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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *CYNOMETRA IRIPA* KOSTEL.

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ABSTRACT

Antimicrobial activity of aerial parts of *Cynometra iripa* Kostel was evaluated against *Bacillus cereus, Staphylococcus aureus, Pseudomonas aerunginosa, and Salmonella typhimurium.* Ethanolic and methanolic extracts of plant parts were screened for antibacterial activity. Methanolic extracts of leaf, stem, young seed, mature seed and seed coat showed a high antibacterial activity against *Pseudomonas aerunginosa* as compared to ethanolic extracts products. Preliminary tests for different chemical groups showed the presence of polyphenols, tannins, flavonoids and saponins.

KEY WORDS

Cynometra iripa, Antibacterial activity, Saponins, Tannins, Flavonoids, Polyphenols.

INTRODUCTION

Natural products are preferred for biologically screening based on ethno medical use of plants, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world[1,2,3]. Drug resistance to pathogenic microorganisms has been reported in literature[4, 5] therefore antimicrobials may have a significant clinical value in treatment of resistant microbial strains[6]. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds and they provide a rich source of saponins, tannins, flavonoids, and polyphenols[7]. Therefore, it was thought worthwhile to screen mangrove species for the presence of antibacterial potential to combat the pathogenic bacteria.

Cynometra a Linnaean genus of approximately 75 species is widely distributed through out the tropics[8]. *Cynometra iripa* Kostel is critically endangered species of mangroves a member of Fabaceae and occurring along the West Coast of Maharashtra. There are much more variations in the heights of *Cynometra* trees which are recorded from 1.5m to tall as 9m[9]. *Cynometra iripa* is characterized by distally curved sepals, bent style and fruits with a prominent lateral beak. The tree gets its common name from its hard, wrinkly seed pods as 'wrinkle pod mangrove'.

MATERIAL AND METHOD

The fresh leaves, stem, young seeds, mature seeds of *C. iripa* Kostel were collected from estuaries of Sindhudurg district of Maharashtra. The collected samples were washed with distilled water properly for removing adhered soil and other particles and initially air dried under shade, then in oven and

powdered. Then the powder was subjected to Soxhlet extraction using ethanol and methanol. The extracts were evaporated till reddish black, slightly solid gummy material was obtained and then preserved at $5^{O}C$ in air tight bottles. The phytochemical tests were carried out following the standard methods [10,11,12,13,14]. Antibacterial activity of ethanol and methanol extracts was determined by cup plate diffusion method[15]. The wells of 7mm were made in the media using sterile cork borer and inoculums containing 10^{6} colony forming units (CFU)/ml were spread on solid plates.

The test extracts were poured in the wells with the help of sterilized pipette. Each well was filled with 0.1 ml extract. Controls were maintained with methanol, ethanol, sterile water and Streptomycin (Nicholas Parimal India Limited Jogeshwari, Mumbai.) as standard antibiotic, in place of the extract at recommended doses. The plates were then kept at $4^{O}C$ for facilitating maximum diffusion and then were transferred in an incubator ($37^{O}C$) and kept for 12-18 hours to allow the growth of microorganisms. Then the diameter of zone of inhibition around wells was measured. The experiments were carried out in three replicates and the mean readings were recorded.

RESULTS AND DISCUSSION

Antibacterial activity of ethanolic and methanolic extracted products of *Cynometra iripa* were examined and found to exhibit a good antibacterial activity at 0.1 ml against most of the studied gram positive and gram negative organisms. The results are depicted in the Table 1.

Among the pathogenic bacteria, *Bacillus cereus* was highly susceptible to ethanolic seed coat extract while *Staphylococcus aureus* showed high sensitivity to ethanolic extract of leaf, stem and methanolic extract of mature seed. *Pseudomonas aeruginosa* was highly susceptible to the methanolic extract of leaf and stem and *Salmonella typhimurium* was highly susceptible to the ethanolic extract of leaf and stem. In the present study, some of the bacterial strains showed resistance to crude extracts, whereas the fractions showed a broad spectrum activity against multiple strains which can be correlated to the masking of antibacterial activity by the presence of some inhibitory compounds or factors that might be present in the extract. The variations in antibacterial activity of extracts may be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Similar observations were made by Vlachose *et al.*[16] who found that fractionation of crude extracts tested enhanced their activity against both Gram negative as well as Gram positive pathogens. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz. polyphenols, tannins, flavonoids, saponins (Table 2) which have been reported for various antimicrobial activities[17,18].

The high concentration of polyphenols, tannins, flavonoids, saponins in leaf, stem and mature seeds can be correlated with impressive antibacterial activity of their ethanolic and methanolic extracts. Whereas low concentrations of phytochemicals in young seed and seed coat proves to have moderate or relatively low antibacterial potential, except the ethanolic seed coat extract which has been effective against *Staphylococcus aureus.* The present study was conducted to develop newer lead for better and safer chemotherapeutic agents of the plant origin from West Coast of Maharashtra. Further studies are needed to purify the active components of antimicrobial importance. From the work results, it is suggested that the methanolic extract of leaf and stem of *Cynometra iripa* shows higher antibacterial activity against *Pseudomonas aeruginosa*. Further studies are under probe.

Table 1.

In vitro screening of crude ethanolic, methanolic extract of Cynometra iripa Kostel on growth of pathogenic bacteria.

Extract	Zone of Inhibition (Diameter in mm)							
	Bacillus cereus		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Salmonella typhimurim*	
	E	M	E	M	E	M	E	M
Leaf	2	3	4	3	8	10	9	7
Stem	4	3	4	2	8	11	8	4
Young seed	4	4	3	2	6	8	6	5
Mature seed	3	4	3	4	8	9	7	5
Seed coat	5	3	1	2	5	7	2	5
Sterile water	00		00		00		00	
Streptomycin	22		26		22		27	
Ethanol	2		2		3		2	
Methanol	3		3		2		2	

E = Ethanolic extract, M=Methanolic extract

Table 2.
Phytochemical screening of a mangrove species Cynometa iripa Kostel.

Phytochemicals	Parts of *C. iripa*				
	Leaf	Stem	Young seed	Mature seed	Seed coat
Polyphenols	++	+++	+	+	+
Tannins	++	+++	+	++	+
Flavonoids	+++	++	+	++	+
Saponins	++	++	+	++	+

+ Low, ++ moderate, +++ high conc.

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