berry. The tree consists of branched tap root system. Different parts of this plant have been used in indigenous system of medicine since ancient times (Iwasa, 1997, Kundu, 2011). The local inhabitants utilize the plant parts in various forms such as medicines, food, fuel, fodder, timber, agricultural implements, etc.

The presence of various types of phytochemicals such as tannins, alkaloids, glycosides, volatile oils etc. showed that the plant possesses considerable medicinal importance. The storage organs of the plant contains these active principles (Khyade *et al*., 2009, Tiwari *et al*., 2010). *S. oleosa* is an important multipurpose ethnomedicinal plant with basic and applied importance to civil as well as tribal society.

2.1 Study Area

The Gorakhpur region is situated along the banks of river Rapti between 26°5'-27°29' N latitude and 84°4' -84°26' E longitudes in Uttar Pradesh. The Kushmi forest is located in Gorakhpur region with luxuriant vegetation. The map of India showing Uttar Pradesh, Gorakhpur region (33) and the study area, Kushmi forest (fig.1, 2, and 3).

The *S. oleosa* found in the region is widely used as traditional medicine in home of various land dwellers and tribals. Reports also revealed that the plant is used as folk medicine by local and tribal people for the treatment of leucorrhea (Sahu *et al*., 2010), rheumatism (Rout *et al*., 2009, Mali, 2012) and skin diseases (Merlin and Narasimhan, 2009).The present study is undertaken to characterize the ethnobotanical and biofunctional properties of the plant as well as to analyse the phytochemical constituents of leaves of this plant. The various parts of the plant shown in fig. 4, 5 6, 7 and 8.

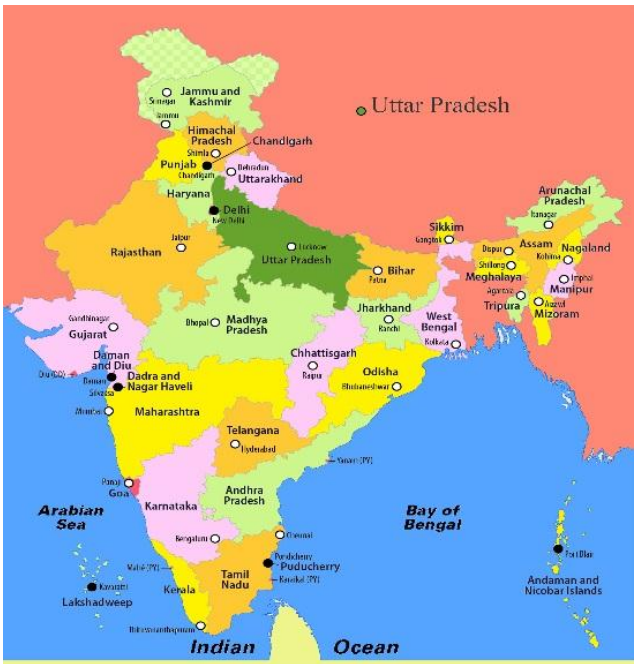


Fig 1 State and Union territories of India

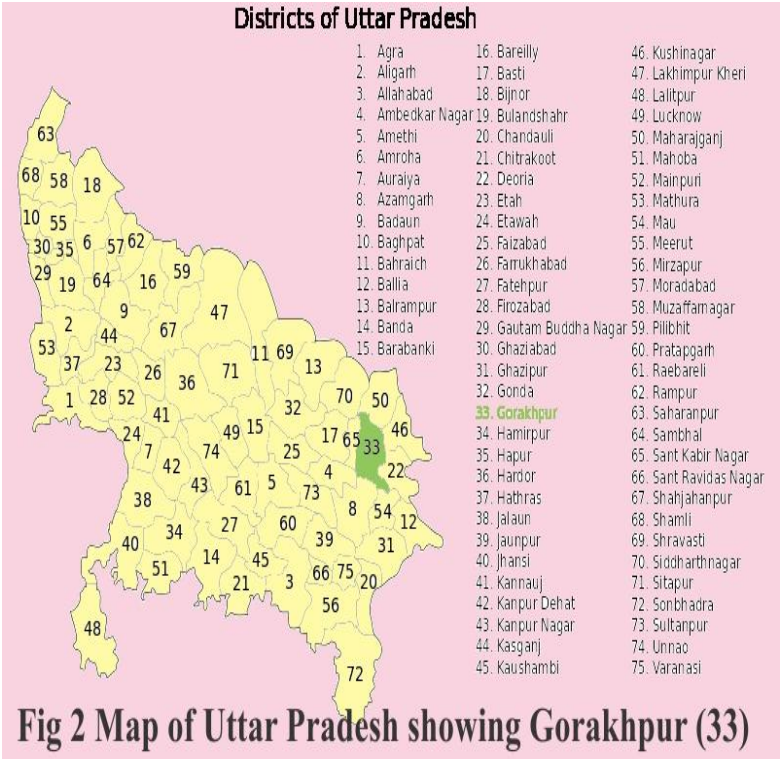


Fig 2 Map of Uttar Pradesh showing Gorakhpur (33)

Fig 3 Map of Gorakhpur showing Kushmi Region


Fig: 4 Young roots of *S.oleosa*


Fig: 5 Leaflets of *S. oleosa*


Fig: **6** Mature fruits of *S. oleosa*


Fig:7 Fruit pulp of *S. oleosa*


Fig:8 Seeds of *S. oleosa*

3. MATERIALS AND METHODS

3.1 Collection and Identification: The mature leaves were collected from Kushmi forest, Gorakhpur in the month of September-October 2014 and 2015 and identified with the expertise available in the Department and from the Herbarium, Department of Botany, DDU Gorakhpur University, Gorakhpur.

3.2 Ethnobotanical study: Extensive survey for collection of samples were carried out in the year 2014-15 and 2015-16 in the Kushmi forest and its adjoining areas. A number of local villagers, hamlets and Vaidyas were contacted to gather the information about the ethnomedicinal uses of plant. The areas under survey visited several times to collect the plant specimen/sample. The samples were collected for herbarium preparation and for scientific identification by using standard procedures (Jain and Rao, 1977).

3.3 Extraction of Plant Sample: The fresh leaves were washed well with running tap water and then with distilled water. The sample was shade dried for a period of 8-10 days at an ambient temperature of 34ºC, then the dried sample were pulverized. The powdered sample were extracted with different solvents viz. distilled water, ethanol, methanol and chloroform in Soxhlet Apparatus. The extract was concentrated using rotatory evaporator and was used for further phytochemical analysis. Phytochemical analysis viz. Carbohydrates by Benedict's test (Bhandary *et al*., 2012) and Molisch's test (Sofowara, 1993); Protein by Biuret test (Somkumar and Kamble, 2013), Xanthoproteic test (Somkumar and Kamble, 2013); Saponin by Foam test (Salehi surmaghi *et al*., 1992); Tannin and Phenolic compound by Ferric chloride test (Aiyegoro and Okoh, 2010); Alkaloids by Hager's test (Bhandary *et al.*, 2012) and Dragendroff's test (Sheela, 2013); Glycosides by Legal's test (Harborne, 1973) and Keller Killani test (Ajaiyeobu, 2002); Phytosterols by Salkowski test (Palanisamy *et al*., 2012); Steroids and Terpenoids by Libermann Burchard's test (Harborne, 1973, Thamaraiselvi *et al*., 2012); Coumarin by Fluorescence test (Geisman, 1962) and Flavanoids by Alkaline Reagent test (Sharma *et al*., 2013).

3.4 Percentage Yield in Different Solvents: The percentage yield in different solvents were determined according to Okigbo (2009):

$$\text{Percentage yield} = \frac{\text{Weight of sample extract obtained (g)}}{\text{Weight of powdered sample used (g)}} \times 100$$

3.5 Preliminary phytochemical Analysis of Prepared Crude Extracts (PCE)

3.51 Test for Carbohydrates

Benedict's test: The prepared test solution boiled in the water bath along with the few drops of Benedict's reagent (alkaline solution containing cupric citrate complex). The formation of reddish brown precipitate showed a positive result for the presence of carbohydrate (Bhandary *et al*., 2012).

Molisch's Test: The extract was dissolved in distilled water and filtered. Then, two drops of alcoholic α-naphthol was added to the test tube containing filtrate. Appearance of a violet ring at the junction confirmed the presence of carbohydrates (Sofowara, 1993).

3.52 Test for Protein

Biuret Test: One drop of 2 per cent of copper sulphate was added to 2 ml of the test solution. Then, 1 ml of 5 per cent ethanol was added followed by addition of excess of potassium hydroxide pellets. The presence of protein was indicated by formation of pink to violet colour in the ethanolic layer (Somkumar and Kamble, 2013).

Xanthoproteic Test: Few drops of concentrated Nitric acid was added to the extract. The presence of protein was indicated by formation of yellow colour (Somkumar and Kamble, 2013).

3.53 Test for Saponin

Foam Test: Five hundred mg of the extract was added to 2 ml of distilled water and then shaken together. If the produced foam remained for 10 minutes, it indicated the presence of saponin (Salehi surmaghi *et al*., 1992).

3.54 Test for Phenolic Compound

Ferric Chloride Test: Fifty mg of the extract was mixed with 5 ml of distilled water and few drops of neutral 5 per cent ferric chloride solution were added. The formation of dark green or bluish black colour indicated the presence of phenolic compound (Aiyegoro and Okoh, 2010).

3.55 Test for Tannin

Ferric Chloride Test: Ten ml of the extract was filtered through filter paper and treated with few drops of 1 per cent ferric chloride ($FeCl_3$) reagent. The formation of a bluish black colour indicated the presence of tannin (Aiyegoro and Okoh, 2010).

3.56 Test for Alkaloids

Dilute hydrochloric acid was added to the extract, dissolved and filtered. The prepared filtrate was used for further test.

Hager's Test: A few drops Hager's reagent (saturated picric acid solution) was added to the prepared filtrate. The appearance of yellow precipitate showed the presence of alkaloid (Bhandary *et al.*, 2012).

Dragendroff's Test: Few drops of Dragendroff's reagent (Potassium Bismuth Iodide solution) was added to the filtrate. The presence of alkaloids was confirmed by formation of orange- brown to red precipitate (Sheela, 2013).

3.57 Test for Glycosides

Legal's Test: The extract was treated with sodium nitroprusside containing sodium hydroxide and pyridine. An appearance of pink to blood red colour indicated a positive test for presence of cardiac glycosides (Harborne, 1973).

Keller Killani Test: Glacial acetic acid (2 ml) containing one drop of $FeCl_3$ solution was added to 5 ml of the extract. To this solution, added 1 ml of concentrated sulphuric acid. A brown ring formed at the junction is the characteristics of cadinolides. Below this ring a violet ring appeared and along with it a bluish green coloured ring would also appear in acetic acid layer which indicated the presence of cardiac glycosides (Ajaiyeobu, 2002).

3.58 Test for Phytosterol

Salkowski Test: The extract was mixed with chloroform and filtered. Then, few drops of sulphuric acid is added and filtrate is allowed to stand for sometimes. A positive result for the presence of triterpenes would be indicated by formation of golden brown colour (Palanisamy *et al*., 2012).

3.59 Test for Steroids

Libermann Burchard's Test: Few ml of chloroform was added to the extract and filtered. Then, few drops of acetic anhydride was added to the filtrate, boiled and cooled. The formation of dark pink or red colour by addition of concentrated sulphuric acid indicated a positive result for presence of steroids (Harborne, 1973).

3.60 Test for Terpenoids

Libermann Burchard's Test: Few ml of chloroform was added to 1 ml of extract. Then, treated with acetic anhydride and few drops of sulphuric acid. The formation of dark green colour indicated the presence of terpenoids (Thamaraiselvi *et al*., 2012).

3.61 Test for Coumarin

Fluorescence Test: Five ml of the extract filtrate was taken in a test tube and covered with a filter paper saturated with sodium hydroxide. Then, the test tube containing filtrate was boiled in a water bath for 10 minutes. The filtrate was then, exposed to UV light. A green bright yellow colour indicated the presence of Coumarin (Geisman, 1962).

3.62 Test for Flavonoids

Alkaline Reagent Test: Few drops of sodium hydroxide solution were added to the extract. An intense yellow colour was formed which become colourless on adding dilute acid. The result revealed the presence of flavonoids (Sharma *et al*., 2013).

4. RESULTS AND DISCUSSION

The Ethnomedicinal uses of various parts of *S. oleosa*, the month of availability and the formulation of various parts used in traditional medicine viz., root, bark, leaves, seeds, flowers and fruits were given in Table 1. The account of

ethnomedicinal uses of *Schleichera oleosa* against various diseases like arthritis, leucorrhea etc. as well as in treatment of various disorders related to tendons, nerves, joints, body inflammations, itches, burns, in curing cold fever and as appetite stimulant.

Table 1: Ethnomedicinal uses of various biofunctional parts of *Schleichera oleosa* (Lour.)Oken

Plant parts used	Month of Availability	Ethnomedicinal uses	Formulation
Root	Whole year	1. The aqueous root paste extract is applied externally to cure any disorders related to muscles, tendons, joints, bone fractures and nerves. 2. The paste of root is also used cure female disorders such as leucorrhea.	Externally in the form of paste.
Bark	Whole year	1. The paste is applied externally to reduce fever, skin diseases, body pain, fractured bones, sprains and also as an astringent. The external application of the paste on wound is also effective in healing it. 2. The bark decoction is considered as a cure for malaria and also reduces abnormal swelling of tissues. 3. The powdered bark is taken orally to reduce the inflammations of intestine and also provide relief from ulcers. 4. The bark along with water is used in reducing the infestation caused by mites.	Externally in the form of paste. Taken in the form of decoction. Bark powder is taken orally. Aqueous extract is applied externally.
Leaf	Whole year	1. The juice of the leaves is mixed with honey and taken orally to reduce helminthes. 2. Leaf in the form of paste is used treat various diseases caused by bacteria.	Juice is taken orally. Leaf paste is applied externally.
Seed	March-August .	1. The seed oil is massaged externally on the body for 15 minutes to reduce cold fever. 2. The oil is also applied to keep body the warm during winter season. It is also used to relieve body pain and for proper circulation of blood in the body. 3. Oil is also applied on joints to treat arthritis, skin to reduce itching, burns and oil also relieves constipation. 4. The oil is used to treat the defects related to excessive deposition of uric acid in blood. 5. The seed paste is applied on the body of animals to protect them from worms. 6. The seed oil makes the hair thick and promotes its growth, when applied on it. 7. Oil also improves strength and immunity.	Oil is applied externally on different parts of body.
Flower	January-August	1. The flower paste is used as a cure for snake bite. 2. Flowers are applied on the hair as a hair tonic.	Externally in the form of paste.
Fruit	March-August	1. Fruits are eaten in severe cases of hyperthermia and valued as appetite stimulant. 2. Fruit paste is used in skin itching.	Fruits are eaten and paste is applied externally.

The survey revealed the percentage of ethnomedicinal uses of the seeds, bark, roots, fruits, flowers and leaves as represented in fig. 9. The maximum use of seeds (35%), followed by bark (25%), roots (16%), fruits (14%), flowers (6%) and leaves (4%). The colour, consistency and percentage yield of leaf extract in different solvents viz., aqueous, ethanol, methanol and chloroform shown in table 2.

Phytochemical Analysis of extracts of leaves of *S. oleosa* shown in table 3.The phytochemical analysis of leaves of *Schleichera oleosa* revealed the presence of various types of phytochemicals such as carbohydrates, tannins, saponins. The carbohydrate, phenolic compound, tannin, and coumarin were present in three extracts viz. aqueous, methanolic and ethanolic leaf extract (a typical photograph for test for phenolic compound and tannin in leaves of *S. oleosa* is shown in fig. 12 and fig. 13.), saponin is present in ethanolic and methanolic extract (a typical photograph of test for saponin in leaves of *S. oleosa* is shown in fig. 11), while glycoside is found in aqueous and ethanolic leaf extract. The presence of phytosterols was noticed in ethanolic and methanolic extracts (a typical photograph of phytosterol test in leaves of *S.oleosa* shown in fig. 14), steroids in methanolic and chloroform extract and terpenoids in methanolic and chloroform extract (The photograph of test for steroids and terpenoids shown in fig. 15). Alkaloid and flavonoid is found only in ethanolic extract. The protein was totally absent in all extracts. The basic group structure of some of the bioactive molecules of the plant shown in fig. 16.

The ethanolic extract of the plant is generally more potent in comparison to other solvent extracts of the leaves. This is probably due to active biomolecules of the leaves dissolve more readily in and were better extracted in ethanol than other solvent extracts. The preliminary phytochemical test is helpful in finding chemical constituents of leaves that may lead to their quantitative estimation and bioprospection.

The result indicate that the leaves of *S. oleosa* is a promising herbal reservoir containing important phytochemicals which yielded diverse secondary metabolites that were responsible for many medicinal and pharmaceutical properties.

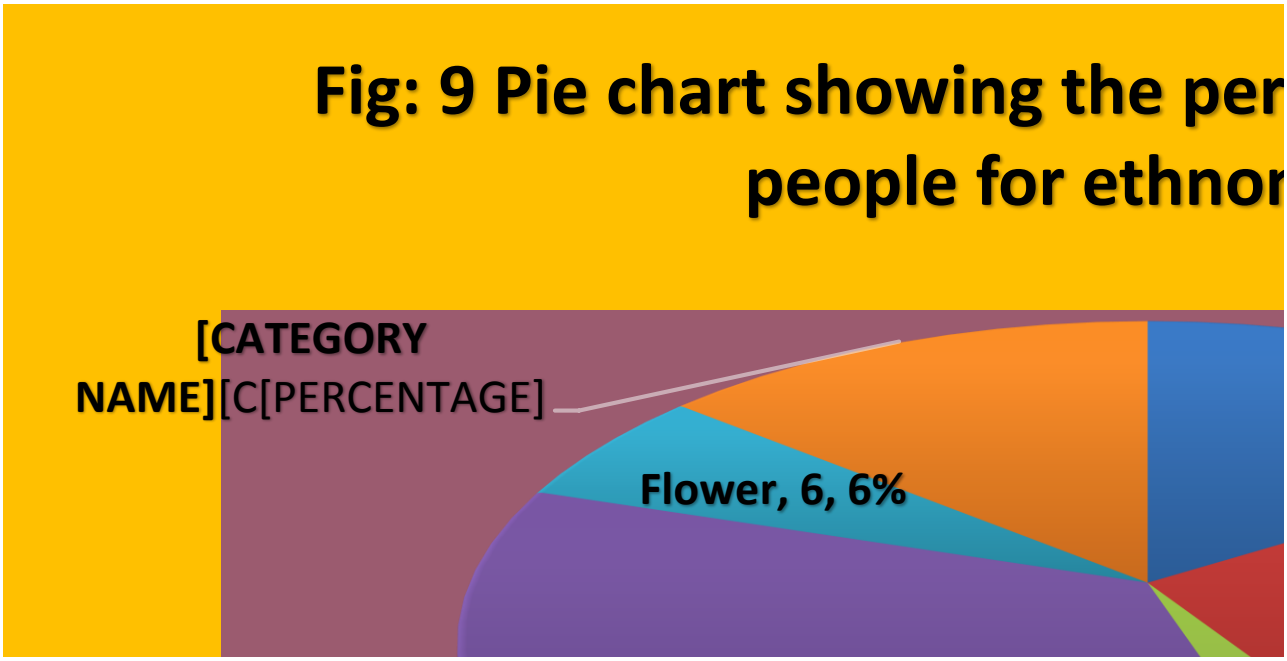


Table-2: Colour, consistency and percentage yield of the leaf extract in different solvents

S.No.	Solvents	Colour of extract in solvent	Consistency	Percentage yield (%)			
				R_1	R_2	R_3	Average
1.	Aqueous	Dark red	Non-sticky	6.15	6.3	6.45	6.3
2.	Ethanol	Intense green	Non-sticky	16.3	16.45	16.15	16.3
3.	Methanol	Dark green	Non-sticky	15.75	15.9	16.15	15.93
4.	Chloroform	Greenish black	Slightly sticky	10.6	11.5	12.25	11.45

Where, R_1= 1st replicate, R_2 = 2nd replicate, R_3 = 3rd replicate.

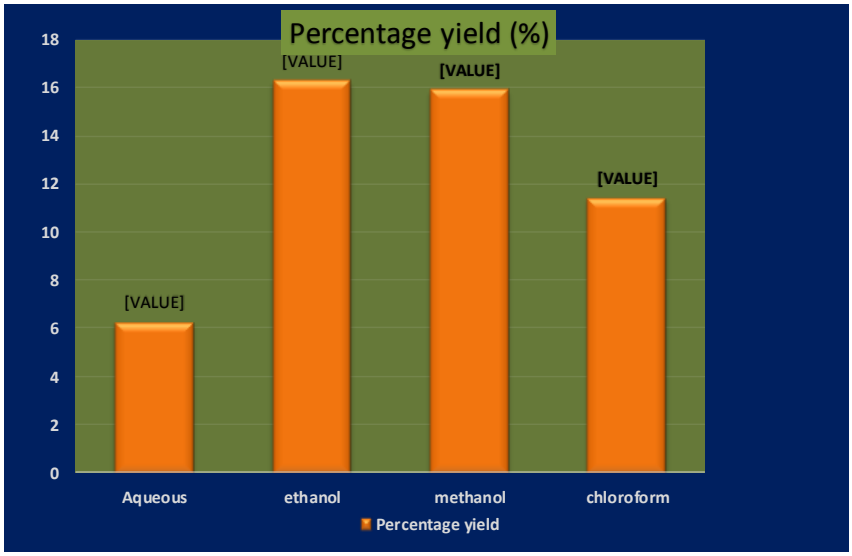


Fig 10: The graph represents percentage yield of leaf extract in different solvent.

Table-3: Phytochemical analysis of extracts of leaves of *Schleichera oleosa* (Lour.) Oken

S.No.	Phytochemicals	Test Performed	Leaf Extracts in Different Solvents			
			Aqueous	Ethanol	Methanol	Chloroform
1.	Carbohydrate	Benedict's test	+	+	-	-
		Molisch's test	+	-	+	-
2.	Protein	Biuret test	-	-	-	-
		Xanthoproteic test	-	-	-	-
3.	Saponin	Foam test	-	+	+	-
4.	Phenolic compound	Ferric chloride test	+	+	+	-
5.	Tannin	Ferric chloride test	+	+	+	-
6.	Alkaloid	Hager's test	-	+	-	-
		Dragendroff's test	-	-	-	-
7.	Glycoside	Legal's test	+	+	-	-
		Keller Killani test	+	+	-	-
8.	Phytosterol	Salkowski test	-	+	+	-
9.	Steroids	Libermann Burchard test	-	-	+	+
10.	Terpenoid	Libermann Burchard test	-	+	-	+
11.	Coumarin	Fluorescence test	+	+	+	-
12.	Flavonoid	Alkaline Reagent test	-	+	-	-

Fig: 11 Test for Saponin (Foam test)

Fig: 12 Test for phenolic compound (Ferric chloride test)

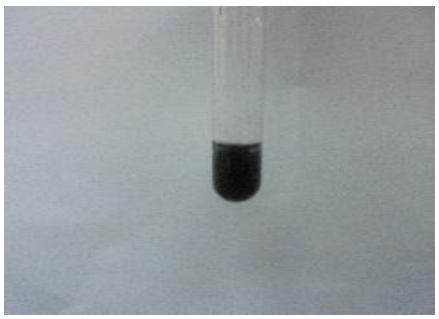


Fig: 13 Test for Tannin (Ferric chloride test)

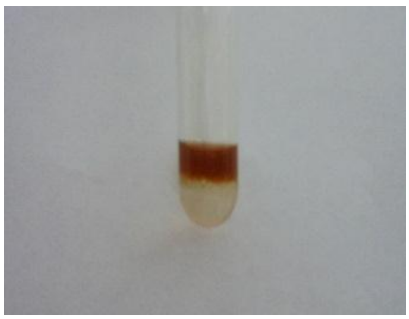


Fig: 14 Test for phytosterol (Salkowski test)

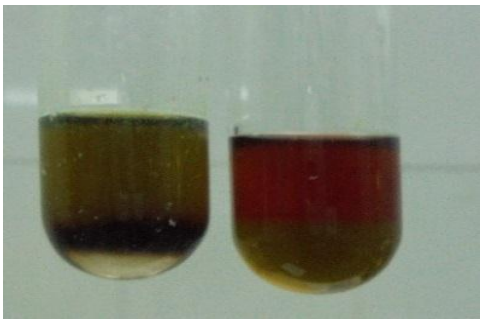
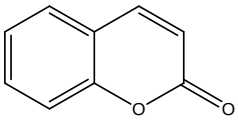
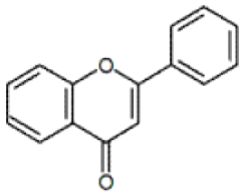


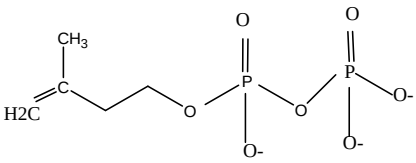
Fig: 15 Test for steroid (left), test for terpenoid (right) (Libermann Burchard's test)



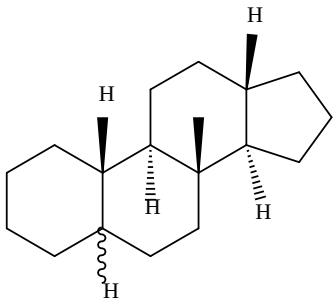
(a) Coumarin



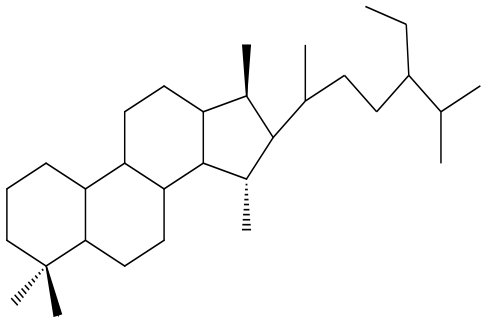
(b) Flavonoid



(c) Terpenoid



(d) Steroid



(e) Phytosterol

Fig. 16 Basic group structure of some of the bioactive compounds of *Schleichera oleosa* (a) Coumarin (b) Flavonoid (c) Terpenoid (d) Steroid (e) Phytosterol

5. CONCLUSION

The present ethnomedicinal study on the plant revealed that the local people and forest dwellers used the different parts of the plant in manufacturing crude drugs to treat their different diseases. The preparations are also very beneficial to local people due to secondary metabolites. The presence of secondary metabolites provide a new avenue to explore the knowledge of the medicinal plant for bioprospection thorough investigation is also needed. The phytochemical analysis of leaf extracts showed the potential of secondary metabolites. The ethanolic and methanolic leaf extract contains more phytochemical constituents i.e., carbohydrate, saponin, phenolic compound, tannin, alkaloid, glycoside, Phytosterol, steroids, terpenoids, coumarin, flavonoid and considered to be beneficial for further investigation. It suggests that the bioactive compounds could be used in pharmaceutical research and to develop new drugs for different ailments.

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