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QUALITATIVE AND QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS OF MEDICINAL PLANTS BELONGING TO THE FAMILY FABACEAE

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ABSTRACT:
In the present study *Albizia Lebbeck* L. Benth. (Shirisha) and *Acacia Catechu* L.f. Willd. (Khadira) were chosen for the Qualitative and Quantitative phytochemicals study. In qualitative analysis, six different solvents were used for the preparation of extracts and analyzed for the presence of various phytoconstituents using standard test methods. In quantitative analysis, the hydroalcoholic extracts of these plant species were analyzed for determining the total phenolic content by Folin-Ciocalteu reagent using gallic acid as a standard, total flavonoid content by aluminum nitrate assay using quercetin as a standard, total terpenoid content by colorimetric method using ursolic acid as a standard and total tannin content by titration method. Preliminary phytochemical screening of these plant species revealed the presence of secondary metabolites which were predominantly found in aqueous and ethanolic extract followed by acetone, isopropyl alcohol, and chloroform fractions and rarely observed in petroleum ether. Quantitative estimation shows that the hydroalcoholic extract of *A. lebbeck* contained Phenolic: 34.9 %; Flavonoids: 5.96 %; Terpenoids: 7.131 % and Tannins: 62 %. The results of *A. catechu* showed that it contained Phenolic: 23.3 %; Flavonoids: 6.77 %; Terpenoids: 14.75 %; and Tannins:41 %. Thus, the phytoconstituents can be used for skin care formulations.

Keywords :- Shirisha, Khadira, Fabaceae, Quantitative estimation, Phytochemical screening, and Skin care.

INTRODUCTION :

India has a long history and a strong base for herbal medicine in the Ayurveda system. As per studies, medicinal plants are useful for healing as well as for curing human diseases because of the presence of their phytochemical constituents [1]. The concept of treating skin disease or beauty care through herbs has been mentioned in classical texts. Charak Samhita is the most authoritative text of Ayurveda and deals extensively with skin care. There are many references in Charak Samhita being in use in present days for curing skin diseases and nourishing it. Shirish–*Albizzia Lebbeck* and Khadir–*Acacia Catechu* family Fabaceae (Mimosaceae) are one of them [2]. In many Ayurvedic texts, it is mentioned to use of Shirish and Khadira for various therapeutic purposes like Kustha (skin diseases), Sothahara (anti-inflammatory), Vranaropaka (wound healer), and many more [3,4]. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin, and functional groups into primary and secondary metabolites [5]. There are many active compounds present in *Albizia lebbeck* like flavonoids, phenolic compounds, terpenoids, tannins, etc [6]. Major phytoconstituents present in *Acacia catechu* are catechin, epicatechin, gallate, tannins, alkaloids, quercetin, and kaempferol [7]. The present study was mainly conducted to assess the potential of stem bark extract of *Albizia lebbeck* and *Acacia catechu* qualitatively and quantitatively for the estimation of phytoconstituents.

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MATERIALS AND METHODS :

Collection and authentication of Plant material: The stem bark of *Albizia lebbeck* and *Acacia Catechu* was collected, identified, and authenticated at the Department of Botany, RTMNU, Nagpur.

Preparation of Plant extracts for Preliminary Screening

Maceration: Powdered material of stem bark of *Albizia lebbeck* (L.) Benth. (AL) and *Acacia Catechu* (L.f.) Willd. (AC) 10gm was taken for maceration with 100ml of different solvents viz., water, ethyl alcohol, isopropyl alcohol, acetone, chloroform, and petroleum ether for 15 days at room temperature with vigorous shaking every 24 hrs. The stem bark extracts were filtered using normal filter paper. All the extracts were stored in an amber-colored bottle for preliminary phytochemical analysis.

Qualitative Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to detect the presence of secondary metabolites using standard chemical test methods [8,9,10].

Quantitative Phytochemical analysis

The presence of secondary metabolites from the stem bark of *Albizia lebbeck* and *Acacia Catechu* were quantitatively determined by adopting standard protocols.

Preparation of Plant extracts for quantitative analysis

Soxhlation: For the hydroalcoholic extraction of samples, 50 gm of Powdered stem bark samples of *Albizia lebbeck* (L.) Benth. (AL) and *Acacia Catechu* (L.f.) Willd. (AC) was soxhlated with 350 ml of extraction solvent (7:3 ethanol: distilled water) for 48 hrs. The supernatants were collected and concentrated by solvent recovery distillation and further concentrated and dried on a hot plate. The dried extract was stored at freezing temperature until further use for quantitative analysis.

Determination of Total Phenolic content (TPC)

Preparation of Standard Gallic acid for Calibration curve. Total phenolic content (TPC) in the stem bark extract samples of *Albizia lebbeck* and *Acacia catechu* were determined by Folin-Ciocalteu colorimetric method. The standard gallic acid solution was prepared by dissolving 10mg of it in 10 ml of methanol (1mg/ml). Various concentrations of gallic acid solution in methanol (10, 20, 30, 40, and 50 µg/ml) were prepared from the standard solution. To each concentration, 5ml of 10% Folin-Ciocalteu reagent (FCR) and 4 ml of 7% Na_2CO_3 were added making a final volume of 10ml. Thus, the obtained blue color mixture was shaken well and incubated for 30 min at 40°C in a water bath. Then, the absorbance was measured at 760 nm against blank. The FCR reagent oxidizes phenols in plant extract and changes into a dark blue color, which is then measured by a UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Preparation of samples for Total Phenolic content. Various concentrations of the extracts (50, 100, and 150 µg/ml) were prepared. The procedure as described for standard gallic acid was followed, and absorbance for each concentration of extracts was recorded. The samples were prepared in triplicate for each analysis, and the average value of the absorbance was used to plot the calibration curve to determine the level of phenolics in the extracts. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/gm). The total phenolic content in all the samples were calculated by using the formula:

$Y = MX + C$

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where Y= Absorbance of an unknown sample mg GAE/g dry extract

X= concentration of gallic acid obtained from calibration curve in mg/ml

M= mass of extract in gm

C= intercept of Y [11]

Determination of Total Flavonoid content

Preparation of standard quercetin for calibration curve. Total flavonoid contents in the *Albizia lebbeck* and *Acacia catechu* plant extracts were determined by aluminum nitrate colorimetric assay. A stock solution (10mg/ml) of quercetin was prepared by dissolving 10mg of quercetin in 10 ml of methanol. From that stock solution, 1 ml of quercetin solution was dissolved in 10 ml of methanol. This standard solution was diluted serially to make various concentrations of 10, 20, 30, 40, and 50 µg/ml solutions. 1 ml quercetin of each concentration was added to the test tube. At the same time, 1.5 ml of ethanol, 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1M sodium acetate, and 2.8 ml of distilled water were added to the test tube. After the 40 min incubation at room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415nm. The total flavonoid content was expressed as quercetin equivalents using the linear equation based on the calibration curve.

Preparation of samples for Total Flavonoid content. A stock solution of 10mg/ml concentration in methanol of the extracts was prepared, and they were diluted serially to make different concentrations (50, 100, and 150 µg/ml) solutions. A similar procedure as described for quercetin was followed for the extracts also, and the absorbance was measured by spectrophotometer at 415nm. Readings were taken in triplicate, and the average value of absorbance was used to calculate the total flavonoid content. The flavonoid content was expressed as quercetin equivalent (mg QE/gm) using the linear equation based on the standard calibration curve [12].

Determination of Total Terpenoid content

The accurate quantity of plant extract was dissolved in 25ml of ethanol. 0.2ml of ethanol solution was pipette out in a graduated test tube. The solution was evaporated to dryness in a boiling water bath. 0.3 ml of 5% vanillin/glacial acetic acid(w/v) and 1 ml of perchloric acid solution were added successively. The sample solution was heated at 60°C for 45 min and then cool in an ice-water bath to the ambient temperature. Then, 5 ml of glacial acetic acid was added. The absorbance of the sample was measured at 548nm on spectrophotometer. The same procedure for the preparation of standard ursolic acid was repeated. The percentage of total terpenoid from the calibration curve was calculated [13].

Determination of Tannin content

Quantitative estimation of tannins was performed by titrating the extract with standard potassium permanganate solution following the U.S.S.R.P X method. Briefly, 1.0 gm of extract powder was taken in a 100 ml volumetric flask, and 50 ml hot water was added with constant shaking. This mixture was filtered through Whatman filter paper no. 41. From the filtrate, 10 ml of the resulting solution was pipetted out in a 1000 ml conical flask, in which 750 ml of distilled water and 25 ml of freshly prepared indigosulphonic acid were added. This mixture was titrated against 0.1 N $KMnO_4$ solution. As titration proceeds, the blue color of the indigo-carmine passes through many shades to a golden yellow color with a faint pink tint at the rim. It was taken as the endpoint. This volume of $KMnO_4$ was used to titrate total tannin plus all other related compounds. Blank titration was also performed by titrating 25 ml of indigosulphonic acid in 750 ml of distilled water [14].

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RESULTS AND DISCUSSION :

Qualitative Phytochemical analysis was carried out using the stem bark of *Albizia lebbeck* and *Acacia Catechu* belonging to the family Fabaceae. The results are depicted in the following Tables.

A. lebbeck stem bark showed the presence of tannins, flavonoids, phenols, saponins, terpenoids, alkaloids, carbohydrates, protein, and amino acids in all fractions of aqueous and ethanolic extracts. Whereas, acetone, isopropyl alcohol, and chloroform also showed some traces but were absent in petroleum ether extract. Other findings showed that steroids and alkaloids have marked presence in petroleum ether as compared to the other solvents. With respect to the presence of cardiac glycosides and anthraquinone glycosides, these were found in ethanolic extract but were absent in the other solvents. (Table No.1)

Phytochemical analysis of all six extracts of *A. Catechu* stem bark revealed that the presence of tannins, flavonoids, phenols, saponins, steroids, cardiac glycosides, anthraquinone glycosides, carbohydrates, and protein in all ethanolic fractions followed by aqueous extract. Whereas, amino acid is absent in all the extracts. Also, the qualitative presence of alkaloids and terpenoids was observed in petroleum ether, chloroform, and ethanolic fractions. (Table No.2)

Quantitative phytochemical analysis was carried out with hydroalcoholic extracts of stem bark of *Albizia lebbeck* and *Acacia catechu* for some major phytochemicals such as phenols, flavonoids, terpenoids and tannins. The results are depicted in the following figures (Fig. 1), (Fig.2), (Fig.3) and table (Table No.3)

Results of Comparative Quantitative estimation of some major phytochemicals of *Albizia lebbeck* and *Acacia Catechu* are depicted in the following figures (Fig.4), and (Fig.5).

CONCLUSION :

The present study has shown that the hydroalcoholic extract of *Albizia lebbeck* and *Acacia catechu* is qualitatively and quantitatively rich in some phytochemicals. The plant extracts contain large amounts of Phenols, flavonoids, terpenoids, and tannins. The presence of these phytochemicals may be responsible for various therapeutic activities like antibacterial and anti-inflammatory for skin, and to formulate skin care cosmeceutical preparation.

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Table No.1: Phytochemical Qualitative test of *Albizia Lebbeck* plant extract in six solvents

S.N.	Tests	*Albizia Lebbeck* L. Benth. (AL)					
		AQ	ET	IPA	AE	CF	PE
1.	**Alkaloids Test**						
	Dragendorff's test	+	+	++	+	++	+
	Wagner's test	+	++	+	+	++	+
	Hager's test	+	+	+	+	++	++
	Iodine test	-	++	-	+	++	++
2.	**Flavonoids Test**						
	Shinoda test	+	++	-	-	-	-
	NaOH test	+	+	+	+	-	-
3.	**Tannins Test**						
	Ferric chloride test	++	++	+	+	-	-
	Lead acetate test	+	++	++	+	-	-
4.	**Phenols Test**						
	Ferric chloride test	+	+	-	+	-	-
	Ellagic acid test	++	++	-	+	-	-
5.	**Terpenoid Test**						
	Salkowski's test	+	+	+	+	++	+
6.	**Steroids Test**						
	Salkowski's test	-	+	+	+	+	++
7.	**Cardiac glycoside Test**						
	Keller-Kiliani test	-	+	+	-	+	-
8.	**Anthraquinone glycoside Test**						
	Borntrager test	-	+	-	-	-	-
9.	**Saponins Test**						
	Foam test	+	-	-	+	-	-
10.	**Carbohydrates Test**						
	Molisch's test	+	+	-	-	+	+
	Seliwanoff's test	+	+	-	+	+	+
11.	**Protein Test**						
	Biuret test	+	+	-	+	-	+
12.	**Amino acid Test**						
	Ninhydrin test	+	+	-	+	+	-

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Table No.2: Phytochemical Qualitative test of *Acacia Catechu* plant extract in six solvents

S.N.	Tests	*Acacia Catechu* L.f. Willd (AC)					
		AQ	ET	IPA	AE	CF	PE
1.	**Alkaloids Test**						
	Dragendorff's test	+	+	-	+	+	+
	Wagner's test	+	++	++	-	++	+
	Hager's test	+	++	+	+	++	-
	Iodine test	+	++	-	-	+	++
2.	**Flavonoids Test**						
	Shinoda test	++	++	+	+	+	-
	NaOH test	+	+	-	+	-	-
3.	**Tannins Test**						
	Ferric chloride test	+	++	-	+	-	-
	Lead acetate test	++	++	+	-	-	-
4.	**Phenols Test**						
	Ferric chloride test	+	++	-	+	-	-
	Ellagic acid test	++	+	+	+	-	-
5.	**Terpenoid Test**						
	Salkowski's test	-	+	-	+	+	+
6.	**Steroids Test**						
	Salkowski's test	-	+	-	-	-	-
7.	**Cardiac glycoside Test**						
	Keller-Kiliani test	+	+	-	-	+	-
8.	**Anthraquinone glycoside Test**						
	Borntrager test	-	+	+	-	-	-
9.	**Saponins Test**						
	Foam test	-	+	-	-	-	-
10.	**Carbohydrates Test**						
	Molisch's test	+	+	-	-	+	-
	Seliwanoff's test	-	+	-	-	+	-
11.	**Protein Test**						
	Biuret test	+	+	+	+	+	+
12.	**Amino acid Test**						
	Ninhydrin test	-	-	-	-	-	-

Key: + + Highly Present; + Present; - Absent; AQ-Aqueous; ET- Ethanol; IPA- Isopropyl alcohol; AE-Acetone; CF-Chloroform; PE-Petroleum ether

Table no. 3: Total Phenolic, Flavonoid, Terpenoid and Tannin content present in the hydroalcoholic extract of *Albizia lebbeck* and *Acacia catechu* stem bark

Plant extracts	Total phenolic content (mg of GAE/mg of extract)	Total Flavonoid content (mg of QE/mg of extract)	Total Terpenoid content (mg of UAE/mg of extract)	Total Tannins content (%w/w)
AL. SB-HA	34.9	5.96	7.131	62
AC. SB-HA	23.3	6.77	14.75	41

AL. SB: *Albizia lebbeck* stem bark; AC. SB: *Acacia Catechu* stem bark; HA: Hydroalcoholic

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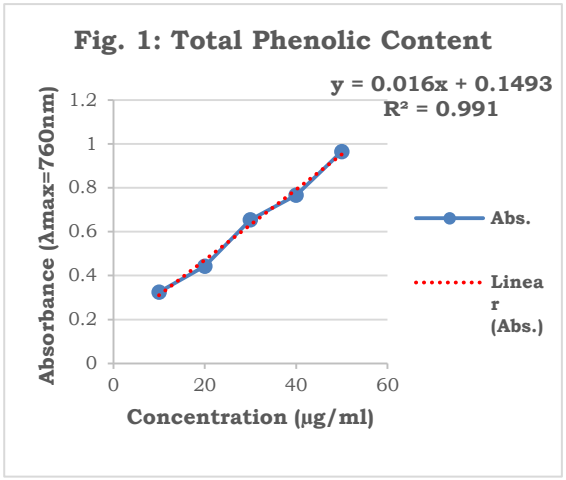
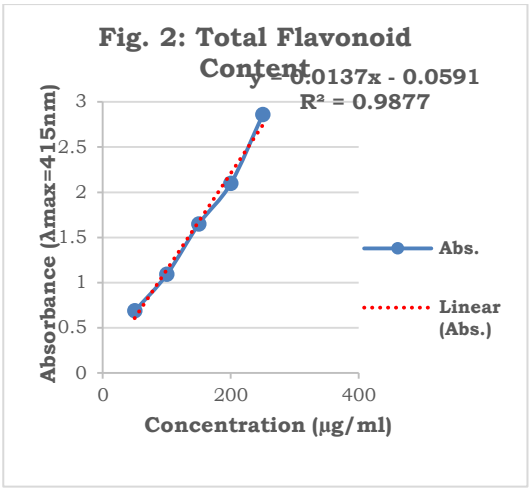


Calibration curve for standard Gallic acid



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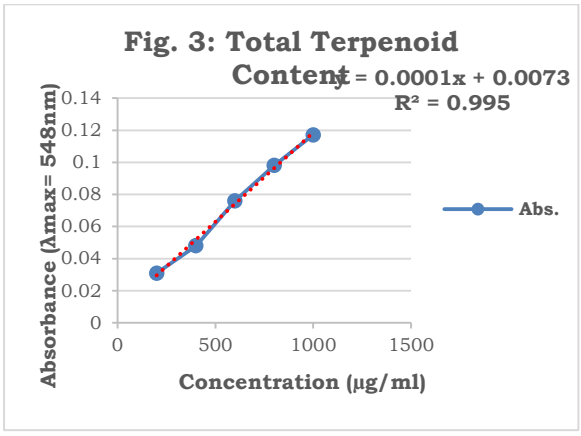
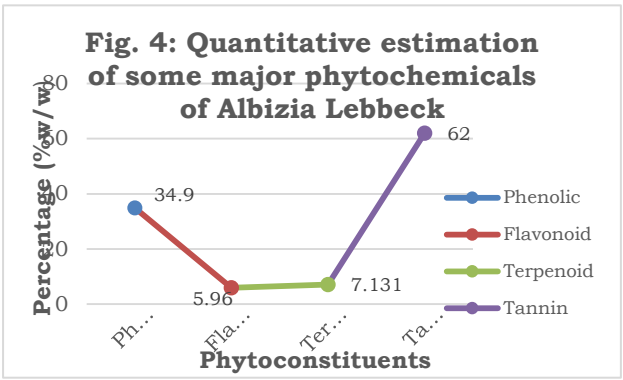
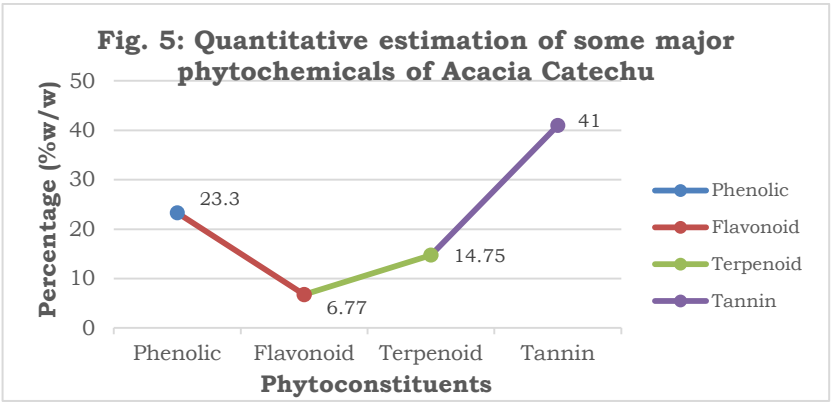


Calibration curve for standard Ursolic acid





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