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Least Concerned Bark and Stipules of Artocarpus Species (Moraceae) – An Effective Antibacterial Agent

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Abstract

The present study aims to evaluate the comparative antibacterial properties of bark and stipule samples of Artocarpus heterophyllus (Jack fruit), Artocarpus hirsutus (Wild jack), and Artocarpus altilis (Bread fruit) extracted with Acetone, Distilled water, Ethanol, Chloroform, Petroleum ether and Benzene against various clinical pathogens like Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Bacillus subtilis. The antimicrobial activity study was carried out using agar well diffusion method. The results obtained indicated that both the samples of the selected three species possess a potent anti-bacterial activity against E. coli, P. aeruginosa, S. typhi and B. subtilis using different extracts. Hence the bark and stipule samples of selected species form a potentially good source of antimicrobial agent and demonstrate its importance in medicinal systems as a good functional medicine.

Keywords: *Artocarpus heterophyllus, Artocarpus hirsutus, Artocarpus altilis,* Bark, Stipule.

Introduction

The genus *Artocarpus* belongs to the family Moraceae and is distributed in India with 18 species[1]. The name *Artocarpus* is derived from the Greek words *artos* (bread) and *carpos* (fruit)[2]. Many species belonging to this family have been used traditionally for their medicinal properties. Three species viz. *Artocarpus hirsutus, Artocarpus heterophyllus* and *Artocarpus altilis* were chosen for the study. Among various species under this genus, the wild jack tree, *Artocarpus hirsutus* Lam. is the only species, whose distribution is constrained to the southern Western Ghats. Also it forms one of the major keystone species of Western Ghats[3,4]. The tree attains a height of about 45 m and girth up to 4.5 m. It is popular as a valuable timber yielding tree along the Malabar Coast[5]. It is also notable for its valuable medicinal properties. Dry leaves are useful in treating buboes and hydrocele. Fruit constitutes a rich source of carbohydrates, ß-carotene and essential aminoacids. Unripe fruits are useful in vitiated conditions of vata and pitta and anorexia. The ripe fruits posses sour, sweet, cooling, appetizer, constipating and aphrodisiac properties. It causes flatulence, colic, tridosa and rakta vitiations. An infusion of the bark is applied to cure small pimples and cracks on the skin, and the powdered bark is used to heal sores. Bark ash mixed with coconut oil is used externally against 'dhobi's itch' and ringworm[6]. Bark paste in coconut oil also can be applied for snake bite[7].

Artocarpus heterophyllus Lam. (jackfruit) is indigenous to tropical Asia[8]. This species is naturally available in the forests of Western Ghats which is believed to be its place of origin[9,10]. It is cultivated in most of the states throughout India. It grows fastly and posses an evergreen, dense canopy with an approximate height of 30 m or more and 30 to 60 cm d.b.h. Each and every part of the jack tree is found to posses some valuable uses[11]. Root extract obtained from the plant is used to treat asthma, skin infections and diarrhea. Wood extract posses sedative property, extract from the pith has the capability to induce abortion[10]. An infusion obtained from bark and mature leaves are utilized for treating gallstones and diabetes. Ash of the leaves is used to heal ulcers[12]. Heated leaves are used for curing wounds[10]. Astringent, acrid, and carminative properties were exhibited by unripe fruits. While ripe fruits are used for treating biliousness and posses laxative, fattening and cooling properties. Seeds obtained from the plant are diuretic and is rich in starch which is given to relieve biliousness and the roasted seeds acquire aphrodisiac property[13].

Artocarpus altilis (Parkinson) Fosberg, commonly referred as breadfruit is cultivated throughout Oceania and mostly along wet tropics. The native of this plant is Malaysia, Papua New Guinea and Philippines. It has been used as an important traditional medicine in Malaysia, Brazil, Dominica and Indonesia, where every part of *A. altilis* has its own importance[14]. Various parts of the plant were reported to possess antitubercular, antiplasmodial, anticancer, and antihypertensive and cytoprotective properties[15-18].

In this study, we aimed to detect a possible inhibitory effect of different extracts of bark and stipule of *Artocarpus heterophyllus, Artocarpus hirsutus* and *Artocarpus altilis* on the growth of various selected human pathogens tested by using agar disc diffusion method.

Material and Methods

Collection of plant material: The bark and stipule samples from three selected *Artocarpus* species were collected from Marthandam, Kanyakumari District, Tamilnadu. The plants

were identified and voucher specimens were deposited to the Herbarium, Department of Botany, Nesamony Memorial Christian College, Marthandam. Each of the samples was shade dried and ground to powder form and stored for further use.

Sample extraction: 500g of powdered bark and stipule samples were weighed and taken separately for the respective species. These samples were extracted with Acetone, Distilled water, Ethanol, Chloroform, Petroleum ether and Benzene individually using Soxhlet's apparatus. The organic extracts obtained were evaporated to dryness by kept open in room temperature. However in case of aqueous extraction, the extract was heated and evaporated to dryness to obtain a semisolid mass using a water bath. This concentrate was later subjected to microbial bioassays.

Microbial Bioassays: Bacterial Isolates and Bioassay: The extracts of Acetone, Distilled water, Ethanol, Chloroform, Petroleum ether and Benzene obtained from the bark and stipules of three selected species were screened against four pathogenic bacterial strains. Standard antibiotic gentamicin is used as a control against the pathogens investigated. The test organisms, *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis* were obtained from "Scudder Research Institute", Nagercoil, Kanyakumari.

Preparation of Inoculum: Stock cultures of selected pathogens were maintained at 4ºC on nutrient agar slants. A loopful of cells from these stock cultures were transferred to Mueller-Hinton broth medium (MHB) and incubated without agitation for 24 hrs at 37 ºC. These cultures were successfully utilized for experimental purpose.

Antimicrobial Suceptibility Test: The antimicrobial susceptibility was tested by agar disc diffusion method using Mueller Hinton Agar medium (MHA). 15-18ml of molten media was poured into each sterile petriplates and was allowed to solidify for about 10 minutes. Inoculum suspension (0.1%) was uniformly swabbed on these sterile petriplates and was allowed to dry. Now the extracts obtained from bark and stipule of selected species (20µl) were loaded on 4mm sterile discs followed by placing it on the surface of medium and the extract was allowed to diffuse. The plates were now kept for incubation at 37ºC for 24 hrs. The petriplates were checked for inhibition zone formation. The zone obtained around the discs was measured with transparent ruler in millimetre. The study was performed in duplicates.

Results and Discussion

The present investigation is focussed on the comparative study of bark and stipules of three tree species of moraceae family and of genus *Artocarpus, A. heterophyllus, A. hirsutus* and *A. altilis*, for their antibacterial activity as shown in figure -1. Acetone, distilled water, ethanol, chloroform, petroleum ether and benzene extracts extracted from the stipule of selected *Artocarpus* species showed antibacterial activity as reported in table–1. In the case of *A. heterophyllus*, there exhibits no activity from the distilled water and petroleum ether extracts. The activity of ethanol extract was found to be higher with *Bacillus subtilis, Salmonella typhi* and *E. coli*. Similar results were reported from the seed extracts of *A. heterophyllus* on *B. subtilis* and *E. coli* species and are due to the presence of phytolectins isolated from it[19]. Also methanolic seed extract is reported to posses excellent cytotoxic effect[20]. Similar studies were also reported in other species like *Punica granatum*[21], *Cinnamomum zeylanicum* and *Trachyspermum ammi*[22]. However little or no activity was observed with acetone, chloroform and benzene extracts. Similarly the action of ethanol and benzene extracts of *A. hirsutus* on all the four selected pathogens is noteworthy. The susceptibility of *Pseudomonas to* the stipule extract of this plant directly points that it can be utilized as a drug against various serious infections like mastitis caused by this organism[23]. Acetone and chloroform extracts exhibits little efficacy and no results in the case of distilled water and petroleum ether extracts. Ethanol and petroleum ether extracts of *A. altilis* shown significant results on all selected pathogens. Same results were also reported in this species by methanol and petroleum ether extracts of leaf samples[24]. Meanwhile, all other extracts treated produced insignificant results.

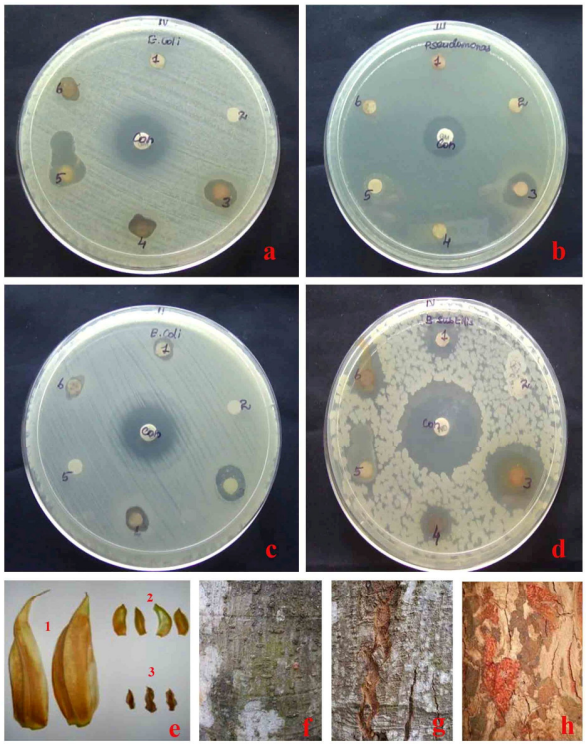

Figure-1

(a) Antibacterial activity of Acetone, Distilled water, Ethanol, Chloroform, Petroleum ether and Benzene extracts of : Bark sample *of A. hirsutus* on *Escherichia coli* (b) Stipule sample *of A. hirsutus* on *Pseudomonas aeruginosa* (c) Bark sample *of A. heterophyllus* on *Escherichia coli* (d) Bark sample *of A. hirsutus* on *Bacillus subtilis* (e) Stipules of (1) *A. altilis* (2) *A. heterophyllus* (3) *A. hirsutus* (f), (g) & (h) Barks of *A. altilis, A. hirsutus* and *A. heterophyllus*

Likewise, Acetone, Distilled water, Ethanol, Chloroform, Petroleum ether and Benzene extracts extracted from the bark of selected *Artocarpus* species possessed prominent antibacterial activity as reported in table–2. From the results, it is revealed that ethanol and benzene extracts from the three selected species possessed most significant results with high inhibition zone formation for all the pathogens investigated. Similar results were also reported from the ethanolic seed extracts of *A. heterophyllus*[19] as well as from methanolic root extracts of *A. communis*[25]. Also the presence of important classes of phytoconstituents was reported from the leaf samples of *A. heterophyllus* and it was assumed to be more therapeutic[26]. In the meantime acetone and petroleum ether extracts subjected also produced satisfactory results. Additionally the results obtained from *A. altilis* were in corroborative with the findings by ethanol and petroleum ether extracts of leaf samples of the same species[24]. Similar antibacterial efficacy studies were also reported in *Pistacia atlantica*[27], *Mimusops elengi*[28] and *Daucus species*[29]. On the other hand, distilled water and chloroform extracts exposed little or no results.

Table-1
Antibacterial assay of stipule extracts of selected *Artocarpus* sp.

Sl. No.	Plant	Microorganisms	Extracts						
			Acetone	Dis.H2O	Ethanol	Chloroform	Petroleum ether	Benzene	Control
1	*Artocarpus heterophyllus*	*E.coli*	9mm	-	9mm	-	-	-	17mm
		Pseudomonas aeruginosa	-	-	-	8mm	-	-	18mm
		Salmonella typhi	-	-	12mm	8mm	-	10mm	21mm
		Bacillus subtilis	12mm	-	15mm	-	-	-	35mm
2	*Artocarpus hirsutus*	*E.coli*	7mm	-	15mm	7mm	-	11mm	20mm
		Pseudomonas aeruginosa	-	-	14mm	-	-	7mm	17mm
		Salmonella typhi	9mm	-	10mm	8mm	-	9mm	23mm
		Bacillus subtilis	-	-	16mm	9mm	-	15mm	34mm
3	*Artocarpus altilis*	*E.coli*	-	-	14mm	-	15mm	-	16mm
		Pseudomonas aeruginosa	-	-	14mm	-	13mm	-	13mm
		Salmonella typhi	-	-	14mm	-	19mm	-	24mm
		Bacillus subtilis	10mm	-	22mm	-	11mm	-	33mm

Table-2
Antibacterial assay of bark extracts of selected *Artocarpus* sp.

Sl. No.	Plant	Microorganisms	Extracts						
			Acetone	Dis.H2O	Ethanol	Chloroform	Petroleum ether	Benzene	Control
1	*Artocarpus heterophyllus*	*E.coli*	9mm	-	13mm	10mm	-	9mm	20mm
		Pseudomonas aeruginosa	9mm	-	14mm	-	-	8mm	20mm
		Salmonella typhi	8mm	-	12mm	10mm	-	11mm	24mm
		Bacillus subtilis	10mm	-	18mm	-	10mm	10mm	34mm
2	*Artocarpus hirsutus*	*E.coli*	-	-	13mm	10mm	14mm	10mm	17mm
		Pseudomonas aeruginosa	9mm	-	13mm	8mm	15mm	9mm	18mm
		Salmonella typhi	-	-	17mm	-	10mm	9mm	23mm
		Bacillus subtilis	14mm	-	24mm	13mm	15mm	14mm	33mm
3	*Artocarpus altilis*	*E.coli*	9mm	-	19mm	-	7mm	9mm	17mm
		Pseudomonas aeruginosa	10mm	-	11mm	-	8mm	7mm	20mm
		Salmonella typhi	15mm	-	9mm	-	12mm	7mm	23mm
		Bacillus subtilis	-	-	8mm	10mm	15mm	10mm	35mm

Conclusion

From the results it is concluded that, the present investigation gives clues and valuable informations for the use of *A. heterophyllus, A. hirsutus* and *A. altilis,* especially its bark and stipules against various diseases caused by the microorganisms investigated. These plant parts also possessed prominent folkloric usage in traditional systems of medicine for curing various ailments. While now a days they are least concerned and too considered as a waste. Further these three selected moraceae species, including an endemic one is commonly found in the home gardens as well as along the road sides of southern Tamilnadu and Kerala. In utility point of view, it can be utilized for its timber, fruits and also for the least concerned bark and stipules as an effective antimicrobial agent. However, phytochemical and pharmacological studies are absolutely necessary for its successful utility in various medicinal systems.

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