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PHYSICOCHEMICAL, PHYTOCHEMICAL PROFILING AND ANTI-MICROBIAL ACTIVITY OF *PTEROCARPUS MARSUPIUM*

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ABSTRACT: *Pterocarpus marsupium* Roxb. (Fabaceae) is a moderate to large deciduous tree, belonging to family Fabaceae. It is considered as an extremely useful source of unique natural products for development of medicines against various diseases and for industrial product development. The present study includes physicochemical parameters like ash value, extractive value, fluorescence analysis, moisture content and preliminary phytochemical screening along with mineral analysis. Antimicrobial activity was also assessed for this plant. The physicochemical constants obtained were within normal levels prescribed by standards. The phytochemical studies revealed the presence of primary and secondary metabolites in various solvent extracts of *Pterocarpus marsupium* bark. The estimated mineral composition was in good amounts which would serve as a device for deciding dosage of ayurvedic drug prepared from the plant. Antimicrobial activity by disc diffusion method indicated the zone of inhibition which ranges from 11-22mm for different extracts. Further evaluation of test samples for minimum inhibitory concentration (MIC) using micro dilution method showed that the methanol extract had exhibited significant activity by inhibiting *S.typhi*, *E.faecalis* at 12.5µg/ml and *A.niger* at 25µg/ml. The present paper will provide the data which is helpful in correct identification, standardization of this medicinal plant prior to carrying out further pharmacological evaluation and also help in preventing its adulteration.

INTRODUCTION: Herbal traditional medicines have obtained appreciable amount of momentum worldwide during the past decade and play a paramount role in health management programs especially in developing countries¹. It is estimated that world's quadrant population *i.e.*, 1.42 billion people are dependent on folk medicines for the treatment of various ailments².

However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the scarcity of documentation, stringent quality assessment and control. There is an internationally increasing demand for documentation of research work accomplished on traditional medicines. With this backdrop, it becomes extremely important to make an attempt towards standardization of the plant material to be used as traditional medicine for proper marketing authorization and approval³. The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. Antibiotic resistance has also become a serious and widespread problem in developing countries, causing high mortality each year⁴.

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These disadvantages undermine the therapeutic utility of the currently available antibacterial drugs and thus necessitating the need for using alternative therapeutic agents from plants origin that are effective against antibiotic resistant bacteria, safe and low cost ⁵. Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth ⁶. Different chemical compounds isolated from the bark exhibit wide pharmacological activities and plays a role in treating the various disorders related to human health.

Pterocarpus marsupium Roxb. is commonly called Red Kino Tree (English), Bijasal (Hindi) and Raktahonne (Kannada). It is native to India, Nepal and Sri Lanka, where it exists in parts of the Western Ghats. Traditionally, the plant material has been used as a cooling external application for headache, inflammations, as antipyretic, anti-helminthic, aphrodisiac, mental aberrations and ulcers. The bark is used for the treatment of stomachache, cholera, dysentery, urinary complaints, tongue diseases and toothache ⁷. The heartwood and bark of *Pterocarpus marsupium* are known for their anti-diabetic activity ⁸.

A variety of flavonoids and their derivatives have been isolated from different parts of the plant. It also showed its antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* ⁹. The literature survey revealed that systemic evaluation of this plant is still lacking. Therefore, the present research work was aimed to evaluate the physicochemical, phytochemical analysis and antimicrobial activity of the *Pterocarpus marsupium* Roxb. bark.

MATERIALS AND METHODS:

Procurement and Identification of Bark material: About 5kg of *Pterocarpus marsupium* bark was collected from village Shadipur, Chincholi in Kalaburagi district, Karnataka, India during the month of June 2015. It was identified by the Department of Botany, Gulbarga University, Kalaburagi.

Preparation of plant extract: Freshly collected bark of *Pterocarpus marsupium* Roxb. was washed thoroughly under running tap water and later

through distilled water, shade dried and then powdered to required particle size. The air dried bark powder (100g) were successively extracted by Hot Soxhlet extraction with solvents of increasing polarity i.e., petroleum ether, chloroform, methanol and distilled water(aqueous).The extracts were dehydrated and preserved in a sterile container for further use.

Physicochemical analysis: The finely powdered bark of *Pterocarpus marsupium* Roxb. was subjected to various physicochemical parameters such as determination of ash value like total ash, acid insoluble ash and water soluble ash, Extractive values like petroleum ether, chloroform, methanol and water soluble, Loss on drying (Moisture content) as per the WHO guidelines on quality control methods for medicinal plant materials and according to the standard methods prescribed in Indian Pharmacopeia ^{10, 11}. Fluorescence investigation was executed according to the method of Kokoski ^{12, 13}.

Phytochemical screening: Petroleum ether, chloroform, methanol and aqueous extracts were subjected to phytochemical analysis for the presence of various secondary phytoconstituents using standard procedure described by Kokatte ^{14, 15}.

Mineral analysis: The minerals like cadmium (Cd), manganese (Mn), zinc (Zn), copper (Cu), potassium (K), magnesium (Mg), vanadium (V), titanium (Ti), calcium (Ca), and molybdenum (Mo) contents of the powdered samples were determined by the standard methods given by NIN ¹⁶. All the determinations were done in triplicates.

Determination of antimicrobial activity:

Test organisms: The antimicrobial activity of *Pterocarpus marsupium* Roxb. bark was assessed against four gram negative bacterial strains; *Salmonella typhimurium* (MTCC 98), *Escherichia coli* (MTCC 45), *Enterobacter aerogenes* (MTCC111) and *Shigella dysenteriae*; two gram positive bacterial strains; *Staphylococcus aureus* (ATCC 29122) and *Enterococcus faecalis* (ATCC 29212), fungal species; *Aspergillus niger* (MTCC 282). The test organisms were supplied by the Department of Microbiology and Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India.

Disc Diffusion Method: The agar well diffusion method was followed to test different extracts of *Pterocarpus marsupium* bark for antimicrobial activity^{17, 18}. The bacterial isolates were grown in nutrient broth for 18 hour and standardized to 0.5 McFarland standards (106 CFU/ml). The fungal isolates grown on Potato dextrose agar (PDA) at 25°C until they are sporulated. The fungal spores were harvested and standardized to 0.1 OD at 600nm. The nutrient agar and potato dextrose agar plates were prepared by pouring 20ml of molten media into sterile petriplates. 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Wells were punched using a sterile 6mm cork borer. 100µl of the crude extract (100 mg/ml) was added into the wells, incubated at 37°C, 24hrs for bacteria and 25°C, 48hrs for fungi. The effects were compared with standard chemotherapeutic agent Cefixime (30mcg, Hi-Media), Piperacillin(30mcg) and Amphotericin B(20mcg) for the gram positive, gram negative bacterial and fungal assays respectively. Dimethyl sulfoxide (DMSO) was used as negative control. Antimicrobial activity was assayed by measuring the diameter of the zone of inhibition formed around the well using standard (Hi-Media) scale. The experiment done in triplicate and the average values were calculated for antibacterial activity.

Minimum Inhibitory Concentration (MIC)

Assay: The minimum inhibitory concentrations (MIC) of different extracts were determined by the broth dilution method¹⁹. In this method, 2ml of nutrient broth and subsequently varying concentrations (100, 50, 25, 12.5, 6.25mgml⁻¹) of plant extracts was added in different test tubes. Standardized inoculums (0.1 ml, 106cfu/ml) of bacterial and fungal suspensions were added to each test tube and incubated at 37°C, 12h for bacteria and 25°C, 24h for fungi. The test tube with the concentration of plant extract at which no detectable growth was observed is considered as the MIC.

RESULTS AND DISCUSSION:

Physicochemical analysis: The nature and colour of petroleum ether extract was found to be oily, greasy yellow, methanol extract to be semi-solid red wine where as chloroform and aqueous extract appeared as solid brown.

An analytical result for total ash was found to be 11.4%. The amount of acid insoluble and water soluble ash were found to be 2.64% and 2.35% respectively. From results, it is clear that the amount of water soluble ash is less than that of acid insoluble ash, whereas the amount of total ash was almost double the quantity of water soluble ash as shown in **Table 1**. The ash content gives an idea about the inorganic content of powdered bark under exploration and thus the quality of the drugs can be evaluated. On the other hand, the water soluble extractive value of the drug was found to be 2.35% which indicates the presence of water soluble components such as sugars, acids and inorganic compounds etc and the alcohol soluble extractive values was found to be petroleum ether (0.5%), chloroform (1.10%), methanol soluble (11.49%) which indicates the presence of polar constituents like phenols, alkaloids steroids, glycosides & flavonoids.

The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore aid in evaluation of definite constituents soluble in a particular solvent²⁰. The results of physicochemical analyses were identified within the acceptable limit which in turn ascertains the quality as well as purity of drug. The moisture content was determined by loss on drying and was found to be 7.2%. The less value of moisture content of drugs could prevent bacterial, fungal or yeast growth through storage²¹.

TABLE 1: PHYSICOCHEMICAL PARAMETERS

S.no.	Parameters	Percentage(%)
1	Total ash value	11.4
2	Acid insoluble ash	2.64
3	Water soluble ash	2.35
4	Petroleum ether soluble extractive	0.5
5	Chloroform soluble extractive	1.10
6	Methanol soluble extractive	11.49
7	Water soluble extractive	2.35
8	Moisture content	7.2

Fluorescence analysis: Fluorescence is the phenomenon exhibited by various chemical components present in the plant material. In the present study, the fluorescence analysis of *Pterocarpus marsupium* bark powder was analyzed under visible light and UV light when treated with different chemicals and solvents.

The exposure of the powder to visible and UV light revealed the development of respective colours as presented in **Table 2**. Fluorescence analysis of powder gives a clue if powder is in adulteration, thus can be used as a diagnostic device for analyzing the adulteration. Presence or absence of

certain important compounds in an extract is determined by color reactions of the compounds with precise chemicals which act as dyes. This method is prerequisite before going for detailed phytochemical investigation²².

TABLE 2: FLUORESCENCE ANALYSIS OF BARK POWDER

Sl no:	Treatment	Observation under Visible light	Observation under UV light
1	Powder as such	Brown	Brown
2	Powder+Distilled water	Brick red	Dark brown
3	Powder+Petroleum ether	Light brown	Fluorescent green
4	Powder+Chloroform	Dark brown	Fluorescent green
5	Powder+Methanol	Red	Dark green
6	Powder+50% HCl	Pale yellow	Fluorescent green
7	Powder+50% HNO ₃	Mustard yellow	Pink
8	Powder+50% H ₂ SO ₄	Brown	Dark green
9	Powder+Picric acid	Yellowish brown	Yellowish green
10	Powder+Ammonia	Reddish black	Dark green
11	Powder+1N NaOH (alcoholic)	Light brown	Fluorescent green
12	Powder+1N NaOH (aqueous)	Reddish black	Dark green
13	Powder+Ferric chloride	Greenish black	Dark green
14	Powder+Acetic acid	Light brown	Fluorescent green
15	Powder+Ethyl acetate	Light brown	Fluorescent green

Phytochemical screening: Carbohydrates, proteins, amino acids, lipids and fats were present in petroleum ether and chloroform extracts whereas, methanol extract has showed positive response for all the metabolites except saponins. Aqueous extract was found to contain carbohydrates, proteins, steroids, saponins, alkaloids and tannins as summarized in **Table 3**.

Phytochemical analysis is useful in detecting the source of pharmacologically active chemical constituents. The plant contains isoflavon glycosides, fixed oil, saponin, tannin, flavonoids, alkaloids, mucilage, resin and polyphenol compounds in various parts of it²³. The phytocomponents procured in this study also correlates with the above report.

TABLE 3: PHYTOCHEMICAL TESTS FOR DIFFERENT EXTRACTS OF PTEROCARPUS MARSUPIUM BARK

Sl.no:	Tests	Petroleum ether	Chloroform	Methanol	Water
1	Test for Carbohydrates				
	A) Molisch test	+	+	+	+
	B) Benedicts test	+	+	+	+
	C) Fehlings test	+	+	+	+
2	Test for Proteins				
	A) Biuret test	+	+	+	+
	B) Ninhydrin test	+	+	+	+
3	Test for Oils and Fats				
	A) Spot test	+	+	+	-
	B) Saponification test	+	+	+	-
4	Test for Phenols				
	A) Phenol test	-	-	+	-
	B) Ellagic test	-	-	+	-
	C) Ferric chloride test	-	-	+	-
	D) Lead acetate test	-	-	+	-
	E) Gelatin test	-	-	+	-
5	Test for Flavonoids				
	A) Flavonoids test	-	-	+	-
	B) Shinoda test	-	-	+	-

	C) Ferric chloride test	-	-	+	-
	D) Lead acetate test	-	-	+	-
6	Test for Steroids				
	A) Salkowaski test	-	-	+	+
	B) Leibermann burchard test	-	-	+	+
7	Test for Saponins				
	A) Foam test	-	-	-	+
	B) Haemolysis test	-	-	-	+
8	Test for Alkaloids				
	A) Mayers test	-	-	+	+
	B) Wagners test	-	-	+	+
	C) Hagers test	-	-	+	+
9	Test for Taninns				
	A) Ferric chloride test	-	-	+	+
	B) Gelatin test	-	-	+	+
10	Test for Glycosides				
	A) Keller-killaini test	-	-	+	-
	B) Conc. Sulphuric acid test	-	-	+	-

+ Presence of the Compound; - Absence of the Compound

Mineral analysis: The AAS elemental analysis of the plant was presented in the **Table 4**. The mineral values were reported as parts per million (ppm). The estimated mineral composition were found in good amounts which would serve as a device for deciding dosage of Ayurvedic drug prepared from the plant. Mineral elements possess a very important role in human nutrition. Though they are required in minute quantities they are essential for proper functioning of the entire human system. This can further be investigated in a wide scale for the purpose of drug development against various deficiencies²⁴.

TABLE 4: MINERAL ANALYSIS

1	Cadmium (Cd)	0.0104
2	Manganese (Mn)	0.0291
3	Zinc (Zn)	0.0351
4	Copper (Cu)	0.0058
5	Potassium (K)	0.3059
6	Magnesium (Mg)	0.5973
7	Vanadium (V)	0.5643
8	Titanium (Ti)	1.7170
9	Calcium (Ca)	3.2022
10	Molybdenum (Mo)	0.1142

ppm- parts per million

Antimicrobial activity: Among the four solvent extracts, maximum inhibition was observed in methanol extract (14-22mm) followed by aqueous (13-19mm), petroleum ether (11-17) and chloroform extracts (13-16mm) as shown in **Fig. 1**. The results were compared with standard Cefixime (14-28mm), Piperacillin (21mm) for gram positive, gram negative bacteria respectively and

Amphotericin B (25–28mm) for fungi. 10% DMSO was used as negative control which showed no inhibitory effect against the tested organisms. The results of MIC assay as shown in the **Table 5**, explains that the methanol extract of *Pterocarpus marsupium* bark has shown significant antibacterial activity for *S.typhimurium* at 12.5µg/ml and antifungal activity for *Aspergillus niger* at 25µg/ml.

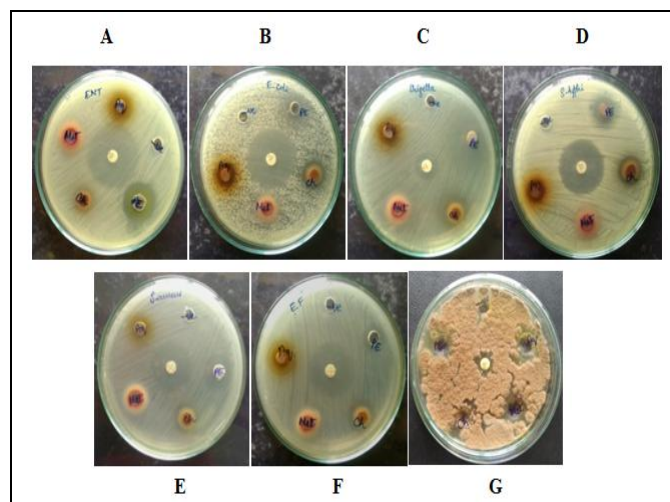


FIG. 1: ANTIMICROBIAL ACTIVITIES OF PTEROCARPUS MARSUPIUM BARK AGAINST A. Enterobacter aerogenes, B. Escherichia coli, C. Shigella dysenteriae, D. Salmonella typhimurium, E. Staphylococcus aureus, F. Enterococcus faecalis, G. Aspergillus niger respectively. PE-Petroleum ether, Ch-Chloroform, Met-Methanol, Aq-Aqueous extracts, Disc-Positive control, -ve-Negative control

On the basis of zone of inhibition and MIC values, *Salmonella typhimurium* was more sensitive to the methanol extract than all other organisms with inhibition zone of 19mm and MIC value of 12.5µg/ml respectively.

Similarly methanol extract of *P.marsupium* bark indicated maximum activity against *Pseudomonas aeruginosa*, *Streptococcus pyrogens* and *Staphylococcus aureus*²⁵. Further, identification and elucidation of active constituents in the plant material is expected to develop novel bioactive antimicrobial compounds which can be useful in designing new drugs active against several infectious micro-organisms.

TABLE 5: MIC OF BARK EXTRACTS ON BACTERIAL AND FUNGAL STRAIN

Test Organism	Strains	PE	CE	ME	AE
<i>Enterobacter aerogenes</i>	MTCC 111	100	50	50	50
<i>Escherichia coli</i>	MTCC 45	100	100	50	50
<i>Shigella dysenteriae</i>	Clinical isolate	100	100	50	100
<i>Salmonella typhimurium</i>	MTCC 98	100	50	12.5	25
<i>Staphylococcus aureus</i>	ATCC 29122	100	100	50	100
<i>Enterococcus faecalis</i>	ATCC 29212	100	50	12.5	25
<i>Aspergillus niger</i>	MTCC 282	100	100	25	50

CONCLUSION: *Pterocarpus marsupium* has a long history of numerous traditional and ethno-botanical applications in diverse cultures. Evaluation of physicochemical parameters is a vital part in the preparation of modern monograph. Thus ash value, extractive values, moisture content and fluorescence studies determined, which signifies standard parameters to ensure the quality and purity of the crude drug. The phytochemical findings of the study confirms the presence of phenolics, flavonoids and other secondary metabolites in plant which are currently of growing interest owing to their functional properties in promoting human health. From our studies, we conclude that the methanol and aqueous extracts of *P.marsupium* bark have exhibited significant antimicrobial activity against microbes. It is essential to mention that the methanolic extract has given the best all-round results. These information will be helpful to differentiate *Pterocarpus marsupium* from the closely related other species and varieties of *Pterocarpus*. Hence, the studied experimental results are useful for further pharmacological and therapeutical evaluation of the extracts along with the standardization of plant material.

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