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Research Article

Pharmacognostic and Preliminary Phytochemical Studies on Leaf Extracts of *Chloroxylon swietenia*

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ABSTRACT

Pharmacognostic studies were conducted on leaf extracts of *Chloroxylon swietenia DC* and were shown in figures1 to 8 and Tables1 to 4. Preliminary Phytochemical analysis of the test extracts of *Chloroxylon switenia* were determined by standard protocols. Thus the qualitative chemical testing indicated that the methanolic extract, hexane, ethyl acetate fractions of *Chloroxylon switenia* leaves were found to possess glycosides, carbohydrates, alkaloids, sterols, flavoniods considerably. All the four extracts does not contain glycosides, proteins, amino acids, fixed oils, gums and mucilages as per the preliminary qualitative tests performed. The results were given in the Table 5.The Quantified phenolic contents of *Chloroxylon swietenia* extracts were ranging from 16.42±0.36 to 26.38±0.18 (mg/gm). The methanolic extract has more phenolic content i.e 26.38±0.18. The alkaloid content ranging from 14.28±0.42 to 30.23± 0.38. The methanolic extract has more alkaloid content i.e 30.28±0.38. The results were showed in Table 6**.**

Keywords: *Chrloroxylon swietenia*, Macroscopy, Microscopy and Powder analysis

INRODUCTION

Chloroxylon swietenia DC (East Indian satin wood) is a medicinal, hardwood and aromatic tree, common in dry deciduous forests throughout Indian peninsula and in Ceylon, native to south India and Sri Lanka and commonly known as Ceylon Satinwood or East Indian Satinwood. In India, it is found wild in dry deciduous forests up to an altitude of 1100 m, extending in the north to the Satpuras and Chota Nagpur. It grows on black cotton soils, metamorphic rocks and bare rocky ground on poor soils, if they are well drained and contain a large portion of sand or gravel. It is a folklore medicinal plant and finds immense application as a phytopharmaceutical formulation for therapeutic use particularly in southern parts of India. *Chloroxylon swietenia* is a folklore medicinal plant that is commonly used for antimicrobial, antifertility, analgesic, insecticidal, antifeedant activities. The whole part of this tree has long been used in the indigenous system of medicine such as the root and bark are used as an astringent. Leaves are applied to wounds, worm infested wound of animals, fungal infection of skin, and for the treatment of inflammation related disorder like pain and rheumatism. Earlier studies have shown that the extract of plant posses antifeedant, antifertility, larvicidal, mosquito repellent, anti-inflammatory, antimicrobial, hepatoprotective and antioxidant activity. Ceylon Satinwood is used in folk medicine in Chattisgarh. In case of a problematic wound, the dried leaves of Ceylon Satinwood are applied on wound in order to increase the healing process. *Chloroxylon swietenia* is a folklore medicinal plant that is commonly used for antimicrobial, antifertility, analgesic, insecticidal, antifeedant activities. The whole part of this tree has long been used in the indigenous system of medicine such as the root and bark are used as an astringent. Leaves are applied to wounds, worm infested wound of animals, fungal infection of skin, and for the treatment of inflammation related disorder like pain and rheumatism. Earlier studies have shown that the extract of plant posses antifeedant, antifertility, larvicidal, mosquito repellent, anti-inflammatory, antimicrobial, hepatoprotective and antioxidant activity. Ceylon Satinwood is used in folk medicine in Chattisgarh. In case of a problematic wound, the dried leaves of Ceylon Satinwood are applied on wound in order to increase the healing process. Stem bark pounded and the juice applied for ophthalmic infection and cataract by Malayalis.

Chloroxylon swietenia plant

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Microscopy

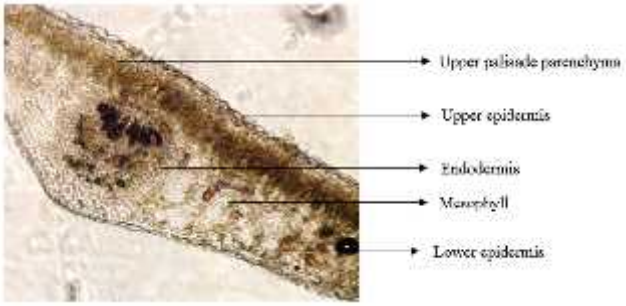

Fig. 1: Transverse Section of Leaf

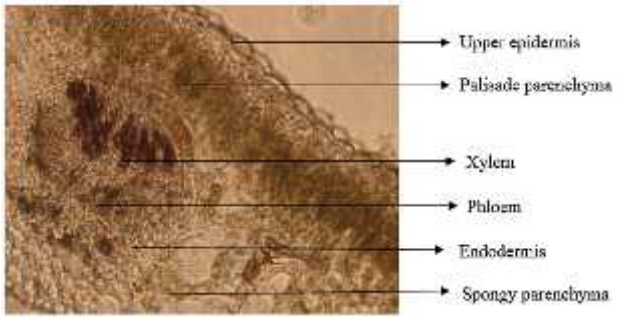

Fig. 2: Ts of Leaf Through Midrib

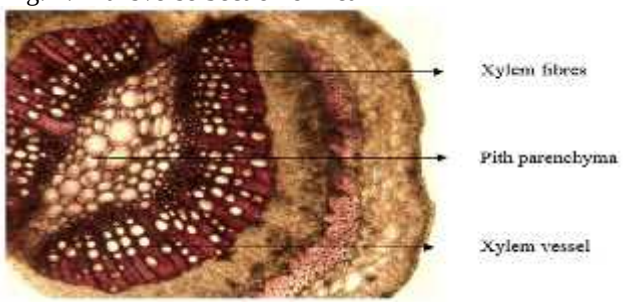

Fig. 3: Transverse Section of Stem

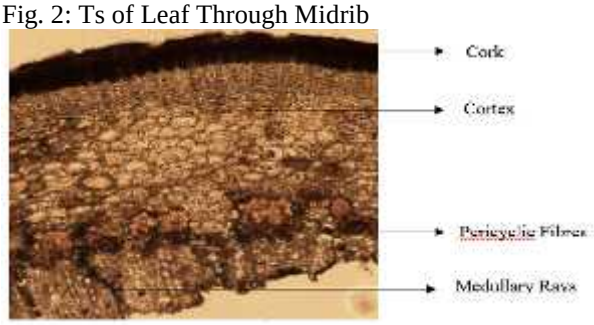

Fig. 4: Transverse Section of Bark

Powder analysis:

Fig.5: Fiber

Fig.6: Fiber

Fig.7: Scalariform vessel

Fig.8: calcium oxalate crystals

Table 1 Physicochemical Parameters

	Parameters	Leaves
1.	Total ash value	1.71 % w/w
2.	Acid insoluble ash	0.29 % w/w
3.	Water soluble ash	0.03 % w/w
4.	Moisture content	7.8 % w/w
5.	Water soluble extractive	20.76 % w/w
6.	Alcohol soluble extractive	28.4 % w/w

Various parts of the plant are traditionally used in rheumatism. The wood produced by the tree is often a golden colour with a reflective sheen. It is used for small luxury items and as a veneer in wooden furniture. It is one of the best-known satinwoods. Chloroxylon is used for

Table 2: Fluorescence Analysis And Consistency

Extracts	Consistency	Day light	UV light
Methanol	Solid	Yellowish brown	Yellow fluorescence
Hexane	Semi solid	Dark green	Green flouroscence
Ethyl acetate	Semi solid	Dark green	Green florouscence

Table 3: details of the extraction

Plant material	Solvent used	Volume of the solvent	Weight of the extract	Percentage yield
Leaves(500g)	Methanol	2 lit	142 g	28.4%

Table 4: Details of fractionation

Weight of the methanolic extract taken (gms)	Hexane soluble extractive(gms), (% yield)	Ethyl acetate soluble extractive (gms), (% yield)	Residual methanolic extractive (gms), (% yield)
100	31.1(44)	1.6(2.37)	50.1(71.4)

Pest Management in Organic Rice Cultivation. The oil is reported to contain monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, coumarins and terpenoids. The sesquiterpene hydrocarbons were found to be the major constituents in both the oils (leaves and stem) were in the ratio of 48.64% and 33.06% respectively.

MATERIAL AND METHODS

Collection and authentication of plant material: The leaves of *chloroxylon swietenia* DC were collected from nearby chowdavaram, Turuvalu village in Visakhapatnam, in the month of February 2013. The plant material (species) was taxonomically identified and authenticated by Prof. M.Venkaiah, Department of Botany, College of Science and Technology, Andhra University, Visakhapatnam. The herbarium voucher specimen (BGR/NA/CS-2013).

Macroscopy: Ceylon Satinwood is a medium-sized deciduous tree, growing to 15-20m tall, with thick, fissured, slightly corky bark. Cylindrical stems having glaucous pinnate leaves alternately arranged and are 15-22 cm long, pinnately divided into 10-20 pairs of oblong, blunt leaflets. The flowers are small, creamy-white, produced in panicles 10-20 cm long. The Buds are round. The fruit is an oblong three-segmented capsule 2.5-4.5 cm long, containing 1-4 seeds in each segment. The wood produced by the tree is often a golden colour with a reflective sheen.

Microscopy: Microscopical techniques provide detailed information about the crude drugs by virtue of its two main analytical uses. Firstly its property to magnify permits the fine structures of minute objects to be visualized and thereby confirm the structural details of the plant drugs under evaluations. Secondly, these techniques can be used in the determination of the optical as well as micro-chemical properties of the crude drug specimen under study. Microscopical observation is based on optical phenomenon, which is governed by the optical system of the microscope and the nature of the light passing through it. This technique is mostly used for qualitative evaluation of organized crude drugs in entire and powdered forms. Microscopic evaluation also covers study of the constituents by application of chemical method to small quantities of drugs in powdered form or to histological section of the drug.

Qualitative anatomical studies were performed. Free hand transverse sections of leaf and stem and root were, studied for different microscopic characters and photographs of the sections were taken with the help of phase contrast Nikon Eclipse 80i microscope.

Powder Analysis: The shade dried leaves of the plant were powdered and powder was passed through 100 # sieve. A small amount of powder was taken onto a microscopic slide, cleared from chlorophyll by heating with chloral hydrate solution and was mounted in 50% v/v glycerol in water. This was then observed under microscope to study the characteristic features.

Physicochemical Parameters: The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials.

Determination of Total Ash: About 2 to 3 grams (accurately weighed) ground leaf powder was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450^0C) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to the air dried powder. The procedure was repeated five times to get constant weight.

Determination of Water Soluble Ash: The Total ash was boiled with 25ml of water for 5 minutes and was filtered through an ash less filter paper (Whatmann No. 41). It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble matter was weighed. The water soluble ash was calculated by subtracting the water insoluble matter from the total ash.

The total ash Determination of Acid Insoluble Ash: obtained was boiled for 5 minutes with 10%w/v dilute hydrochloric acid and filtered through an ashless filter paper (Whatmann No. 41). The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed.

Determination of Loss on Drying: For the determination of loss on drying the following method was followed. About 1-2gm of the powdered leaf was accurately weighed in a glass stoppered weighing bottle which is previously dried for 30mins in the drier. Then, the sample was gently

Table 5: Qualitative phytochemical tests

S. No	Name of the test	Observation		
		Methanolic extract	Hexane fraction	Ethyl acetate fraction
1	Tests for alkaloids			
a)	Mayer's test	++	+	-
b)	Wagner's test	++	+	-
c)	Hager's test	++	+	-
d)	Dragendroff's test	++	+	-
2.	Tests for carbohydrates			
a)	Molisch's test	-	-	+
b)	Fehling's test	-	-	+
c)	Barfoed's test	-	-	+
d)	Benedict's test	-	-	+
3.	Tests for glycosides			
a)	Borntrager's test	++	+	-
b)	Legal's test	++	+	-
c)	Keller-Kiliani test	++	+	-
4.	Tests for saponins			
a)	Foam test	-	-	-
5.	Tests for proteins and amino acids			
a)	Millon's test	-	-	-
b)	Biurettes test	-	-	-
c)	Ninhydrin test	-	-	-
6.	Tests for phytosterols			
a)	Libermann-Burchard test	++	+	++
7.	Test for fixed oils			
a)	Spot test	-	-	-
8.	Tests for phenolic compounds and tannins			
a)	Ferric chloride test	-	-	-
b)	Gelatin test	-	-	-
c)	Lead acetate test	-	-	-
9.	Tests for flavonoids			
a)	Alkaline reagent test	+	+	-
b)	Schinoda test	+	+	-
c)	Zn+HCl test	+	+	
10.	Tests for triterpenoids			
a)	Salkowski test	-	-	-
11.	Tests for gums and mucilages			
a)	Alcoholic precipitation test	-	-	-
12	Test for lignin			
a)	Lignin test	++	-	-
b)	Labat test	+	-	-

++ → *more intense,* + → *less intense,* – → *absence.*

Table 6: Total Phenolic And Alkaloid Content (Mg/Gm)

S.no	Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
1	Methanol	16.42±0.36	14.28±0.42
2	Hexane	20.43±0.42	22.42±0.43
3	Ethyl acetate	26.38±0.18	30.23±0.38

shaken side wise for even distribution and dried in an oven at 100^{O}C to 105^{O}C by removing the stopper. It was cooled and again weighed. The loss on drying was calculated with the reference to the amount of air dried powder taken.

Determination of Alcohol Soluble Extractive: 5 grams of the powder was macerated with 100ml of alcohol of the specified strength in a closed flask for 24hrs, shaking frequently during 6hrs and allowing it to stand for 18hrs. It was filtered rapidly taking precautions against loss of alcohol, and 25ml of the fiterate was evaporated to dryness in a tared bottomed shallow dish at 105^{O}C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried powder.

Determination of Water Soluble Extractive: About 5gms of the powder was added to 50ml of water at 80^{O}C and to it 2gms of keiselghur was added and filtered. 5ml of the filterate was transferred to a tared evaporating dish, 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 two hours and weighed. The percentage of water

soluble extractive was calculated with reference to air dried drug.

Fluorescence analysis and consistency: Fluorescence analysis of the drug was observed in day and UV light (245nm) using various extract of the drug. The drug powder was treated separately with different solutions. The solvents used were 1N sodium hydroxide (aqueous), 1N sodium hydroxide (alcoholic), 1N hydrochloric acid, 50%nitric acid and methanol. Then they were subjected to fluorescence analysis in day and UV light (245nm) using various extracts of the drug.

Extraction (Soxhlet Extraction Process): Freshly collected plant material (leaves) were dried under the shade and the dried material was milled (pulverized) to obtain a coarse powder weighing about 1 kg. The powdered material was then subjected to Soxhlet extraction process with

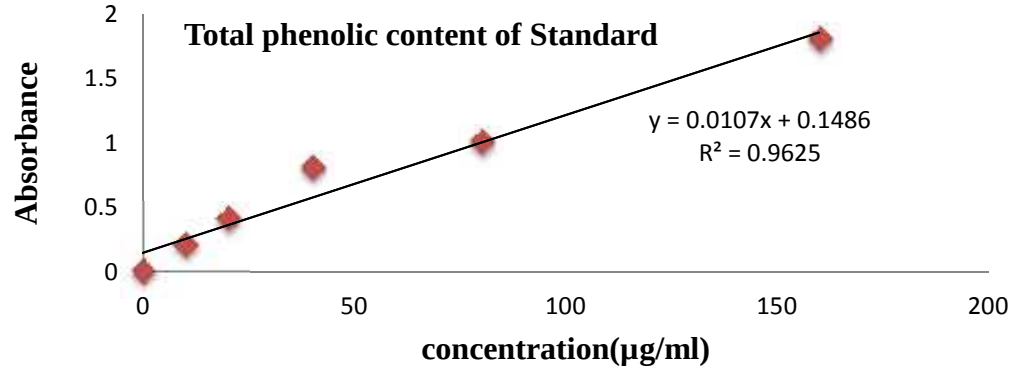

Fig.9: Caliberation Curve of Standard

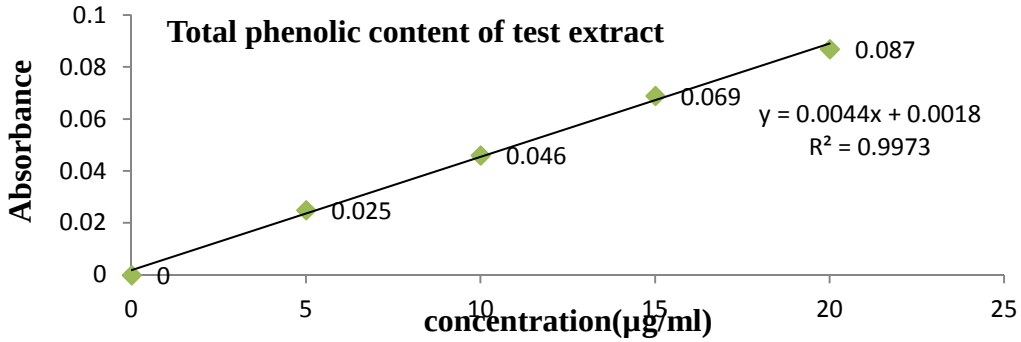

Fig. 10: Caliberation Curve of Test Extract

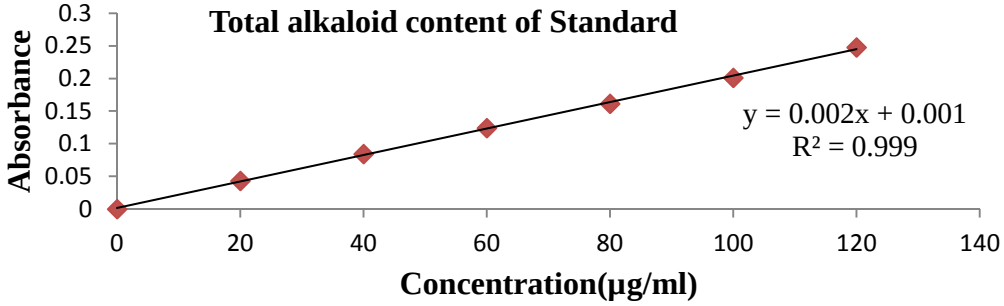

Fig. 11: Caliberation Curve for Standard

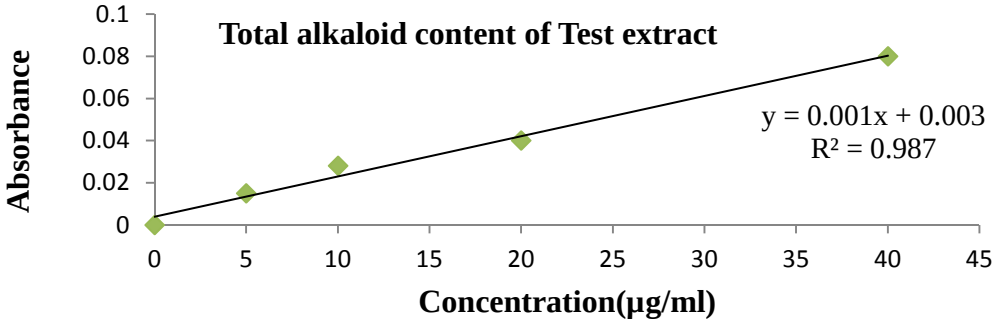

Fig. 12: Caliberation Curve for Test Extract

methanol, and further fractionation was done with hexane, ethyl acetate.

Crude Methanolic Extract: The dried powdered material about 1 kg of the leaves of the plant was extracted successively three times with methanol. The (filtrate) liquid fraction so obtained was collected and concentrated by evaporating under reduced pressure by using rotary evaporator (Buchi R-210, Switzerland) until a soft mass obtained. This extract was kept in the dessicator to remove all traces of the solvent and weighed and the percentage yield was calculated and given in Table From 28.4g of obtained methanolic extract some amount of extract was kept a side for phytochemical and pharmacological investigation and the remaining was subjected to fractionation with hexane, ethylacetate successively using separating funnel.

Hexane Fraction: The methanolic extract was dissolved in water and to this hexane was added and separated by shaking in separating funnel. Now the supernatant (hexane layer) was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210, Switzerland) until a soft mass obtained. The extract was dried in dessicator to remove all traces of the solvent and weighed and the percentage yield was calculated and given in Table 3

Ethyl Acetate Fraction: The lower layer of the separating funnel was taken and to this ethyl acetate was added and separated by shaking in separating funnel. Now the supernatant (ethyl acetate layer) was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210, Switzerland) until a soft mass obtained. The extract was dried in dessicator to remove all traces of the solvent and weighed and the percentage yield was calculated and given in Table3 and 4

Preliminary Qualitative Phytochemical Screening By Chemical Tests [1-7]: The crude methanolic extract, hexane, ethyl acetate fractions obtained as explained earlier were subjected to qualitative chemical tests for the identification of chemical constituents. The results were shown in the table 5. The extracts were dissolved in methanol, hexane, ethyl acetate and the following tests were carried out.

Tests for Alkaloids: About 50 mg of solvent – free fraction was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

Mayer's Test: To 1 ml of filtrate, two drops of Mayer's reagent was added along with the sides of the test tube. If the test is positive, it gives white or creamy precipitate.

Wagner's Test: To 1 ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish-brown precipitate confirms the test as positive.

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

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

Tests for Carbohydrates: About 100 mg of the fraction was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

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

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

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

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

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

Borntrager's Test: To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken. Chloroform layer was separated and 10 % ammonia solution was added to it. Formation of pink colour indicates the presence of anthroquinone glycosides.

Legal's Test: About 50 mg of the fraction was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10 % sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink colour.

Tests for Saponins

Foam or Froth Test: 1 ml of the fraction was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two-centimeter layer of foam or froth that is stable for 10 minutes indicates the presence of saponins.

Tests for Phytosterols and Triterpenoids

Libermann–Burchard test: 1ml of fraction was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet colour at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

Salkowoski Test: Few drops of concentrated sulphuric acid were added to the alcoholic fraction shaken on standing. Red colour in the lower layer indicates the presence of steroids and golden yellow colour indicates the presence of triterpenoids.

Tests for Phenolic compounds and Tannins

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

Gelatin Test: 1 ml of fraction was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10%

sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

Lead acetate Test: 1ml of fraction was dissolved in distilled water and to this 3 ml of 10% lead acetate solution was added to this. A bulky white precipitate indicates the presence of phenolic compounds.
Alkaline reagents: 1ml of aqueous solution of fraction was treated with 10% ammonium hydroxide solution–yellow fluorescence indicates the presence of flavonoids.
Shinoda test or Magnesium – Hydrochloric acid reduction: 1ml of the fraction was dissolved in alcohol and a few fragments of magnesium turnings and concentrated hydrochloric acid were added drop–wise. If any pink or crimson–red colour develops, the presence of flavonol glycoside is inferred.
Test for quinines: To 1 ml of the fraction, 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.
Quantitative Estimation of Total Phenolic And Alkaloid Contents [2]
Quantification of Total Phenolic Content: Total phenolic content was determined using Folin- Ciocalteau reagent. Folin- Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm. The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, the concentration of phenolic content in Gallic acid equivalents using unit's mg/ gm(GAE) was measured. All experiments were performed thrice and the results were averaged and reported in the form of Mean ± S.E.M.
Quantification of Total Alkaloid Content: The plant extract (1mg/ml) was dissolved in 2N HCl and then filtered. The p^H of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5ml of BCG solution along the complex formed was extracted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice and the results were averaged and reported in the form of Mean ± S.E.M.

RESULTS

Macroscopy
Botanical name: *Chloroxylon swietenia*
Common name: satin wood tree
Leaves: paripinnate.
Leaf lets: 10-16 pairs, elliptic or lanceolate, dotted on lower surface, entire, obtuse, unequal base.
Flowers: white and are in axillary and terminal panicles.
Sepals: they are 5 and lobed.
Petals: they are 5-10 and lobed.
Stamens: they are 5, free inserted between the lobes of disc.
Ovary: 3 and is locular, ovules are 4-8 per locule, axile.
Capsules: loculicidal and oblong.
Seeds: Brown and apically winged.

DISCUSSION

Pharmacognostic studies were conducted on leaf extracts of *chloroxylon swietenia DC* and were shown in figures1 to 8 and Tables1 to 4. Preliminary Phytochemical analysis of the test extracts of *Chloroxylon switenia* were determined by standard protocols. Thus the qualitative chemical testing indicated that the methanolic extract, hexane, ethyl acetate fractions of *Chloroxylon switenia* leaves were found to possess glycosides, carbohydrates, alkaloids, sterols, flavoniods considerably.
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