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SCREENING THE PHYTOCHEMICAL CONSTITUENTS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *ACACIA NILOTICA*, *FICUS BENGALENSIS* AND *THESPESIA POPULNEA*

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ABSTRACT
The present study was aimed to investigate the phytochemical constituents, total phenol content, flavonoid contents and antioxidant activities by Ferric Thiocynate assay (FTC), Ferric Reducing Antioxidant power (FRAP) and antimicrobial activity of methanol bark extracts of *Acacia nilotica*, *Ficus bengalensis*, *Thespesia populnea*. Phytochemical analysis showed the presence of carbohydrates, proteins, tannins, alkaloids, flavonoids in the methanol bark extract. The total phenol content (32.13mg TAE/g) was highest in *T. populnea* whereas the total flavonoid content was high (14.45mg QE/g) in *A. nilotica*. *A. nilotica* exhibited higher antioxidant activity when compared to the bark extracts of other plants with 19.245% FIC and 15% FRAP activity. The methanol extract of the barks were screened for antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The highest antibacterial activity was exhibited by *F. bengalensis* against *Staphylococcus aureus* (15mm) and *T. populnea* against *K. pneumoniae* (12mm) at 4mg /ml.

KEYWORDS: Bark, *Acacia nilotica*, *Ficus bengalensis*, *Thespesia populnea*, Phytochemicals, Antioxidant - Antibacterial activity.

INTRODUCTION
Plants are a potential source of various bioactive compounds and from ancient times, plant products are used to cure diseases.[1] Drugs from plants are easily available, efficient, safe, less expensive and seldom have side effects.[2] Phytochemicals are biologically active naturally occurring chemical compounds, which are also known as secondary metabolites are produced by plants have defense mechanism and protects themselves.[3,4] These are not essential nutrients and are not required by the human body for sustaining life but possess properties that help fight various diseases.[5] Plants produces natural antioxidants in the form of phenolic compounds such as flavonoids, tannins etc., alkaloids, saponins glycosides and terpenoids.[6] These natural antioxidants can scavenge oxygen-nitrogen derived free radicals either by donating a hydrogen atom or an electron, activating antioxidant enzymes and thereby inhibiting oxidases.[7] Flavonoids and terpenoids play important role in their ability to reduce oxidative damage there by protect the human body from diseases.[8-12]

Ficus benghalensis belongs to the family Moraceae, and is commonly known as Banyan tree. It possess various properties such as antioxidant scavenging activity, analgesic and antipyretic activities, anti-inflammatory, antidiabetic and ameliorative activity, anti-helminthic, anti-tumor activity, antibacterial activity and is also used for the treatment of skin allergies, fever, vomiting, ulcers and vaginal complaints.[13-19] *Acacia nilotica* (L.) Willd. Ex Del is commonly known as Indian gum Arabic tree, babul, kikar. It is used as timber and for firewood.[20] The bark of the plant is used as astringent, acrid, cooling, styptic, emollient, anthelminthic, aphrodisiac, diuretic, expectorant, emetic and nutritive, to treat cancer and/or tumors (of the ear, eye or testicles), bleeding piles, skin diseases and seminal weakness, wound ulcers, leucoderma, hemorrhage.[21-24]

Thespesia populnea (L.) Linn. (Fam. Malvaceae), commonly called the Portia tree/Umbrella tree cures scabies, psoriasis, skin diseases, ulcer, dysentery, urinary disorders, painful joints, piles and also has depurative, anti-bacterial, anti-inflammatory activity.[25, 26] Therapeutically bioactive compounds are extracted from all parts of a plant body such as the leaves, bark, stem, roots, rhizomes, flowers, fruits and seeds. Hence in the present study stem bark of *Acacia nilotica*, *Ficus bengalensis* and *Thespesia populnea*

were used to study the phytochemical constituents, antioxidant activity and antibacterial activity.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Stem barks of *Acacia nilotica*, *Ficus bengalensis*, *Thespesia populnea* were collected from Nagercoil, Kanyakumari district, Tamilnadu. The stem barks were shade dried and crushed into coarse powder in a mechanical blender and stored in air tight containers. 25g of each plant material was extracted in a Soxhlet with 250ml of methanol. The extracts were collected, evaporated by rotary evaporator and stored in air tight bottles at 4°C.

PHYTOCHEMICAL SCREENING

The phytochemical tests were carried out using the standard procedures to identify the phytochemical constituents.

Test for Flavonoids

To 250 µl of crude extract, 250 µl of 2% NaOH was added. An intense yellow turns colourless on addition of few drops of acetic acid indicated the presence of flavonoids in the sample.[27]

Test for Tannins

To 1 ml of extract was added 2 ml of 5% $FeCl_3$. A dark blue or green black colour indicates the presence of tannins.[28]

Test for Quinines

To 250 µl of extract, 250 µl of concentrated sulphuric acid was added. Appearance of red colour indicated the presence of quinines in the sample.[29]

Test for Alkaloids

(a) Mayer's Test: To 250µl of crude extract, 250µl of 1% Hydrochloric acid and 6 drops of Mayer's reagent was added. An organic precipitate indicated the presence of alkaloids in the sample.[27]

(b) Dragendroff's Test: To 250 µl of crude extract, 250 µl of 1% Hydrochloric acid and 6 drops of Dragendroff's reagent was added. Appearance of red or orange precipitate indicated the presence of alkaloids in the sample.[27]

Test for Steroids

To 250 µl of crude extract, 250 µl of chloroform and 250 µl of concentrated sulphuric acid were added. Appearance of red colour precipitate indicated the presence of steroids in the sample.[27]

Test for Glycoside

Keller-Killani Test: To 250 µl of crude extract, 250 µl of glacial acetic acid and 1 to 2 drops of 2% ferric chloride reagent was added and poured into a test tube containing concentrated sulphuric acid. The presence of brown ring at the interphase indicated the presence of glycoside in the sample.[27]

Test for Proteins

Million's Reagent: To the 250 µl crude extract 6 to 7 drops of Million's reagent was added. The precipitate turns red upon heating.[27]

Test for Carbohydrates

Fehling's Test: 250 µl of Fehling A and 250 µl of Fehling B were mixed together and 500 µl of it was added to 500 µl of crude extract and gently boiled. Appearance of brick red precipitate indicated the presence of carbohydrates.[27]

Benedicts Test: To 250 µl of crude extract, 250 µl of Benedict's reagent was added. The appearance of reddish brown precipitate indicated the presence of carbohydrates.[27]

ESTIMATION OF TOTAL PHENOLIC CONTENT

The total phenol content in the methanol stem bark extract was determined by Folin Ciocalteau method. 500µl of distilled water and 100µl of Folin Ciocalteau reagent was added to 100µl of the extract and incubated for 6 min at room temperature. 1.25ml of 7% Sodium Carbonate was added and the final volume was made up to 5ml with distilled water and incubated for 90 min. The absorbance was measured at 760nm using UV- Vis spectrophotometer. The total phenol content was expressed as mg Tannic Acid Equivalents (TAE) per dry weight of the material using a standard plot of Tannic Acid.[30]

ESTIMATION OF TOTAL FLAVONOID CONTENT

5ml of 0.5ml of 0.1M Aluminum Chloride was added to 200µl of methanol extract and shaken well. This was incubated for 45 minutes at room temperature and absorbance was measured at 415nm using UV- Vis spectrophotometer. A standard plot of Quercetin at varying concentrations was used to evaluate the total flavonoid contents, expressed as mg QE/g dry weight of the material.[30]

FERRIC THIOCYANATE ASSAY (FTC)

120µl of 98% ethanol, 100µl of 2.51% linoleic acid in ethanol and 9ml of 40mM phosphate buffer (pH7) was added to 100µl of methanol extract. 9.7ml of 75% ethanol, 100µl of 30% ammonium thiocyanate and 100µl of 20mM $FeCl_3$ in 3.5% HCl was added and the solution was maintained at dark at 40˚C. The absorbance of the solution was measured at 500nm using UV- Visible spectrophotometer after 3 min. The percentage inhibition was calculated with Tannic Acid as the standard.[30]

% Inhibition = $[(A_{Control} - A_{Sample}) / A_{Control}] \times 100$.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

2.5ml phosphate buffer (0.2M, pH 7) and 1% potassium ferricyanide (2.5ml) was added to 1ml of methanol extract and incubated at 50˚C for 30 min. 2.5ml of 10% trichloroacetic acid was added and centrifuged at 6500

rpm for 10 min. Distilled water (2.5ml) and 0.5ml of 0.1% $FeCl_3$ was added to 2.5ml of the supernatant. The absorbance of the solution was measured at 700nm. The reducing ability of the flower extract was evaluated in terms of percentage by relating to the standard, tannic acid.[30]

% Inhibition = $[(A_{Control} - A_{Sample}) / A_{Control}] \times 100$

ANTIBACTERIAL ASSAY

Bacterial cultures of *Staphylococcus aureus* and *Klebsiella pneumoniae* were obtained from Department of Biotechnology, University of Madras, Guindy Campus, Chennai. Antibacterial activity of stem bark extracts was determined by standard agar well diffusion method. Methanol extracts of the bark (100mg) were dissolved in 900µl of 5% (v/v) DMSO in separate tubes. Streptomycin sulphate (0.2mg/ml) and 5 % (v/v) DMSO were taken as positive and negative control respectively. Suspensions of each test organisms were made in Nutrient broth and adjusted to 0.5cells / ml by matching the turbidity with Mc Farland standard ($1.5x\ 10^{-8}$ cfu/ml). Muller Hinton agar plate was uniformly spread with 100µl of test organisms. A sterile cork borer was used to make wells on the medium. Different concentrations (1mg, 2mg, 3mg and 4mg) of extract were added to each well. A positive and negative control was also made. The plates were incubated at 37˚C for 24 hour and the zone of inhibition was measured to determine the antibacterial activity of the stem bark extracts.

RESULTS AND DISCUSSION

In the present study carbohydrates, proteins, steroids, tannins, alkaloids and flavonoids were present in the methanol extracts of stem barks of *A. nilotica* (Table 1). Similar results have been reported for the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, cardiacglycosides, anthraquinone in the ethanol and ether extracts of *A. nilotica*.[31-33] In contrast, absence of steroids and flavonoids in the stem bark extract have been reported.[34] In the current investigation carbohydrates, proteins, glycoside, tannins, alkaloids and flavonoids were present in the methanol extracts of stem barks of *F. bengalensis* whereas quinines were absent (Table 1). The presence of carbohydrates, tannins, flavonoids, saponins, phenols has been reported similar to the present study.[35,36] The phytochemicals present in the methanol extract of the stem bark of *T. populnea* are carbohydrates, proteins, glycoside, tannins, alkaloids, flavonoids and quinines (Table 1). The alcoholic extract of stem bark of *T. populnea* had carbohydrates, glycosides, tannins, flavonoids, triterpenoids, phytosterols, proteins and lipids/fixed oils similar to the present study.[37] Flavonoids, phenols, steroids, saponins and tannins were present in the n-hexane: ethyl acetate stem bark extract.[38]

Table 1: Phytochemical Constituents of the Methanol Extract of the Stem Bark of *Acacia nilotica, Ficus bengalensis* and *Thespesia populnea.*

Phytochemical tests	*Acacia nilotica*	*Ficus bengalensis*	*Thespesia populnea*
Flavonoids	+	+	+
Tannins	+	+	+
Qunines	+	-	+
Alkaloids	+	+	+
Steroids	+	-	-
Glycosides	-	+	+
Protein	+	+	+
Carbohydrates	+	+	+

Flavonoids show antioxidant activity through radical scavenging or chelating process as they contain hydroxyl group.[39-41] Phenolic compounds act as free radical terminators.[42] In the present study the total phenolic content in *A. nilotica* was 15.039mg TAE/g and the flavonoid content was 14.59mg QE/g (Table 2). Total phenolic contents of 63.75mg/g GAE and flavonoid content of 5.02 mg/g QE have been reported.[43,44] In the present study the FIC of *A. nilotica* was observed as 19.245% and the FRAP capacity was 15.384%. The ethyl acetate extract of *A. ataxacantha* had the highest FRAP capacity (1273.63 µmol AAE g-1).[45] In the present study the total phenolic content in *F. bengalensis* was 26.259mg TAE/g and the flavonoid content was 7.109mg Quercetin equivalents (QE)/g. In a similar study, a total phenolic content of 1.28g100g-1 and total flavonoid content of 1.14g100g-1 in the methanol extract of *F. bengalensis* bark have been reported.[46,16] In the present study the FIC of *F. bengalensis* was 9.286% and the FRAP capacity was 12.257% (Table 2). The methanol extract of *F. bengalensis* latex had an IC50 value of 41µg/ml for FRAP.[47] In the present study the total phenolic content in the methanol extract of the stem bark of *T. populnea* was 32.188 mg TAE/g (Table 2). Similar to the present study, a high phenol content of 10.11 % w/w gallic acid and 10.5 % w/w gallic acid respectively were reported.[48,49] In the current investigation, the total flavonoid content of methanolic stem bark extract was 9.199 mg Quercetin equivalents (QE)/g. The methanol flower extract of *T. populnea* had a flavonoid content of 25.05mg/ml.[50] In the present study the FIC value was 15.939% and the FRAP values was 6.939%. FRAP values of 39.01 Fe (II) micromole / l have been recorded.[51]

Table 2: Total Phenol Content, Total Flavonoid Content and Antioxidant Activity of the Methanol Extract of the Stem Bark of *Acacia nilotica, Ficus bengalensis* and *Thespesia populnea*

Plant source	Total Phenol content	Total flavonoid content	FIC	FRAP
Acacia nilotica	15.09 ± 0.723	14.459 ± 0.037	19.245 ± 0.256	15.384 ± 0.26
Ficus bengalensis	26.259 ± 0.618	7.109 ± 0.112	9.286 ± 0.200	12.257 ± 0.047
Thespesia populnea	32.188 ± 0.080	9.199 ± 0.336	15.939 ± 0.056	6.939 ± 0.043

In the present study, methanolic stem bark extract of *A. nilotica* had a zone of 12mm against *Staphylococcus aureus* when compared to *K. pneumoniae* (11mm) (Table 3). Antimicrobial activity of *A. nilotica* stem bark extract against *Staphylococcus aureus* and four other microorganisms have been documented.[52] The methanolic stem bark extract of *F. bengalensis* had a zone of 15mm against *Staphylococcus aureus* whereas it was 9mm for *K. pneumoniae* (Table 2). *F. bengalensis* (1 mg/ml) inhibited the growth of *S. aureus* and *K. pneumoniae* (12,13mm) similar to the findings of the present study.[16,31] In the present study, methanolic stem bark extract of *T. populnea* had a zone of 13mm against *Staphylococcus aureus* and 12mm against *K. pneumonia* (Table 3). Similar results of maximum antibacterial activity against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* were reported.[52,53]

Table 3: Antibacterial Activity of the Methanol Extract of the Stem Bark of *Acacia nilotica, Ficus bengalensis* and *Thespesia populnea.*

Plant source	*K. pneumoniae*	*Staphylococcus aureus*
	Zone of Inhibition (mm)	
Acacia nilotica	11	12
Ficus bengalensis	9	15
Thespesia populnea	12	13

CONCLUSION

The results of the present study indicates that the barks of *Acacia nilotica*, *Ficus bengalensis*, *Thespesia populnea* contain polyphenols and were active against Gram positive and Gram negative bacteria. The high phenol and flavonoid content can be attributed to their high antioxidant activities. Further studies to characterize the bioactive molecule responsible for these activates needs to be undertaken. The findings of the current investigation indicates that the barks of these plants are a good source of antioxidants.

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