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Physicochemical and phytochemical investigation of different fractions from hydroalcoholic extract of *Tectona grandis* (Linn) barks

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ABSTRACT

The objective of the present work is to carry out the physicochemical investigation of powder drug and phytochemical screening of different fractions obtained from hydroalcoholic extract of Tectona grandis (Linn) barks. Physicochemical analysis was done to determining total ash, acid insoluble ash, water soluble ash, sulphated ash, extractive value, moisture content, crude fibre content and foaming index. The inorganic elements determination and fluorescence analysis was performed as per the standard methods. Phytochemical investigations were carried out on different fraction to determine various active constituents present in it by using standard preliminary phytochemical tests. The inorganic element, such as calcium, magnesium, sodium, potassium, iron, copper, zinc and manganese were found in the hydroalcoholic extract of barks. Florescence analysis of the fractions were reported which gives the sensitivity of the chemical in different chemical reagents. Preliminary phytochemical screening of different fractions of hydroalcoholic extract showed the presence of the phytoconstituents like alkaloids, glycoside, proteins and amino acid, carbohydrates, flavonoids, terpenoids, tannins, saponins and sterols. However the volatile oil, fixed oil and mucilage were found to be absent. The physiochemical examination of the barks of Tectona grandis Linn (Saguan) can be used as rapid, inexpensive botanical identification and standardization. The phytochemical data obtained from the results of the Tectona grandis fractions may be useful for further pharmacological evaluation.

Key words: *Tectona grandis* (Linn.), physicochemical analysis, phytochemical screening, methanolic fraction, flavonoids.

INTRODUCTION

Tectona grandis Linn (Family: Verbenaceae) is known as Teak in English, Sagwan in Hindi and Saguan in Odia. It is a large to very large deciduous tree, 25-35m in height with light brown grey bark having sallow longitudinal furrows, fluted and buttressed base and characteristically quadrangular channelled branches; leaves simple, opposite, broadly elliptical or ovate, acute or acuminate, coriaceous, rough above, stellately-grey tomentose beneath, possessing minute glandular dots, main nerves 8-10 pairs; flowers many, white, small, sweet scented, in large erect terminal branched tomentose cymose panicles; fruits hard, bony, irregularly globose drupes enveloped by light brown bladder-like calyx; seeds usually 1-3, ovate, marble white. It is distributed in South and South-East Asia; indigenous to India, Burma and western parts of Thailand. The bark is astringent, acrid, cooling, anthelmintic, depurative and is useful in bronchitis, hyperacidity, dysentery, diabetes, leprosy and skin diseases [1, 2]. Ethanolic (70%) extracts of dried bark possess antiulcer, smooth muscle relaxant, CNS stimulant and CNS depressant activity. It is also reported to have nitric oxide scavenging activity [3, 4] However, only a few phytochemical have been reported on this plant barks in the literature. Betulin, betulin aldehyde, betulinic acid and lupeol isolated from the stem barks whereas a new 1, 4-anthraquinone derivative-9, 10-dimothoxy-2-methyl-anthra-1, 4-quinone along with dehydro-α-lapachone, lapachol, tecomaquinone I and tectoquinone are isolated from heart wood [5]. In the light of

skimpy data on phytochemical study and in view of traditional and medicinal importance the present work has been planned to investigate the physicochemical study of powder barks and phytochemical screening of different fractions obtained from hydroalcoholic extract of *Tectona grandis* (Linn) barks.

MATERIALS AND METHODS

Collection of Plant Material

The fresh barks were collected in the month of December 2011 from Sadeipur village of Jagatsinghpur district (Odisha), India and authenticated at Department of Botany, Utkal University, Odisha. A voucher specimen number IPT/PC/HM-29/2011 was deposited in the institute museum for further reference. The samples were carefully observed for presence of foreign materials, washed with distilled water and shed dried for a period of 30 days. The dried barks were made to coarse powder by a mechanical grinder and then passed through number 40 sieve mesh. The barks powder was processed in such a way that they are useful for carrying out powder studies as well as phytochemical analysis.

Organoleptic characteristics study

The Organoleptic characteristics such as colour, odour, taste, texture, shape and size of *Tectona grandis* (Linn) barks were observed [6].

Physicochemical investigation of powder barks

Coarse powder of the barks was used to perform quality control tests such as total ash, acid insoluble ash, water soluble ash, sulphated ash, extractive matter, moisture content, crude fibre content, foaming index etc.

Determination of ash values

Tectona grandis (Linn) barks powder was analysed for total ash, acid insoluble ash and water soluble ash and sulphated ash according to standard procedures [6]**.**

Determination of extractive value

For determination of extractable matter, different solvents viz. petroleum ether, ethanol and water were used as the solvents for extraction by maceration method. For preparation of petroleum-ether extract, about 4.0 g of coarsely powdered air dried material was accurately weighed and taken in a glass stoppered conical flask. 100 ml of petroleum ether was added and weighed to obtain the total weight including flask. The conical flask was corked and set aside for 24 h with shaking frequently. The solvent was then filtered through a dry filter paper taking care of not to lose any solvent and 25 ml of this filtrate was transferred to a tarred flat bottom dish and evaporated to dryness on a water bath. The solvent was dried at 105ºC for 6 h and cooled in a desiccator for 30 minutes, was weighed without delay. The content of extractable matter in mg per gram of air dried material was calculated. Ethanol and water extractable matter were also determined as per the procedure described above [6].

Determination of foaming index

About 1gm of coarse powder was weighed accurately and transferred to a 500 ml conical flask containing 100ml of boiling water. Moderate boiling was maintained for 30 minutes, cooled and filtered into a 100ml volumetric flask and sufficient water was added through the filter to dilute the volume. The decoction was poured into 10 stoppered test tubes (height 16cm, diameter 16 mm) in successive portion of 1 ml, 2 ml, 3 ml etc. up to 10 ml and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered the tubes and shaken in lengthwise motion for 15seconds, two shakes per second. Allowed to stand for 15 min and measured the height of the foam. The results are assessed as follows.

- If the height of the foam in every tube is less than 1cm, the foaming index is less than 100.
- If the height of the foam is 1cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1cm in every tube, the foaming index is over thousand. In this case the determination was repeated using a new series of dilutions of the decoction in order to obtain a result the foaming index Calculate using the following formula: 1000/a

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed [6].

Determination of moisture content

The dishes were washed with detergent and dried at 105^{o}C overnight. The dishes were then placed in desiccators for cooling and then weighed. Accurately weighed quantity (2.0 g by difference) of the powdered barks was placed into a weighed dish. It was placed in 105^{o}C oven overnight (with lids open until a constant weight loss). The dishes were removed, placed in desiccators and cooled. The cooled dishes were taken out of desiccators and weighed as quickly as possible [7].

Calculation:

$$\text{Moisture content (\%)} = \frac{\text{weight of fresh sample} - \text{weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

Estimation of crude fibre

The ground sample (2g) was extracted with petroleum ether to remove fat (initial boiling temperature 35-38^{0}C and final temperature, 52^{0}C). Then 2 g of dried sample were boiled with 200 ml of sulphuric acid for 30 min with bumping chips. It was then filtered through muslin cloth and washed with boiling water until the washings were free from acid. The residue was boiled with 200ml of sodium hydroxide for 30 minutes, filtered through muslin cloth again and washed with 25ml of boiling sulphuric acid. The residue was removed and transferred to pre-weighed ashing dish ('W1', g). Dried the residue for 2 h at 130 ± 2^{o}C, then cooled in a desiccator and weighed ('W2', g). It was ignited for 30 min. at 600 ± 15^{o}C, cooled in a desiccator and reweighed ('W3', g) [8].

Calculation:

Loss in weight = (W2 - W1) - (W3 - W1)

$$\text{\% crude fiber content} = \frac{\text{On the ignition}}{\text{weight of sample (g)}} \times 100$$

Fluorescence analysis

A small quantity of dried and finely powdered barks sample was placed on a grease free microscopic slide and added 1-2 drops of a freshly prepared solution such as 1M sodium hydroxide, 1M sulphuric acid, 1M hydrochloric acid. It was mixed by gentle tilting the slide and was left for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in daylight, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in various radiations were recorded [9]. The fluorescence analysis of different fractions obtained from the hydroalcoholic extract of *Tectona grandis* (Linn) barks in daylight, short (254 nm) and long (365 nm) UV radiations also observed thereafter.

Test for inorganic elements

The ash was prepared from the hydroalcoholic extract of *Tectona grandis* (Linn) barks. To the ash 50% v/v HNO_3 was added and it was kept for 1 hour or longer. It was filtered & with filtrate test for calcium, magnesium, sodium, potassium, iron, copper, zinc, manganese and chromium was performed [10].

Preparation of barks extract and fractions

The dried barks powder (2kg.) was defatted with petroleum ether (60-80°C) and then extracted with hydro-alcoholic (water: ethanol, 50: 50) solution for 72 h using a soxhlet apparatus. The liquid extract was concentrated under vacuum to yield dry extract. The dried extract fractionated successively with n-butanol, chloroform, ethyl acetate and methanol. All the fractions were concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) using a rotary evaporator. The above fractions were studied for their colour, consistency and yield values and are reported. The fractions were stored in a closed bottle and kept in refrigerator until tested. The four fractions were then subjected to phytochemical screening.

Preliminary phytochemical analysis

Pharmacological activity of herbal drug depends upon the type of constituents present in it. The different fractions obtained from hydroalcoholic extract of *Tectona grandis* (Linn) barks were screened to determine the presence of phytoconstituents by using different chemical tests as per standard procedures [11, 12].

RESULTS AND DISCUSSION

Organoleptic characteristics

The organoleptic characteristics such as colour, odour, taste, texture, shape and size of *Tectona grandis* (Linn) barks are given in **(Table 1)**. Outer surface of barks appear brown in colour, however Inner surface of barks appear light

brown to buff in colour.The bark possesses astringent or acrid taste, characteristic odour with coarse texture. It has been quadrangular, fluted shape with 5-12 mm thick in size.

Table 1: Organoleptic characters of *Tectona grandis* (Linn) barks.

Organoleptic Characters	Descriptions
Colour	Outer surface of barks appear brown in colour. Inner surface of barks appear light brown to buff in colour.
Odour	Characteristic
Taste	Astringent or Acrid
Texture	Coarse
Shape	Quadrangular, fluted
Size	5-12 mm thick

Physicochemical and phytochemical parameters of plant material

The analytical parameters of dried powdered barks studied such as ash values, extractive values, moisture content, crude fibre content, foaming index. The data obtained from the above studies are shown in **(Table 2).** The physicochemical analysis presented in this research will be beneficial in determining plant adulteration with other species.

Table 2: Physicochemical parameters of *Tectona grandis* (Linn) barks.

Sl. No.	Parameters	Values in %
1	**Ash Value**	
	Total Ash	6.98
	Acid insoluble ash	3.1
	Water soluble ash	1.48
	Sulphated ash	7.3
2	**Extractive value**	
	Pet ether	2.1
	Ethanol	7.8
	Aqueous	13.5
3	**Moisture content**	9.4
4	**Crude fibre content**	25.20
5	**Foaming index**	Less than 100

Fluorescence analysis

Fluorescence analysis of powder barks had been carried out in daylight and under U.V. light. The powders were treated with different organic solvents and solutions were again observed in normal daylight and under U.V. light. The fluorescence analysis of different fractions also observed and is given in **(Table 3).** The analysis shows the presence fluorescence, which may be attributed due to the presence of some phytochemicals under different light conditions.

Table 3: Fluorescence analysis of *Tectona grandis* (Linn) bark powder

Sl. No.	Reagent	Visible/Day light	UV 254 nm	UV 366nm
1	Powder drug as such	Light brown	Greyish brown	Yellowish brown
2	Powder + 1M NaOH	Yellowish brown	Dark Yellowish	Dark brown
3	Powder +1M H_2SO_4	Brown	Dark brown	Blackish brown
4	Powder +1M HCl	Brownish yellow	Light brown	Dark brown
Fluorescence analysis of different fractions from hydroalcoholic extract of *Tectona grandis* (Linn) barks				
I	N-Butanol	Pale green	Dark green	Pink
II	Chloroform	Greenish brown	Green	Pinkish green
III	Ethyl acetate	Pale brown	Yellowish brown	Dark yellow
IV	Methanol	Brown	Dark brown	Dark green

Table 4: Determination of inorganic element in hydroalcoholic extract of *Tectona grandis*(Linn) bark powder

Sl. No.	Test for	Inference
1	Calcium	+
2	Magnesium	+
3	Sodium	+
4	Potassium	+
5	Iron	+
6	Copper	+
7	Zinc	+
8	Manganese	+
9	Chromium	-

Inorganic element

Inorganic elements found in the ash of hydroalcoholic extract are calcium, magnesium, sodium, potassium, iron, copper, zinc and manganese reported in **(Table 4).** The inorganic elemental analysis is important as these elements play an important role in a physiological process involved in plant and animals.

Physical parameters of fractions

The colours, consistency of the four fractions were studied and the percentage yield of each fraction was determined. The results are depicted in **(Table 5).** The percentage yield obtained from n-butanol, chloroform, ethyl acetate and methanol fractions were 2.1, 3.2, 7.89, and 11.82% respectively. From the fractionation it was found that methanol fraction may contain more quantity of phytoconstituents than other fractions. Further, this indicates that the plant may contain higher quantities of polar phytoconstituents than non-polar phytoconstituents.

Table 5. Physical parameters of different fractions from hydroalcoholic extract of *Tectona grandis* (Linn) barks

Sl. No.	Fraction	Colour	Consistency	Yield % w/w
1	n-Butanol	Pale green	Waxy	2.1
2	Chloroform	Greenish brown	Greasy	3.2
3	Ethyl acetate	Pale brown	Sticky	7.89
4	Methanol	Brown	Sticky	11.82

Preliminary phytochemical analysis

All the four fractions were screened for phytochemical investigation by different phytochemical tests to check the presence or absence of a group of phytochemical constituents. These phytochemical tests shown the presence of proteins, carbohydrates, alkaloids, saponins, tannins, flavonoids, steroids, tri-terpenoids, glycosides etc. present in different fractions as mentioned in **(Table 6).** The n-butanol and chloroform fractions gave positive tests for tri-terpenoids and phytosterol; Ethyl acetate fraction gave positive results for alkaloids, tannins & polyphenols, tri-terpenoids and saponin; methanol fraction were found to contain alkaloids, carbohydrate, protein, glycoside, saponin, flavonoids and tannins & polyphenols. Preliminary phytochemicals analysis relieves the presence of more phytoconstituents in methanolic fraction than other fractions.

Table-6: Preliminary phytochemical analysis of different fractions from hydroalcoholic extract of *Tectona grandis* (Linn) barks

Phytoconstituents	n-butanol	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	+	+
Carbohydrates	-	-	-	+
Gums and mucilages	-	-	-	-
Proteins and amino acids	-	-	-	+
Tannins & polyphenolic	-	-	+	+
Steroids and sterols	+	+	-	-
Tri-terpenoids	+	+	+	-
Saponins	-	-	+	+
Flavonoids	-	-	+	+
Glycosides	-	-	-	+

CONCLUSION

The present work focuses on the physicochemical analysis of powdered barks and phytochemical screening of different fractions obtained from hydroalcoholic extract of *Tectona grandis* (Linn). As there was no phytochemical evaluation of different fractions has been established in this folklore valued drugs so the present work is carried out to investigate the phytochemicals present in it, which can be exploit for further pharmacological screening. The physicochemical data obtained from the results are useful in determining the authenticity of the procured plant part. The chemical classes present in these collected fractions are alkaloids, carbohydrates, proteins, tannin & phenolic compounds, steroids, tri-terpenoids, saponins, flavonoids and glycosides stand as a group of major importance in the new drug discovery. From the above phytochemical screening it was found that the methanolic fraction possesses more number of phytoconstituents than other fractions so it would be useful for evaluation of various pharmacological activities. The presence of phytoconstituents like flavonoids and polyphenols in the methanolic fraction also gives the inference that it can be pharmacologically more active than other fractions.

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