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EVALUATION OF TANNIN CONTENTS OF SOME INDIGENOUS PLANT SPECIES IN KOLLI HILLS, ANCHETTY AND SATHYAMANGALAM FORESTS

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Abstract

Tannins are complex phenolic substances of plant origin. These pale yellow to light brown amorphous substances are secondary metabolic products of plants which are considered unimportant to the producer plants. But, the results of many research activities have proved the importance of tannins. They play a role in protection from predation and in plant growth regulation. They are used in photography, dyeing, in tanning leather, in clarifying wine and beer and as an astringent in medicine. It was interesting to know that the two species of *Albizzia* (*A. odoratissima* and *A. lebbeck*) had nearly equal amount of tannin in their bark. Similarly, the two species of *Gardenia* possessed (*G. gummifera* and *G. latifolia)* nearly equal amount of tannin in their bark and the barks of the two species of *Terminalia* (*T. bellerica* and *T. chebula*) also contained nearly equal amount of tannin in their barks. Tannin was also noticed that the bark of *Terminalia bellerica* contained more amount of tannin (93.36 ± 0.03) than its seed (85.76 ± 0.03). Similarly, the bark of *Gardenia latifolia* possessed more quantity of tannin (93.36 ± 0.03) than its seed (86.05 ± 0.03) and fruit rind (85.96 ± 0.03). The antimicrobial activity was also studied and the study depicts that the methanol extracts of bark of *P. marsupium* can be used as a potential source of antimicrobial agents.

Key words: *Albizzia*, *Terminalia*, *Gardenia*, Tannin, Phytochemicals and Antimicrobial activity**.**

1. Introduction

Tannins are commonly found in both Angiosperms and Gymnosperms. They are distributed in many families of dicotyledons and monocotyledons. Many families of dicot also contain tannin-free species. The best known families of which almost all species contain tannin are: Arecaceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae etc., for dicot and Najadaceae and Typhaceae in monocot. Some families like Boraginaceae, Cucurbitaceae, Papaveraceae etc., contain no tannin-rich species. The amount of tannins present in plants varies greatly from one species to another. Many plant organs serve as biological source of tannins. Tannins are found in stem bark, heart wood, root, leaf, young shoot, fruit, fruit rind etc.

Tannins are mainly located in the vacuoles or surface wax of plants. Tannins are secondary metabolites of plants. They are non-nitrogenous and phenolic in nature. They have a property to tan animal skin to convert to leather or hide. Conversion imparts resistance to water, heat and abrasives. They can be extracted using water-acetone/alcohol mixture. They have a property to precipitate gelatin and heavy metals. Chemically, tannins contain mixture of complex organic

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substances in which polyphenols are present, generally with O-dihydroxy or O-trihydroxy groups on a phenyl ring. They have fairly high molecular weight and unlike alkaloids, are devoid of nitrogen. Tannins form colloidal solutions with water and are non-crystalline substances. In solution, they show acidic reaction due to phenols. Complex tannins are macro-molecules with many sugar molecules. There are three major classes of tannins. They are, i) Hydrolysable tannins, ii) Non- hydrolysable tannins (Condensed tannins) and iii) Pseudo tannins. Hydrolysable tannins on heating with hydrochloric or Sulphuric acids yield gallic or ellagic acid. E.g., Myrobalans and Pomegranate bark. Non-Hydrolysable tannins on heating with hydrochloric acid yield Phlobaphenes like Phloroglucinol. E.g., Black catechu and Kinos. Pseudotannins are low molecular weight compounds associated with other compounds. They do not answer gold beater skin test unlike hydrolysable and condensed tannins. E.g., Tea and Coffee. They have catechins which give rise to the peculiar odour.

The study of Phytochemicals is Photochemistry. Phytochemicals are chemicals derived from plants and are also described as large number of secondary metabolic compounds found in plants. These compounds have multiple functions and the prior function was to protect the plants from insect attacks and diseases. They also exhibit a number of protective functions for human consumers. Photochemistry is a branch of chemistry concerned with plants, their chemical composition and processes. Photochemistry is the branch of biochemistry dealing with plants and plant processes. Photochemistry can be considered as sub-fields of Botany and Chemistry. Activities can be led in botanical gardens or in the wild with the aid of Ethno botany. Selenium, which is abundant in many fruits and vegetables, is involved with major metabolic pathways, including thyroid metabolism and immune function (Brown and Arthur, 2001). Selenium is an essential nutrient and co-factor for the enzymatic synthesis of glutathione, an endogenous antioxidant (Papp *et al.,* 2007). There are many phytochemicals in clinical trials for a variety of diseases at present. There should be sufficient scientific agreement to conclude an effect of phytochemical on any disease.

Food processing techniques may destroy the phytochemicals in freshly harvested plant foods. The processes include cooking also. Absence or deficiency of phytochemicals in processed foods may contribute to increased risk of preventable diseases. (Lieu, 2004; Rao and Rao, 2007). But, a converse example may exist in which lycopene, a phytochemical present in tomatoes, is unchanged in content or made more concentrated by processing to juice or paste, thereby maintaining good levels of bioavailability (Agarwal *et al.,* 2001; Dewanto *et al.,* 2002).

2. Materials and Methods

The tannin content was studied in the bark, leaf, or fruit of 23 plants collected from Kolli kills (Namakkal Dt.), Anchetty reserve forest (Krishnagiri Dt.) and Sathyamangalam reserve forest (Erode Dt.). The Flora of Madras (Gamble, 1936) and the Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification of the plants.

Sample collection and preparation

The barks, leaves and fruits were collected from healthy plants early in the morning. The barks were stripped off with the help of a knife without making any damage to the trunk of the tree. The leaves and fruits were handpicked. The collected parts of the plant were washed in water, freed from extraneous matter and packed in air-tight plastic bags. After returning to the Botany laboratory of the college (Kandaswami Kandar's College, Velur and Namakkal Dt.), the barks, leaves and fruits were chopped in to small pieces and placed in trays and shade dried for about 10 days and they were powdered separately using an electric mixer. The powders were stored in separate airtight plastic containers, for further studies (tannin tests, phytochemical analysis and screening of antimicrobial activity).

Two gram of powdered plant material was taken in 50 ml test tube and 20 ml petroleum ether was added. Then the solution was transferred to

water bath and heated for 4 hours at 40 ˚C. After 4 hours the temperature was increased to 60 ˚C and heated for 4 hours. After 8 hours the solution was filtered with the help of two folded muslin cloth into a petriplates. Then, the filtered sample was shade dried at room temperature. The dried samples were packed in air-tight plastic bags and stored in a cool, dry place for further studies.

Qualitative Analysis

Phytochemical Analysis

The phytochemicals in each sample was determined qualitatively by following the standard method (Odebiyi and Ramstard, 1978; Waterman 1993).

Test for Tannins

To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

Test for Saponins

Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

Test for Flavonoids

A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal was added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

Test for Steroids (Salkwoski's test)

Five drops of concentrated sulphuric acid (H_2SO_4) was added to 2 ml of each extract and observed for red coloration.

Test for Glycosides

To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

Test for Alkaloids

To 4 ml of extract filtrate, a drop of Mayer's reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is positive.

Test for Anthraquinones

One gram of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl_4 then CCl_4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red colour. One gram of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for phenolic compounds

Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

Quantitative Analysis

Tannins

A quantity of 500 mg of plant sample was weighed and transferred to 50 ml flask. Then, 50 ml of distilled water was added and stirred for 1 hrs. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the filtered sample was pipette into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm wavelength within 10 min (Harborne *et al.*, 1973; Tyler, 1994).

Saponins

Twenty gram of each ground plant samples were put into a conical flask and 100 ml of 20 % ethanol was added to the plant sample. The said sample is heated over a water bath for 4 hrs at about 55 °C with continuous stirring. The

extracted mixture was then filtered and the residue was then re-extracted again with 200 ml of 20 % ethanol. The collective residues are reduced to 40 ml over a hot water bath. The concentration was then transferred to a separating funnel and 20 ml of diethyl ether was added to the plant extract and shaken vigorously. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. 60 ml of n- Butanol was added and combined n-Butanol extract were washed twice with 10 ml of 5 % sodium chloride. The remaining solution was then heated on water bath and after evaporation; the samples were dried in oven to a constant weight.

Flavonoids

Ten gram of the plant sample was extracted with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered and the filtrate was then transferred into a water bath. The solution was evaporated to dryness and weighed to a constant weight (Mattila and Hellstrom, 2007).

Alkaloids

Five grams of the plant sample was prepared in a beaker and 200 ml of 10 % Acetic acid (CH_3CO_2H) in ethanol (C_2H_5OH) was added to the plant sample. The mixture was covered and allowed to stand for 4 hrs. The mixture was then filtered and the extract was allowed to become concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid, which was then dried and weighed.

Phenols

Plants sample was boiled for 15 min with 50 ml of Diethylether $(CH_3CH_2)_2O$. Five ml of the sample was pipette into 50 ml flask and 10 ml of distilled water was added. Then, 2 ml of NH_4OH solution and 5 ml of concentrated amyl alcohol (CH_3 $(CH_2)_3CH_2OH$) was added to the mixture. The sample was made up to the mark and left to react for 30 min for colour development and measured for 505 nm wave length using a spectrophotometer (Tyler, 1994; Harborne *et al*., 1973).

Antimicrobial activity by Agar well diffusion assay (Perez, *et al*., 1990)

The agar well diffusion method was used for the inhibitory effects of *Pterocarpus marsupium* bark extract. The dried extract was reconstituted with Dimethyl sulfoxide (DMSO) to obtain a stock solution of 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml were prepared. Mueller Hinton agar plates were prepared and the plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Five wells (6 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. Up to 25 µl to 100 µl of each concentration of the extract were respectively introduced into the wells using sterile automatic pipettes, with the stock in one well. It was allowed to diffuse at room temperature for 2 hrs and the plates were incubated to 37 °C for 24 hrs. Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant. Five clinical pathogens were used for the study (*Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Streptococcus* sp.).

3. Results and Discussion

In the present investigation the qualitative analysis (presence of tannins in different organs (mostly bark) was performed in 23 species belonging to 20 genera and 15 families. Quantitative analysis (tannin percentage) was performed and the antimicrobial screening against 5 clinical pathogens were done in the plant species with highest amount of tannin.

Qualitative analysis –Tannins

The Table - 1 represents the qualitative analysis of tannins present in 23 species. The presence of tannin was confirmed by the change in colour observed in the extract solution by the

addition of ferric chloride solution. The dark blue or green black coloration confirmed the maximum amount of tannins (+++) and the medium coloration confirmed the medium amount of tannin content (++) and the pale coloration was observed in few species and it was concluded that the presence of tannin was in less amount (+). From the qualitative analysis, it was found that the maximum colour change of the extract was found in 12 species (+++), medium colour change in 9 species and pale coloration in 2 species. It was interesting to note that the bark extracts showed dark coloration than the fruit rind and seed extracts (Table - 1).

Quantitative analysis (Tannin estimation)

The Table - 2 depicts the amount of tannins (given in percentage) in 23 species of angiosperms studied. It was found that the bark of *Pterocarpus marsupium* possessed the maximum amount of tannin (99.42 ± 0.03), followed by *Ficus tomentosa* (95.76 ± 0.03), *Terminalia chebula* (94.13 ± 0.03), *Terminalia bellerica* (93.36 ± 0.03), *Soymida febrifuga* (93.36 ± 0.03), *Gardenia latifolia* (93.36 ± 0.03), *Shorea roxburghii* (93.36 ± 0.03), *Gardenia Gummifera* (93.17 ± 0.03), *Cinnamomum iners* (91.92 ±0.03), *Commiphora caudata* (91.53 ± 0.06), *Diospyros melanoxylon* (91.25 ± 0.03) and *Bauhinia racemosa* (90 ± 0.06). All the above 12 species possessed good tannin amount i.e., above 90 % (in their bark).

The barks of *Alianthus excelsa* (89.13 ± 0.04), *Butea monosperma* (88.94 ± 0.04), *Albizzia odoratissima* (88.26 ± 0.07), *Madhuca indica* (88.17 ± 0.04), *Buchanania axillaris* (87.88 ± 0.06), *Albizzia lebbeck* (87.59 ± 0.04), *Boswellia serrata* (87.59 ± 0.04), *Manilkara hexandra* (85.67 ± 0.03) and *Mundulea sericea* (83.84 ± 0.04), had fairly good tannin amount (80 to 90 %). Only two species namely *Holarrhena pusbescens* (78.46 ± 0. 04), and *Melia dubia* (77.78 ± 0.03) possessed less amount of tannin in their barks when compared to the other 21 species. It was interesting to know that the 2 species of *Albizzia* (*A. odoratissima* and *A. lebbeck*) had nearly equal amount of tannin in their bark. Similarly, the 2 species of *Gardenia* possessed (*G. gummifera* and *G. latifolia)* nearly equal amount of tannin in their bark and the barks of the 2 species of *Terminalia* (*T. bellerica* and *T. chebula*) also contained nearly equal amount of tannin in their barks. It was also noticed that the bark of *Terminalia bellerica* contained more amount of tannin (93.36 ± 0.03) than its seed (85.76 ± 0.03). Similarly the bark of *Gardenia latifolia* possessed more quantity of tannin (93.36 ± 0.03) than its seed (86.05 ± 0.03) and fruit rind (85.96 ± 0.03) (Table - 2).

From the above results we can conclude that the bark of the plants possess more amount of tannins than the seeds and fruit rinds. Of the 23 species studied, *Pterocarpus marsupium* Roxb. (Fabaceae) possessed the maximum amount of tannin (99.42 ± 0.03) in their bark. So, we decided to find out the other secondary metabolites namely Saponins, Flavonoids, Steroids, Glycosides, Alkaloids, Anthroquinones and Phenolic compounds in this species. It was noted that the amount of tannins was the highest (1.42 mg/g) among all the other secondary metabolites (Table - 3 and 4).

The different concentration of the phytochemical extract of the bark of *Pterocarpus marsupium* possessed antibacterial activity, against the organisms *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Streptococcus* sp. The inhibitory activity was found to be concentration dependent. The highest zone of growth inhibition was shown by methanol extract (100 µl) against the five clinical pathogens, of which the maximum zone of inhibition was against the pathogen *Staphylococcus aureus* (Table - 5). The antimicrobial activities of methanol extracts against the five clinical pathogens may be attributed to the presence of tannins. The study depicts that the methanol extracts of bark of *P. marsupium* can be used as a potential source of antimicrobial agents.

Ramaya Subramanian (2010) has studied the antimicrobial activity of bark and leaf extracts from *Pterocarpus marsupium* Roxb. Hexane, ethyl acetate and methanol extracts were tested against

four selected Gram positive and Gram negative bacteria. The antimicrobial activity was tested using a modified disc diffusion method (Ncube *et al.*, 2008). Ethyl acetate and methanol extracts were found to be more active towards the organisms tested than hexane extract. The growth analysis revealed that the methanol extract showed maximum inhibition zone against the pathogens *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes*. In the present study only methanol extract was used against 5 pathogens and the results confirmed the formation of inhibition zone against the clinical pathogens. The maximum zone of inhibition (conc.100 µl) was found against *Staphylococcus aureus* (15 mm) and *Pseudomonas aeruginosa* (14 mm) followed by *E.coli* (12.4 mm), *Klebsiella pneumoniae* (12 mm) and *Streptococcus* sp. (10 mm).

Gayathri and Kannabiran (2009) have evaluated the antimicrobial activity of *Hemidesmus indicus, Ficus bengalensis* and *Pterocarpus marsupium* (Roxb) against pathogenic bacteria *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia* in an *in vitro* condition. Aqueous extracts of roots and barks of the three plant species were tested for antimicrobial activity using the zone of inhibition method. The results of the study were positive suggesting that the aqueous extracts of the three plants species had significant antibacterial activity against pathogenic bacteria.

In the previous studies, *Pterocarpus marsupium* was considered to be one of the important medicinal plants in diabetes management. Different stem bark samples (apical bark, middle bark and mature inner bark) were analyzed with respect to phytoconstituents, total reducing sugars, total sugars, amylase, total polyphenols etc. The preliminary phytochemical analysis indicated the presence of pharmacologically active phytoconstituents, phenols, tannins, flavones, flavonoids, alkaloids, terpenoids and cardiac glycosides.

The saponins were absent in all the three bark Samples (Mankani *et al.*, 2005). In the present study, the phytochemicals were analyzed quantitatively in the stem bark extract of *Pterocarpus marsupium*. The result showed the presence of the secondary metabolites Tannins, Saponins, Flavonoids, Steroid, Alkaloids, Glycosides and Phenolic compounds. Tannins were found to be in large quantity and Saponins and Flavonoids were very less in quantity (Table - 4).

Simon Mole (1993) has given a brief account on tannins. Tannins are commonly found in both gymnosperms as well as angiosperms though they are distributed in species throughout the plant kingdom. He studied the distribution of tannin in about 180 families of dicotyledons and 44 families of monocotyledons. The results were based on the tests conducted to find out the ability of tannins to precipitate proteins. The best known families of which all species tested contain tannin are Aceraceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae, Dipterocarpaceae, Ericaceae, Grossulariaceae, Myricaceae for dicots and Najadaceae and Typhaceae in monocots. 73 % of the species (N = 22) contain tannin in the family of Acacias, Mimocaceae contain only 39 % tannins (N = 28). Among Solanaceae rate drops to 6 % and 4 % for the family Asteraceae. Some families like the Boraginaceae, Cucurbitaceae, Papaveraceae contain no tannin-rich species. In the present study, it was found that tannins are found in families like Anacardiaceae, Burseraceae, Caesalpinaceae, Combretaceae, Dipterocarpaceae, Ebenaceae, Fabaceae, Lauraceae, Meliaceae, Mimosaceae, Moraceae, Rubiaceae, Sapotaceae and Simaroubaceae in good amount in their barks.

Table - 1: Qualitative analysis – Tannins

Botanical Name	Tannins
Ailanthus excelsa Roxb. SIMAROUBACEAE	++
Albizzia odoratissima Benth. MIMOSACEAE	++
Albizzia lebbeck Benth. MIMOSACEAE	++
Bauhinia racemosa Lam. CAESALPINIACEAE	+++
Boswellia serrata Roxb. BURSERACEAE	++
Buchanania axillaris (Desr.) Ramamoorthy. ANACARDIACEAE	++
Butea monosperma (Lam.) Taub. FABACEAE	++
Cinnamomum iners Reinw. LAURACEAE	+++
Commiphora caudata Engl. BURSERACEAE	+++
Diospyros melanoxylon Roxb. EBENACEAE	+++
Ficus tomentosa Roxb. MORACEAE	+++
Gardenia gummifera Linn. RUBIACEAE	+++
Gardenia latifolia Ait. RUBIACEAE	+++
Holarrhena pubescens Wall. APOCYNACEAE	+
Madhuca indica J.F. Gmel. SAPOTACEAE	++
Manilkara hexandra (Roxb.) Dubard. SAPOTACEAE	++
Melia dubia Hiern. MELIACEAE	+
Mundulea sericea (Willd.) A. Chev. FABACEAE	++
Pterocarpus marsupium Roxb. FABACEAE	+++
Shorea roxburghii G.Don DIPTEROCARPACEAE	+++
Soymida febrifuga Adr. Juss. MELIACEAE	+++
Terminalia bellerica Roxb. COMBRETACEAE	+++
Terminalia chebula Retz. COMBRETACEAE	+++
Seed of *Gardenia latifolia* Ait. RUBIACEAE	++
Fruit rind of *Gardenia latifolia* Ait. RUBIACEAE	++
Seed of *Terminalia bellerica* Roxb. COMBRETACEAE	++
Fruit rind of *Terminalia bellerica* Roxb. COMBRETACEAE	++

Tannin content: +++ = Abundant; ++ = Normal and + = Less

Table - 2: Tannin estimation

Botanical Name	OD Value 1	OD Value 2	OD Value 3	Total	Average	Percentage
Ailanthus excelsa Roxb. SIMAROUBACEAE	0.113	0.11	0.109	0.332	0.110	89.13±0.04
Albizzia odoratissima Benth. MIMOSACEAE	0.122	0.117	0.108	0.347	0.115	88.26±0.07
Albizzia lebbeck Benth. MIMOSACEAE	0.129	0.124	0.122	0.375	0.125	87.59±0.04
Bauhinia racemosa Lam. CAESALPINIACEAE	0.104	0.096	0.092	0.292	0.097	90±0.06
Boswellia serrata Roxb. BURSERACEAE	0.129	0.131	0.129	0.389	0.129	87.59±0.04
Buchanania axillaris (Desr.) Ramamoorthy. ANACARDIACEAE	0.126	0.114	0.116	0.356	0.118	87.88±0.06
Butea monosperma (Lam.) Taub. FABACEAE	0.115	0.112	0.11	0.337	0.112	88.94±0.04
Cinnamomum iners Reinw. LAURACEAE	0.084	0.085	0.083	0.252	0.084	91.92±0.03
Commiphora caudata Engl. BURSERACEAE	0.088	0.085	0.077	0.25	0.083	91.53±0.06
Diospyros melanoxylon Roxb. EBENACEAE	0.091	0.09	0.089	0.27	0.09	91.25±0.03
Ficus tomentosa Roxb. MORACEAE	0.044	0.041	0.041	0.126	0.042	95.76±0.03
Gardenia gummifera Linn. RUBIACEAE	0.071	0.071	0.069	0.211	0.070	93.17±0.03
Gardenia latifolia Ait. RUBIACEAE	0.069	0.069	0.069	0.207	0.069	93.36±0.03
Holarrhena pubescens Wall. APOCYNACEAE	0.224	0.222	0.22	0.666	0.222	78.46±0.04
Madhuca indica J.F. Gmel. SAPOTACEAE	0.123	0.12	0.119	0.362	0.120	88.17±0.04
Manilkara hexandra (Roxb.) Dubard. SAPOTACEAE	0.149	0.148	0.146	0.443	0.147	85.67±0.03
Melia dubia Hiern. MELIACEAE	0.231	0.232	0.231	0.694	0.231	77.78±0.03
Mundulea sericea (Willd.) A. Chev. FABACEAE	0.168	0.167	0.163	0.498	0.166	83.84±0.04
Pterocarpus marsupium Roxb. FABACEAE	0.006	0.009	0.009	0.024	0.008	99.42±0.03
Shorea roxburghii G.Don DIPTEROCARPACEAE	0.069	0.069	0.07	0.208	0.069	93.36±0.03
Soymida febrifuga Adr. Juss. MELIACEAE	0.069	0.067	0.069	0.205	0.068	93.36±0.03
Terminalia bellerica Roxb. COMBRETACEAE	0.069	0.069	0.07	0.208	0.069	93.36±0.03
Terminalia chebula Retz. COMBRETACEAE	0.061	0.063	0.063	0.187	0.062	94.13±0.03
Seed of *Gardenia latifolia* Ait. RUBIACEAE	0.145	0.144	0.145	0.434	0.144	86.05±0.03
Fruit rind of *Gardenia latifolia* Ait. RUBIACEAE	0.146	0.146	0.145	0.437	0.145	85.96±0.03
Seed of *Terminalia bellerica* Roxb. COMBRETACEAE	0.148	0.146	0.145	0.439	0.146	85.76±0.03
Fruit rind of *Terminalia bellerica* Roxb. COMBRETACEAE	0.167	0.167	0.168	0.502	0.167	83.94±0.03

Table - 3: Phytochemical analysis of *Pterocarpus marsupium* Roxb.

Phytochemicals	Sample - 1
Tannins	+++
Saponins	+
Flavonoids	+
Steroids	++
Glycosides	+++
Alkaloids	+++
Anthroquinones	++
Phenolic compounds	+++

Table - 4: Quantitative and Phytochemical analysis of *Pterocarpus marsupium* Roxb.

Phytochemicals	Sample - 1 (mg/g)
Tannins	1.42
Saponins	0.23
Flavonoids	0.30
Steroids	0.78
Alkaloids	0.92
Glycosides	0.95
Phenolic compounds	0.87

Table - 5: Antimicrobial activity of *Pterocarpus marsupium* Roxb.

Pathogen	Zone of inhibition (mm in dm) in various concentration				
	25	50	75	100	Antibiotic (kanamycin)
P. aeruginosa	6	10	10.8	14	8
S. aureus	7	11	11.2	15	8
K. pneumoniae	4	5.2	8	12	6.5
E. coli	5.4	8	8.2	12.4	7.0
Streptococcus sp.	8	8	9.2	10	8

4. Conclusion

The results of various researches proved that tannins are one of the important secondary metabolites useful to plants, humankind and animals. The tannins present in fruit serve as a natural defense against microbial infections. This property is used in food processing to increase the shelf-life of certain foods such as cat fish fillets. Most of the tannin products are used as an astringent. Tannins also possess antimicrobial properties against many microbial pathogens. In the present study, though the effect of the methanol extract showed antibacterial activity against the five selected pathogens, it may be effective on many more human pathogens and therefore further investigations may be carried out. The pharmacological activities of tannins include antibacterial, antidiabetic, antifertility, anti-inflammatory, anti-oxidant, anti-tumour and antifungal activities. Tannins are used in dyeing, tanning and ink manufacture. They have the property of converting putrescible hides and skins into an imputrescible form. Tannins are widely used in leather industry. Mostly infusion of the bark of *Cassia auriculata*, *Acacia decurrens,* fruits of *Terminalia bellerica* and *T. Chebula* are used. The results of the present study showed that the bark of *Pterocarpus marsupium* possess abundant tannin in their bark (99.42 ± 0.03). Therefore, we can use the bark of *Pterocarpus marsupium* for tanning which will produce better results. This plant is not common and therefore we can increase its population by afforestation and reforestation programs.

5. References

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