PHYTOCHEMICAL SCREENING AND MICROBICIDAL ACTIVITY OF STEM BARK OF *PTEROCARPUS MARSUPIUM*

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Abstract

Bactericidal potential of methanolic extract of stem bark (Apical bark, middle bark and Mature bark) of *Pterocarpus marsupium* was evaluated with respect to pathogenic bacteria *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoneae, Salmonella typhi, Proteus mirabilis and Micrococcus sp.* The methanolic extract of apical stem bark was effective than the middle bark and mature bark in inhibiting the growth of all bacteria. The bacterium *Staphylococcus aureus* was most sensitive among all the bacterial species studied. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, flavonols, phenols and terpenoids. Saponins were absent in all the bark samples.The concentrations of these phytoconstituents was higher in the apical stem bark than the middle and mature stem bark. The percent extract yield was maximum in apical stem bark. Thus, in the pharmacological point of view, it is important to study the biochemistry of apical bark in order to isolate and screen the new pharmacological active principals which can be useful in designing of new drugs active against various infectious micro-organisms like bacteria, fungi and viruses etc.

Key words: Bactericidal potential, *Pterocarpus marsupium*, phytochemical analysis, percent extract yield and phytoconstituents.

Introduction:

Plants are regarded as the pharmaceutical factories of natural origin for most of the drugs used by human beings. Plants medicines are highly important in the lives of human. As India is the largest producer and consumer of the medicinal drugs and is rightly called the botanical garden of the world [1]. Most of the plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives and pesticides. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [2]. Large number of primary metabolites acts as precursors of pharmacologically active metabolites in pharmaceutical compounds for the synthesis of drugs. Biotechnologically derived and synthesized medicines have renewed interest to pay attention on herbalism [3]. Traditionally, bark products have been particularly prominent as sources of medicines and raw materials. Different chemical compounds isolated from the bark exhibit wide pharmacological activities and plays role in treating the various disorders related to human health.

Pterocarpus marsupium (Roxb.) is a deciduous tree, commonly called as Indian Kino tree or Malabar Kino, belonging to the family fabaceae. The bark exudes a red gummy substance called 'Gum Kino' when injured. *Pterocarpus marsupium* is distributed in deciduous forest throughout the India [4]. Heart wood is astringent, bitter, acrid, cooling, anti-inflammatory, depurative, haemostatic, constipating and rejuvenating [5]. Bark is useful in vitiated condition of *kapha* and *pitta*, elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentery, cough and grayness of hair. Aqueous infusions of the bark possess antidiabetic potential [6]. The powdered bark is mixed with *Schleichera oleosa* and taken with cold water to treat dysentery [7]. The juice of the bark is applied in the mouth [8].Wood of the tree is useful in making the water glasses of the diabetic patients [9].

Materials and Methods

Collection and processing of the plant material

Different bark samples (Apical bark, middle bark and mature inner bark) of *Pterocarpus marsupium*, were collected from the hilly regions of Kolhapur district. The bark was collected in the month of May 2009.

The bark samples were cut into pieces, sun-dried then oven dried at 60^0C. Dried bark samples were ground into powder and stored in an air tight plastic container.

Microorganisms

Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa, Klebsiella pneumoneae, Salmonella typhi, Proteus mirabilis and Micrococcus sp. were used for testing antibacterial activity of bark extracts. The test organisms used in this study were obtained from the department of Microbiology, Shivaji University, Kolahapur, Maharashtra, India. The bacterial strains were cultured on nutrient agar slants. The cultures were maintained by subculturing periodically and preserved at 4ºC until further use.

Preparation of the extract

Oven dried 10g of powdered bark material was weighed accurately and placed in soxhlet extraction chamber which was suspended above the flask containing 100mL of 80% methanol and below a condenser. The flask was heated and the methanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded at certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and methanol was evaporated by using rotary evaporator. The weight of the residual extract was measured and percent yield was calculated. The residue of the extract was dissolved in 25ml of pure methanol and stored in air tight glass vials at 4^0C until further use [10].

$$\text{Extract yield \%} = \frac{W1}{W2} \times 100$$

Where

W1= Net wt of powder in grams after extraction

W2= total wt of wood powder in grams taken for extraction.

Qualitative screening of phytochemicals

Different extracts were screened for the presence of alkaloids, glycosides, flavonoids, flavanols, phenols, saponins and terpenoids by using standard protocols [11], [12].

Preparation of the media

Accurately weighed 28g of nutrient agar (Himedia) was dissolved in the 1000ml of distilled water. The medium was sterilized under 15Lb pressure for 15 minutes in an autoclave. 30ml of this sterilized semisolid nutrient agar medium was poured in pre-sterilized 90mm glass petriplates under aseptic conditions in laminar flow. The plates were allowed to cool at room temperature to solidify the medium.

Determination of antibacterial activity by agar well diffusion method

Agar well diffusion method described by Perz *et al*., [13] was employed to determine antibacterial activity. Well of 10mm diameter was prepared with sterilized cork-borer. Standared antibiotics Chloramphenicol at 50µg/ml were served as positive control and Methanol as negative control. The plates inoculated with different bacterial species were incubated at 37^0C in incubator for 24 h and the zone of inhibition was measured (Diameter in mm). All of the experiments were performed in triplicate. The results are reported as the average of 3 experiments.

RESULTS AND DISCUSSION

Qualitative analysis of methanolic extract of bark revealed the presence of some secondary metabolite alkaloids, glycosides, flavonoids, flavanols, phenols, and terpenoids (Table No. 2). The methanolic extract showed the absence of saponins in all the three samples. The concentration of these phytoconstituents was

intensely higher in the apical stem bark than the middle bark and mature bark. The apical stem bark shown higher percent extract yield than the middle and mature bark on main trunk (Table No.1).

Antibacterial activity of stem bark extract of *Pterocarpus marsupium* against different bacterial pathogens is displayed in table No. 3. From the Table No. 3 it is clear that the bactericidal potential of apical stem bark extract was maximum than middle and mature bark against all the bacterial species studied except the bacterium *Pseudomonas aureginosa*. The bacterium *Bacillus subtilis* and *Pseudomonas aeruginosa* were inhibited at a minimum inhibitory concentration of 50 µL while inhibition by mature bark extract in case *Bacillus subtilis* was observed at minimum concentration of 100 µL. The MIC for *Staphylococcus aureus* was at 100 µL concentration while for *Micrococcus sp Proteus mirabilis, Klebsiella pneumoneae, Salmonella typhi* and *Escherichia coli* was observed at 200 µL concentration. The inhibitory effect was increased with increase in extract concentration and showed maximum inhibition at 300 µL extract concentration. At maximum extract concentration among the three bark samples, the apical stem bark highly inhibited the growth of *Bacillus subtilis* (8.50 ± 0.55mm). In case of *Staphylococcus aureus* the inhibition by apical and middle bark extract was more or less similar i.e. 8.17 ± 0.75 and 8.00 ± 0.89mm respectively. The growth of bacteria *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoneae,* and *Micrococcus sp* was significantly inhibited by apical stem bark extract and exihibited the inhibition zones 5.83 ± 0.41mm, 9.83 ± 0.71mm, 6.17 ± 0.98mm, 5.33 ± 0.16mm, 4.03 ± 0.51mm and 4.48 ± 0.21mm respectively. The inhibitory effect was observed in the order *Pseudomonas aeruginosa* > *Staphylococcus aureus* >*Bacillus subtilis* > *Salmonella typhi* > *Escherichia coli* > *Klebsiella pneumoneae* > *Proteus mirabilis* > *Micrococcus sp.* The inhibition by negative control methanol was zero while the standard antibiotic Chloramphenicol was inhibited the growth of all the bacterial species effectively at low concentration of 50µg/ml with the zone of inhibitions ranging from 11.17mm to 21.50mm.

Phenolics and polyphenols present in the plants are known to be toxic to the microorganism [14]. Tannin from *Dichrostachys cinerea* root bark possesses antibacterial activities against *S. aureus, E. coli* and *P. aeruginosa* [15]. In vitro studies by Chung *et al*., [16] showed that tannins with different structure inhibited the growth of the microorganism. Flavonoids have been reported to have both antibacterial and antifungal activities [17]. Bijase *et al*., [18] reported that isoflavonoids from methanolic extract of root bark and stem bark of *Bolusanthus specious* exhibits antibacterial activity. The bark extract was found to be containing tannin glycosides, alkaloids, steroids and Flavonoids which are biologically active [19]. Among the most of the phytoconstituents which posses potent antibacterial activity, alkaloids also exhibit microbicidal action [20]. In our study all the three bark samples revealed the presence of secondary metabolite. The different rates of inhibition observed may be probably due to the quantity of the phytochemicals present in the extracts [21]. In young apical bark there might be synthesis of some new active principles which are present in low concentration in the middle and mature bark predicting less inhibitory activity. Another reason to low bactericidal potential exhibited by middle and mature bark can be explained by the fact that the active principle(s) present in the apical bark might have undergone metabolism during ageing of the bark and the byproducts might be possessing less activity reflecting low bactericidal potential of middle and mature bark or might be present in inactive form imparting no activity to these bark samples.

Conclusion:

The new active principles present in the apical bark may be active against widely spreading human diseases like diabetes, hepatitis, AIDS etc. Thus, in the pharmacological point of view, it is important to study the biochemistry of apical bark in order to isolate and screen the new pharmacological active principals which can be useful in designing of new drugs active against various infectious micro-organisms like bacteria, fungi and viruses etc.

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Table No. 1. Percent Extract Yield

Plant name	**Bark sample**	**Summer**
Pterocarpus marsupium	Apical bark	23.21%
	Middle bark	22.63%
	Mature bark	21. 17%

Table No. 2 Phytochemical analysis of methanolic extract of bark of *Pterocarpus marsupium*

Sample	Phenols	Flavones	Flavonoid	Tannin	Terpenoids	Saponin	Alkaloids	Cardiac glycosides
1	+++	+++	+++	+++	+++	-	+++	+++
2	++	++	++	++	++	-	++	++
3	+	+	+	+	+	-	+	+

1-Apical bark; 2-Middle bark and 3- Mature bark
+++: Present in high concentration , ++ : Present in moderate concentration and + : Present in low concentration
'-': Absent

Table No : 3 Antibacterial activity of stem Bark of *Pterocarpus marsupium*

Minimum inhibitory concentration Zone of inhibition (Diameter in 'mm')*								
Microorganisms	**Bark sample**	25µL	50 µL	100 µL	200 µL	300 µL	M	C (50µg/ml)
Bacillus subtilis	**1**	o.oo	2.30 ± 0,41	3.50 ± 0.55	5.67 ± 0.52	8.50 ± 0.55	o.oo	21.50 ± 1.05
	2	o.oo	1.75 ± 0.66	3.33 ± 0.52	5.67 ± 0.52	7.33 ± 0.48	o.oo	
	3	o.oo	0.00	1.33 ± 0.52	2.17 ± 0.41	4.83 ± 0.75	o.oo	
Staphylococcus aureus	**1**	o.oo	o.oo	1.83 ± 0.75	4.50 ± 0.84	8.17 ± 0.75	o.oo	11.17 ± 0.98
	2	o.oo	o.oo	1.33 ± 0.63	3.83 ± 0.75	8.00 ± 0.89	o.oo	
	3	o.oo	o.oo	1.33 ± 0.52	2.83 ± 0.41	7.83 ± 0.75	o.oo	
Escherichia coli	**1**	o.oo	o.oo	o.oo	3.67 ± 0.52	5.83 ± 0.41	o.oo	16.50 ± 0.55
	2	o.oo	o.oo	o.oo	1.50 ± 0.55	3.33 ± 0.52	o.oo	
	3	o.oo	o.oo	o.oo	1.67 ± 0.53	3.50 ± 0.55	o.oo	
Pseudomonas aeruginosa	**1**	o.oo	2.64 ± 0.43	4.83 ± 0.52	6. 67 ± 0.75	9.83 ± 0.71	o.oo	14.33 ± 0.5
	2	o.oo	1. 87 ± 0.79	4.67± 0.52	6.47 ± 0.52	8.60 ± 0.50	o.oo	
	3	o.oo	1. 56 ± 0.65	3.67 ± 0.75	5.10 ± 0.55	7.67 ± 0.52	o.oo	
Salmonella typhi	**1**	o.oo	o.oo	o.oo	2.50 ± 0.55	6.17 ± 0.98	o.oo	16.67 ± 1.21
	2	o.oo	o.oo	o.oo	1.33 ± 0.52	3.83 ± 0.75	o.oo	
	3	o.oo	o.oo	o.oo	1.17 ± 0.39	3.00 ± 0.63	o.oo	
Klebsiella pneumoneae	**1**	o.oo	o.oo	o.oo	3.48 ± 0.09	5.33 ± 0.16	o.oo	14.85± 1.17
	2	o.oo	o.oo	o.oo	1.88 ± 0.29	2.77 ± 0.57	o.oo	
	3	o.oo	o.oo	o.oo	1.23 ± 0.11	1.65 ±0.83	o.oo	
Proteus mirabilis	**1**	o.oo	o.oo	o.oo	3.01 ± 0.63	4.03 ± 0.51	o.oo	13.70 ±0.49
	2	o.oo	o.oo	o.oo	1.69 ± 0.07	2.53 ± 0.22	o.oo	
	3	o.oo	o.oo	o.oo	1.21 ± 0.83	1.96 ± 0.78	o.oo	
Micrococcus sp	**1**	o.oo	o.oo	o.oo	3.77 ± 0.44	4.48 ± 0.21	o.oo	16.63 ± 0.23
	2	o.oo	o.oo	o.oo	1. 60 ± 0.57	2.9 ± 0.87	o.oo	
	3	o.oo	o.oo	o.oo	1. 23 ± 0.31	1.91 ± 0.57	o.oo	

1: Apical Bark, 2: Middle Bark and 3: Mature Bark C: Chloramphenicol M: Methanol; *: Agar well diffusion method
Values are mean ± SD of three replicates