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Pharmacognostic Screening, Phytochemical Evaluation and In- Vitro free radical Scavenging Activity of *Acacia leucophloea* Root

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ABSTRACT

Objective: Pharmacognostic Screening and evaluate the *in-vitro* free radical scavenging activity of roots *Acacia leucophloea*. **Methods:** Pharmacognostic Standardization, Physico-chemical evaluation of the roots of *Acacia leucophloea* was carried out to determine its macro-and microscopical characters and also some of its quantitative standards. Microscopical studies were done by using trinocular microscope. Microscopically, root showed cork, cortex, stellar region and calcium oxalate crystals. Petroleum ether, ethanol, aqueous extracts of *Acacia leucophloea* were prepared, with successive extraction in soxhlet apparatus. Each extract was selected to study the free radical scavenging activity by superoxide scavenging assay method. **Results:** It was found that aqueous extract contained carbohydrates, glycosides amino acids flavonoids, tannins, alkaloids, steroids; ethanolic extract contained glycosides amino acids flavonoids, tannins, alkaloids, steroids. Ethanolic extract of *Acacia leucophloea* shows maximam inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity compared to ethanolic extract), while Petroleum ether extract showed poor inhibition of superoxide scavenging activity. **Conclusion:** The present study on pharmacognostic standardization, physico and phytochemical evaluation of *Acacia leucophloea* root might be useful to supplement information about its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

1. Introduction

Acacia leucophloea (Roxb.) Willd. (Syn. *Mimosa leucophloea*) (Mimosoideae) is a large thorny tree attaining heights of 35 m and diameters at breast height of 100 cm [1]. Its native range through South and Southeast Asia is non-contiguous. Its largest continuous distribution is arid India through Sri Lanka, Bangladesh, Burma and much of Thailand [2]. Bark of plant is used as antimicrobial, anthelmentic, expectorant and blood purifier. It is also used to treat skin diseases (leprosy), ulcer, gum bleeding, mouth ulcer, dry cough, dysentery, diabetes fever and Snake-bite [3]. Its gum and decoction of Bark is used for contraception and menstrual complaints [4]. Inner bark is used to manufacture dyes and tannins [5].

Literature revealed that pharmacognostic studies have not been reported for the roots of this plant. Therefore the main aim of the present work is to study the macro, microscopic and some other pharmacognostic characters and physic-chemical standards of roots of *Acacia leucophloea* which could be used to explore this plant.

2. Material and Method

2.1 Collection of Plant Material

The plant specimens for the study were collected from the Bastar region of Chhattisgarh, India, and were positively identified and authenticated by the Botanist Dr. N. Shiddhamallaya, Regional Research Institute (Ay.), Central council for research in Ayurveda and Siddha, Ashoka pillar, Jayanagar, Bangalore. A voucher specimen no. is (RRCBI/mus.5-27). Care was taken to select healthy fully grown plant and normal organs. The samples of different organs were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities and dried in sunlight.

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2.2 Preparation of Extracts

The roots of *Acacia leucophloea* were collected and shade dried. The dried root were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60 80℃), ethanol (64.5 − 65.5℃) and distilled water. The extracts were concentrated under reduced pressure (bath temp 50℃). The dried extracts were stored in airtight container in refrigerator.

2.3 Macroscopical characterization

Macroscopical studies of root were done by naked eye and shape, color, taste and odor of roots were determined and reported.

2.4 Microscopical characterization

2.4.1 Sectioning

Selected samples of the dried root were stored in a solution containing formalin (5 ml), acetic acid (5 ml) and 70% v/v ethyl alcohol (FAA) (90 ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the method (Sass, 1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (50-60 ℃m.p.) until tertiary-Butyl alcohol solution attained super saturation. The specimens were casted into paraffin blocks. The paraffin-embedded specimens were sectioned with the help of Senior Rotary Microtome, RMT-30 (Radical Instruments, India). The thickness of the sections was kept between 10 and 12 μm. The de-waxing of the sections was carried out as per the procedure [6]. The section was stained with phloroglucinol -hydrochloric acid (1:1) and mounted in glycerin. A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder [Sieve mesh 60] of the dried roots was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol-hydrochloric acid (1:1) solution, acetic acid and iodine solution to determine the presence of Sclerenchymatous interfasicular tissue, parenchymatous tissue and xylem vessels [7].

2.4.2 Photomicrograph

Microscopic descriptions of selected tissues were supplemented with micrographs. Photographs of different magnifications were taken with Nikon Lab Photo Microscopic unit. For normal observations, bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property under polarized light they appear bright against dark background [8].

2.4.3 Physico-chemical evaluations

Physicochemical parameters of *A. leucophloea* root powder were determined [9] and reported as total ash, water-soluble ash, acid-insoluble ash, and sulphated ash values. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content and pH was also determined.

2.4.4 Preliminary phytochemical Screening

The coarse root powder of *A. leucophloea* (25 g) was subjected to soxhlet for successive solvent extraction. Extract were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents [1].

2.4.5 Superoxide scavenging activity

Petroleum ether, aqueous and ethanolic extracts were screened for anti-oxidant activity using superoxide free radical scavenging activity in dose and time dependent manner [5]. The assay was based on the capacity of the samples to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contains 50 mM phosphate buffer, pH 7.6, 20 μg riboflavin, 12 mM EDTA, 0.1 mg/3 ml NBT, added in that sequence. The reaction was started by illumination the reaction mixture with different concentrations (5-100 μg/ml) of samples for 15, 30 and 45 min. The absorbance was measured immediately after illumination at 590 nm and ascorbic acid was used as standard drug. Percentage inhibition and IC50 were calculated (Results are shown in Fig. 4).

3. Results

3.1 Macroscopical Study

The root was long, about 18-25 cm in length and 2-2.5 cm in thickness. Surface was brown in colour, but inside reddish in colour. Surface was rough, slightly some marking are prominent, outer layer was peelable in mature roots. Fractures were slightly fibrous; easily breakable by hand (Fig. 1). It had slightly bitter taste and agreeable odor.

Figure 1: External morphology of *A. leucophloea* root.

3.2 Microscopical Study

T. S. of the root was circular in out line showed outer cork, secondary cortex, stellar region and well developed stolen and abundant phloem fibers mean the phloem region.

Cork: Outer cork was many layered, slightly brown (reddish brown) in colour, thin walled, filled with brown to red cell content. Cork cambium was single layered (Fig. 2 a & 2 b).

Secondary cortex: It was many layered, made up of thin walled parenchymatous cells, round to polygonal thin walled and some of the cells filled with simple starch grains. In the cortex region, one to two layered stone cells were presented. Stone cells were polygonal to rectangular with small lumen. Some of the cortex cells were filled with reddish cell content predominately (Fig. 2 a & 2 b).

Phloem: Near the phloem region that is after cortex region, phloem fibres were presented abundantly in groups. Phloem

well developed with many layers of thin walled cells. Cambium was single layered (Fig. 2 a & 2 d).

Xylem: It was well developed with xylem vessels prominently. Medullary ray cells were uni to biseriate. Some of the xylem vessels were also filled with reddish content prominently (Fig. 2 a & 2 d).

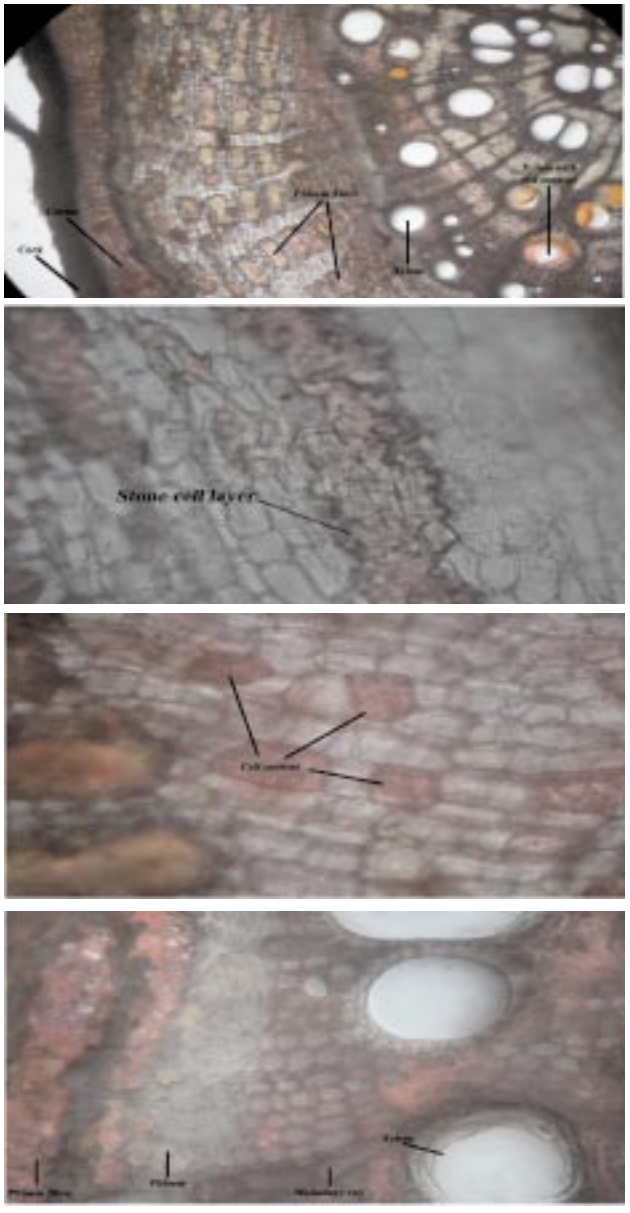

Figure 2: Microscopical view of T. S. of *A. leucophloea* root.
a: Microscopical view at 10xX10x,
b: Microscopical view enlarged at10xX40x,
c: Microscopical view of cortex region showing reddish cell content at 10xX40x,
d: Microscopical view of vascular region enlarged at 10xX40x.

Powder Microscopy: Powder of the root was light pink in colour, agreeable in odor, slightly bitter in taste, rough & fibrous in touch and rough in texture. Powder when treated, showed the presence of starch grains, fragments of abundant xylem vessels with simple pits, fibres, crystal fibres, xylem fibres in bundle, phloem fibres, medullary ray cells, cork cells, parenchyma cells with red cell content, different tissues with abundant xylem vessels with pitted thickenings, parenchyma, and stone cells singly having polygonal diagnostic characters. Abundant phloem fibres in groups, abundant crystal fibres and abundant xylem fibres were also observed (Fig. 3 a & 3 e)

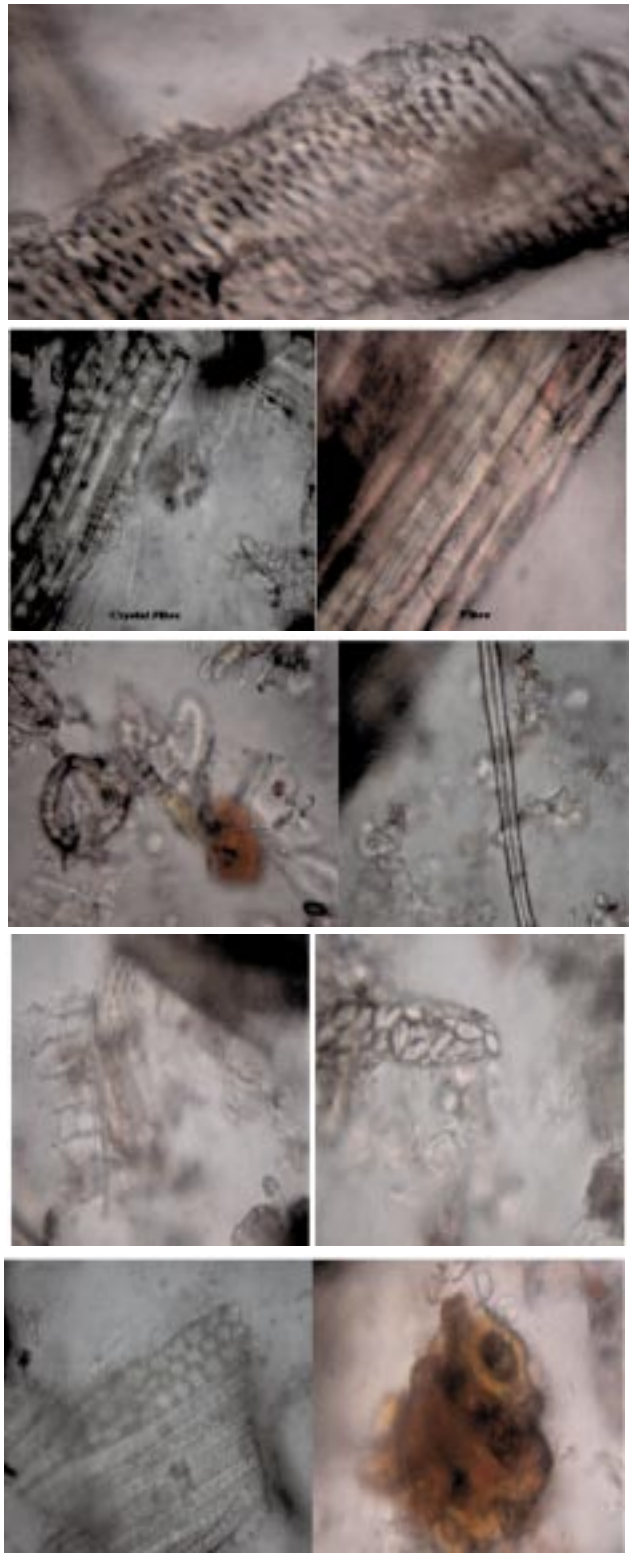

Figure 3: Powder microscopy of *A. leucophloea* root at 10xX40x.
a: xylem vessel with pitted thickenenings,
b: crystal fibre and fibre,
c: stone cell and Phloem fibre,
d: cork cell and starch grains,
e: medullary ray cells and parenchyma containing red cell content.

3.3 Physicochemical Parameters

A. leucophloea root powder showed the presence of total ash – 8.90% w/w, acid–insoluble ash – 3.58 % w/w, water–soluble ash – 2.34 % w/w, water–soluble extractive – 2.34 % w/w, alcohol–soluble extractive – 4.70 % w/w, moisture content – 4.30 % and pH– 6.8 (Table 1).

Table 1

Physicochemical Analysis of Acacia leucophoea root.

Physicochemical parameter	Value (%) Mean±S.E.
Total Ash	8.90% w/w
Acid insoluble ash	3.58 % w/w
Water soluble ash	2.34 % w/w
Water soluble extract	2.34 % w/w
Ethyl alcohol soluble extract	4.70 % w/w
Moisture content	4.30 %
pH	6.8

* w/w: weight/weight.

* Total ash is approximately 2 times and 4 times more than acid insoluble and water soluble ash respectively. Ethanol soluble extractive is approximately 2 times higher than water soluble extractive. Moisture content is less then 7 % and pH is 6.8.

3.4 Preliminary Phytochemical Studies

Phytochemical analysis showed the presence of terpene in petroleum ether and chloroform extract. Alcohol extract showed positive report for alkaloids, terpenes, flavanoids and tannins (Table 2). T.L.C. of Petroleum–ether (60–80 ℃) extract of drug on Silica gel 60 F254 precoated sheets using Benzene: Methanol (19:1) showed nine spots at Rf –0.02, 0.06, 0.12, 0.18, 0.26, 0.42, 0.52, 0.59, 0.89 in Iodine vapor. In the chloroform extract, using Chloroform: Methanol (19:1), nine spots at Rf –0.06, 0.13, 0.14, 0.23, 0.46, 0.54, 0.63, 0.73, 0.86 and in ethanol extract, using full Ethyl acetate solvent system only four spots at Rf –0.06, 0.18, 0.33, 0.35 were observed using same viewing medium. (Table 2)

3.5 Free radicals scavenging activity

Ethanolic extract of *A. leucophloea* had showed 57.6±0.62 % inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (55.3±0.48% compared to ethanolic extract), while Petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed dose and time dependent inhibition of superoxide scavenging activity. The results are reported in Table 3 and shown in Fig. 4.

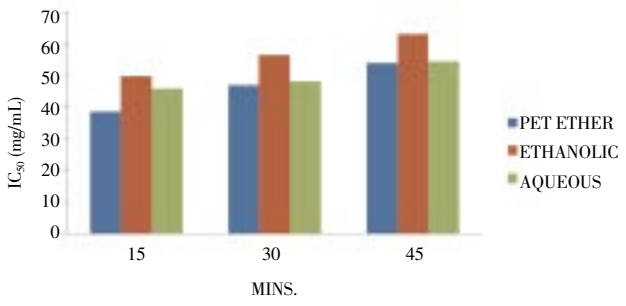

Fig. 4: IC50 of tested extracts

Table 2

Phytochemical Analysis of *Acacia leucophloea* root.

Test for constituent	Petroleum ether extract	Chloroform extract	Ethyl alcohol extract
Alkaloid	–	–	+
Steroid	–	–	–
Terpene	+	+	+
Flavanoids	–	–	+
Glycoside	–	–	–
Sugars	–	–	–
Saponin	–	–	–
Tannin	–	–	+
Colour & Consistency	Light Yellow oily	Yellow gum	Reddish Brown gum

* +: Present. – : Absent

* Phytochemical analysis showed the presence of terpene in petroleum ether and chloroform extract. Alcohol extract showed positive report for alkaloids, terpene, flavanoid and tannins.

Table 3

Percentage inhibition of superoxide free radical scavenging activity of petroleum, ethanolic and aqueous extracts

S. No.	Concentrations (μg/ml)	% Inhibition								
		Minutes								
		15			30			45		
		Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous
1.	5	26.8±0.28	37.0±0.32	33.6±0.22	32.9±0.34	40.4±0.38	38.7±0.39	39.4±0.44	51.4±0.49	43.1±0.33
2.	10	31.5±0.31	44.8±0.49	38.4±0.27	39.7±0.39	54.9±0.45	42.4±0.47	47.6±0.45	62.8±0.58	48.2±0.51
3.	25	38.6±0.32	47.8±0.53	42.7±0.39	48.3±0.54	57.5±0.55	48.3±0.49	53.6±0.57	61.8±0.66	52.8±0.57
4.	50	45.0±0.52	57.6±0.62	55.3±0.48	54.9±0.53	61.6±0.59	50.9±0.52	60.4±0.63	68.4±0.65	60.0±0.63
5.	100	50.6±0.47	61.2±0.51	59.7±0.53	59.7±0.59	68.6±0.61	60.5±0.64	68.5±0.67	70.8±0.69	67.8±0.58

Data are mean±S.D of three measurements. Statistical analysis was performed by the Student's t–test and by ANOVA

4. Discussion

The macroscopic study of root indicated that its colour, odor and taste might be an important characteristic feature for identifying the plant. The anatomy of the root was studied by taking transverse section. Transverse section of the root showed slightly brown (reddish brown), many layered Outer cork filled with brown to red cell content [10]. Secondary cortex was made up of thin walled parenchymatous cells, round to polygonal thin walled and some of the cells filled with simple starch grains. One to two layered stone cells were observed. Reddish cell content were presented in some of the cortex cells predominately. Phloem was constituted with many layers of thin walled cells [11]. Some of the xylem vessels were filled with reddish content prominently. Powder microscopical examination showed the presence of crystal fibres, xylem fibers in bundle, phloem fibers, medullary ray, and cork cells, parenchyma cells with red cell content, tissues with abundant xylem vessels with pitted thickenings, parenchyma, and Stone cells [12].

Total ash was approximately 2 times and 4 times more than acid insoluble and water-soluble ash respectively. Ethanol soluble extractive was approximately two times higher than water-soluble extractive.

Phytochemically, root was found to contain alkaloids, terpenes, flavanoids and tannins. T.L.C of petroleum ether and chloroform extract showed nine spots using Benzene: Methanol (19:1) and Chloroform: Methanol (19:1) respectively while ethanol extract showed four spots using Ethyl acetate.

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14 % w/w [13]. Pharmacognostic standardization including physico-chemical evaluation in Table-1 and 2 is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs [14]. Since the plant, *Acacia leucophloea* (Roxb.) willd is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug.

In the present study, aqueous and ethanolic extracts were selected as they contain alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds. This may have active constituents for producing the free radical scavenging effect [15].

Free radicals are produced under certain environmental condition and during normal cellular function in the body. These molecules are missing an electron, giving them an electric charge. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. Other antioxidants works against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage [16]. This investigation revealed that the *A. leucophloea* Contains pharmacologically active substance such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the Superoxide scavenging activity [17].

The Pharmacognostic constants for the roots of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

Conflict of interest statement

We declare that we have no conflict of interest.

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