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Evaluation of Phytochemical and Antioxidant Activity of *Albizzia lebbeck* Bark Extract

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Abstract: ***The aim of the present study was Evaluation of Phytochemical and Antioxidant Activity of Albizzia lebbeck Bark Extract. The plant has potential antioxidant activity due to presence of alkaloids, tannins, saponin, glycosides and flavonoids. The antioxidant activity were determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The phytochemical study showed that the plant have alkaloids, flavonoids, tannins, glycosides, alkaloids were found using different solvent.***

Keywords: *Albizzia lebbeck; Phytochemical constituent; Antioxidant Activity*

1.Introduction

Albizia lebbeck (L.) Benth. (Family: Fabaceae) is the important medicinal tree usually found in Asia. It is generally known as Shiris in Hindi. it is mostly distributed in tropical and subtropical area of India, Andaman Island, Myanmar, tropical Africa, Asia and northern Australia. This plant is extensively used in the Indian traditional system of medicine due to varied phytochemicals and Ayurvedic research due to their excellent medicinal values.

It is traditionally it also used as anti-diarrhoeal, anti-asthmatic, anti-dysenteric, anti-inflammatory, anti-fertility, antiseptic, and anti-tubercular. It also used in the treatment of wounds, gonorrhoea, leucorrhoea, bronchitis, leprosy, paralysis, helmenth infection and other genital diseases.

Albizia lebbeck gives potent physiological and pharmacological activities. Because of presence of phytoconstituent like, melacacidin, D-catechin, β-sitosterol, albizia hexoside, betulnic acid and echinocystic acid glycosides.[1,2]

1.2 Nomenclature[4]

Family: Fabaceae - Mimosoideae

Synonyms:

English: Siris Tree, Lebbeck Tree
Hindi: Siris, Shiris
Sanskit: Bhandi, sitapuspa, sukapriya
Urdu: Siris
Bengali: Sirish, Siris
Gujrati: Shirish
Kannad: Bagey, Bage Mara, Hombage
Malyalam: Vaka, Nanmenivaka
Marathi: Siris
Oriya: Sersuan, Sirisha
Punjabi: Sirish, Sareehn
Tamil: Vakai
Telgu: Dirisena

1.3 Botanical Description

Albizia lebbeck can attain a height up to 30 m and diameter up to 1 m. Generally 15-20 m tall and 50 cm diameter tree is more often. The colour of the bark is grey-violet with rusty brown breathing pores, rough and fissured. The leaves are bi-pinnate and hairy; leaves produce 2-4 pairs of pinnae, each of these with 2-11 leaflets.

The stalked are short, glabrous glands are raised on the surface and elliptic to circular, on the upper side of the stalk close to the base and between most pairs of leaflets. After the new leaves the white, heavily scented flowers appear shortly, with the stamens free above the corolla, large heads around 18-36 mm across excluding the stamens, heads around 5-7.5 cm long, Arising separately or in the small groups in the in the leaf axils and in terminal panicles; stamens 30-40, yellowish-green on top side, white underside, up to 5 cm long; the flower-stalks up to 5 mm long; the seeds brown, orbicular, flat, or elliptic, long up to 8-10 x 6-7 mm; transversely placed with 6-12 in each pod.

A. lebbeck is hermaphroditic. In its natural habitat, the flowering occurs between the month of September to October; the pods are mature then its remain long period on the tree and are available May-July. Again the flowering is occur suddenly from march to May and fruits from May to August. Flowers are bisexual. [3,21]

1.4 Taxonomic Hierarchy[19]

Kingdom: Plantae
Sub-kingdom: Tracheobionta
Super-division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Genus: Albizia
Species: A. lebbeck (L.) Benth

1.5 Phytochemistry

The phytochemical profile of this plant reveals the Bark contains tannins, d-catechin d-leucocyanidin and it yield 7 compounds Including frieedlan-3-one-and y-sitosterol. The leaves contain echinocystic acid, flavon, vicenin II and β-sitosterol. Flowers conten triterponiods saponons labbekanin D and 4 saponins glycosides lebbckannins D, F, G & H. Mature leaves of *Albizzia lebbeck* contained keto acids including phosphor enolpyruvate, glyoxalate, oxalacetate and α-oxoglutarate; vicenin-2, reynoutrin, rutin, myricitrin and robinin from leaves. It also have alkaloids, flavonoids, tanins, saponins.

It conten highest amount of Potassium and copper is lowest. The Arginine and lysine are present in excessive amounts in seeds while glutamic acid and aspartic acid are present in the highest concentrations in pods due to presence of amino acid. While the linoleic acid detected as the major fatty acid in pod and seed oil, α-tocopherol determined as the major tocopherol component in oil. In vitro antioxidant assays such as ferric reducing antioxidant power, total radical-trapping antioxidant parameter, and Trolox equivalent antioxidant capacity that showed the antioxidant potential.[11,12]

1.6 Medicinal values

The plant contains various important chemical saponins, flavanoids, alkaloids, glycosides and proteins. That possesses antiallergic, anti-inflammatory, analgesic, nootropic, anti spermatogenic and antimicrobial properties. Albizzia lebbeck is an important source of D-catechin, 13-sitosterol, melacacidin,, albizia hexoside and betulinic acid which are effective as antiseptic, anti-dysenteric, antitubercular and used in bronchitis, leprosy, paralysis and helmenth infection also.

Its flowers conten aeromatic substance that gives sweet-smelling oil having p-nitro-benzoate, benzyle alcohol and benzzoic acid. In traditional medicine, the flower emollient is used as a poultice to be applied to boils. The hot aqueous stem bark decoction and its butanolic fraction was found effective in the anti-allergic activity. Saponin from pods and roots has spermicidal activity. The bark is used medicinally to treat inflammation. In addition, anti-protozoal, hypoglycemic, anticancer and analgesic properties have also been reported in this plant.

The *Albizia lebbeck* plants are known to be useful in various other ways, the leaves are use as a good fodder with 17-26% crude protein. It is an excellent fuel wood species with a calorific value of 5200 kcal/g. They are efficiently used for timber, interior moulding, parquet, furniture, panelling, turnery, general construction and for making agricultural implements and mine props. The trunk of tree excreted a reddish gum that is used as an adulterant of gum Arabic. In India locally the bark is used in India for tanning fishing nets or dyestuff. For the preparation of soap highly the dried and pounded bark can be used.

A. lebbeck is a good nitrogen fixing tree without specificity for Rluzobium and native strains are nearly always capable of producing an abundance of nodules. Nitrogen-rich leaves are valuable as green manure enhancing the fertility of the soil. In India *A. lebbeck* is often planted along roads and in home gardens as an ornamental tree.[9,23]

2.Material and Methods

2.1 Collection of Plant Material

Bark of ***Albizia Lebbeck*** was collected from the adjoining area of barpali (Dist – Bargarh, Odisha) in the month of November 2022.

Figure 1: Tree of Albizzia lebbeck Tree

Figure 2: Bark of Albizzia lebbeck

2.2 Pharmacognostic Studies

2.2.1 Macroscopy

Macroscopic character of barks:

Macroscopic characters of barks were done by evaluation of shape, colour, taste and odour, in special feature like touch and texture etc. Shown in **table. 1**

2.3 Phytochemical Investigation

2.3.1 Processing of the Plant Material

After collection of barks sample, it was cut into small pieces for better drying as we know evaporation increases with the incensement of the surface area. After that it was shade dried for several days. The dried bark was then crushed by mechanical cutter, which was well cleaned

before crushed to avoid cross contamination. The dried crushed bark was then used for extraction with suitable solvents.

On the basis of polarity petroleum ether, acetone and ethanol are for successive extraction. Then all the solvent were used in order of increasing polarity in successive manner using a Soxhlet extraction apparatus.

2.3.2 Extraction of Plant Material

Solvent used for extraction in order of increasing polarity were;

1. Pet. Ether (40°-60° C)
2. Acetone (56° C)
3. Ethanol (78. 37° C)
4. Aqueous

On the basis of polarity petroleum ether, acetone and ethanol are for successive extraction. Then all the solvent were used in order of increasing polarity in successive manner using a Soxhlet extraction apparatus.

About 100 gm of dry crushed material was extracted with petroleum ether by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 10 hrs. The petroleum ether extract was then concentrated to a dry mass using vacuum distillation. A yellowish Green residue **(2.19 gm)** was obtained. Subsequently the dried powder was extracted with Acetone for 15 hrs. The Acetone extract was then concentrated and a blackish brown residue **(14.38 gm)** was obtained.

Then extracted with ethanol for 15 hrs. The ethanolic extract was filtered and concentrated by vacuum distillation. A reddish brown residue was obtained **(5.2 gm).** After ethanolic extraction was dried then extracted with distilled water by simple maceration.

The maceration was continued for 15 hrs and then extract was concentrated by vacuum distillation. A deep brownish colour residue **(6.97 gm)** was obtained **(Table-2).** [2,3,6]

2.4 Phytochemical Analysis of Extract

Phytochemical analysis of the different extract of stem bark was carried out using standard methods. The plant materials were checked for the presence of various active constituents. Like carbohydrates, protein, alkaloids, glycosides, flavonoids, tannins, fixed oils and fats, resins and phytosterols (Table-3).

Materials: Water, Alcohol, 70 % alcohol, extracts, Reagents: Mayer's, Wagner's, Sulphuric acid, 10 % Ammonia, Pyridine, Sodium nitropruside, Acetic anhydride, Ferric chloride, 10 %, 5% Lead acetate, 10 % Ammonium hydroxide solution. [2,3,6]

2.4.1 Determination of Total Phenolic Content

Principle

The total phenol content of Different extract of Albizia lebbeck was determined by the Folin-Ciocalteu reagent using gallic acid as standard.

The Folin Ciocalteu reagent is the mixture of phosphomolybitate and phosphotungstate. This method use for measures the amount of substance needed to inhibit the oxidation of the reagent. [14,17,22]

Instrument

Shimadzu UV Visible spectrophotometer - Model 1601

Reagents

7.5 % sodium carbonate
1N Folin-Ciocalteu reagent

Procedure

An aliquot quantity of gallic acid was weighed and dissolved in methanol to prepare1mg/ml stock solution. The different concentration of standard solution (20, 40, 60, 80 and 100 µg/mL) was taken in to 10 ml volumetric flask; make the volume up to 1 ml with methanol. To this solution, 2.5 ml of Folin-Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate were added and final volume was made up to 10 ml with methanol. Then the tube was incubated for 30 minutes at room temperature. The absorbance was read at 760 nm spectrometrically. To 1 ml of plant extracts in methanol were mixed with 2.5 ml of Folin-Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate were added and final volume was made up to 10 ml with methanol and the absorbance was read at 760 nm after the incubation at room temperature for 30 min. Then the calibration curve was plotted by using various absorbance of gallic acid. Then a linear regression equation was find out and by using this equation the amount of phenolic compounds was determined.

The total phenolic content was expressed as mg gallic acid equivalents (GAE)/g of extract using the formula,

$C = cV/M$

Where

C=total content of phenolic compounds in mg/g GAE,
c=the concentration of gallic acid (mg/ml),
V=Volume of extract,
m=the weight of pure plant extract

Total phenolic content in different extracts of *Albizia lebbeck (Benth) Bark* in terms of gallic acid equivalents (Table-4)

3.Result

Table No 1: Macroscopy of *Albizia Lebbeck*

Part of plant	Morphology	Observation
Bark	Colour	Dark brown
	Odour	Characteristic
	Taste	Slightly astringent
	Texture	Rough texture

Table No 2: Percentage Yield of *Albizia Lebbeck* Bark extracts

Extract	Colour	% yield
Petroleum ether	Yellowish Green	2.19
Acetone	Blackish brown	24.38
Ethanol	Reddish brown	5.2
Water	Blackish brown	6.97

Table No 3: Qualitative Phytochemical Screening of *Albizia Lebbeck*

	Phytochemical Test	Pet-Ether	Acetone	Ethanol	Water
1	**Test for Carbohydrates**				
	Molisch's test (General test)				
	Fehling's test	-	+	+	+
	Benedict's test	-	+	+	+
	Barfoed's test	-	+	+	+
	Selwinoff's test	-	_	_	_
2	**Test for Proteins**				
	Biuret test (General test)	+	+	+	+
	Millon's test	+	+	+	+
	Xanthoprotein test	+	+	-	-
3	**Test for Amino Acids**				
	Ninhydrin test (General test)	-	-	-	+
4	**Test for Steroid**				
	Salkowski reaction	+	+	+	+
5	**Test for Glycosides**				
	Legal's test (cardiac)	-	+	+	+
	Borntragers test (anthraquinone)	-	+	+	+
	Foam test (saponin)	-	+	+	+
6	**Test for Flavonoids**				
	Shinoda Test	-	+	+	+
7	**Test for Alkaloids**				
	Dragendorff's test	-	+	+	+
	Mayer's test	-	+	+	+
	Hager's test	-	+	+	+
	Wagner's test				
8	**Test for Tannins and Phenolic Compound**				
	5 % FeCl3 solution	-	+	+	+
	Lead acetate	-	+	+	+
	Potassium dichromate	+	+	+	+
9	**Test for Enzyme**				
	Oxidase	-	-	-	-
	Dehydrogenase	-	+	+	+

'+' presence '-' absence

Table No 4: Total Phenolic Content of Albizia lebbeck Bark Extracts

Extracts	Amount of total phenolic content in terms mgGAE/g of extract
Petroleum Ether	21.23 ± 0.02
Acetone	33.16 ± 0.01
Ethyl Alcohol	43.29 ± 0.01
Aqueous	42.27 ± 0.01

The value was obtained by calculating the Mean of three readings ± Standard deviation.

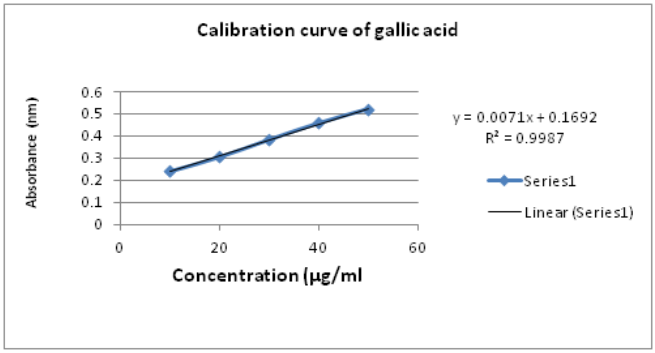

Figure 3: Calibration curve of Gallic acid

Table No 5: Total flavonoid Content of Albizia lebbeck Bark Extracts

Extracts	Amount of total flavonoids content in terms mgGAE/g of extract
Petroleum Ether	15.7±0.09
Acetone	23.23±1.03
Ethyl Alcohol	39.29±1.03
Aqueous	45.69±1.04

The value was obtained by calculating the Mean of three readings ± Standard deviation.

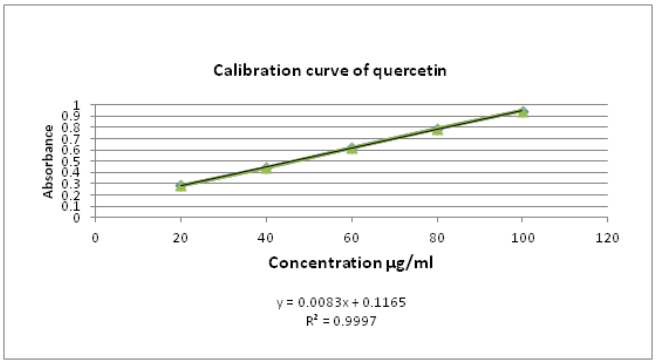

Figure 4: Calibration curve of Quercetin

Table No 6: Free radical scavenging activity of extracts by DPPH method

Concent Ration (µg/ml)	% Inhibition				
	Pet-Ether	Acetone	Ethanol	Water	Ascorbic acid
20	9.15	9.95	19.43	11.73	18.07
40	13.27	15.30	26.63	18.07	27.80
60	18.43	19.95	31.05	22.00	38.07
80	22.98	24.77	37.71	26.46	43.79
100	26.91	29.86	45.39	30.66	49.59

Table No 7: IC50 Values of different extracts

Parameter	Pet-Ether (µg/ml)	Acetone (µg/ml)	Ethanol (µg/ml)	Water (µg/ml)	Ascorbic acid (µg/ml)
IC50	30.28	28.98	22.43	27.98	20.60

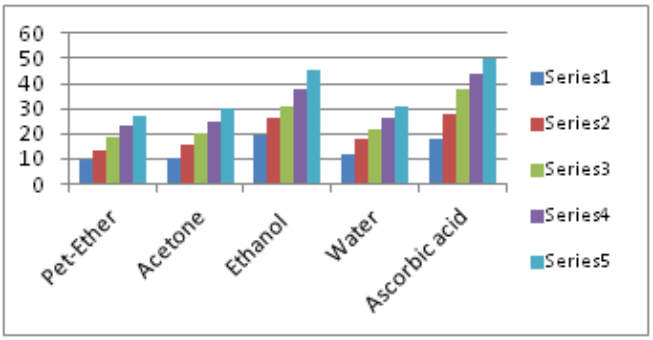

Figure 4: Estimation of DPPH radical scavenging activity

4.Conclusion

The present study showed that the Bark extract of *Albizzia lebbeck* possess Anti-oxidant activity. The Ethanolic extract of the bark possess highest antioxidant activity (IC50 value 22.43 µg/mL) compared to pet-ether, Aceton and water extracts. Flavonoids, tannins, saponins, alkaloid and steroids are the main chemical constituent that are present in the Bark of Albizzia lebbeck which may be responsible for Anti-oxidant activities.

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