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Full Length Research Paper

Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L.f

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***Semicarpus anacardium* L.f. is a medicinal plant that belongs to the family of Anacardiaceae and is used by tribals of Similpal Biosphere Reserve, Orissa, India for various ailments. Aqueous and organic solvent extracts of the plant were screened for antimicrobial (disc diffusion method) and phytochemical properties. The petroleum ether (PEE) and aqueous extract fractions (AQE) showed inhibitory activity against *Staphylococcus aureus* (10 mm) and *Shigella flexneri* (16 mm) at 100 mg/ml, respectively. While chloroform extract showed inhibition against *Bacillus licheniformis*, *Vibiro cholerae* and *Pseudomonas. aeruginosa*. The ethanol extract showed inhibition to *Pseudomonas aeruginosa* and *S. aureus*. The oils extracted from nuts did not exhibit any antimicrobial activity. Alkaloids, tannins, saponins, flavonoids, anthraquinone and volatile oils were detected in nuts aqueous extracts with only triperpenoid in PEE and steroid in both PEE and chloroform extracts. The phytochemicals such as alkaloids, flavonoids, tannin and anthroquinone were present in oils extracted from nuts. The antibacterial activity of the nut extracts of *Semicarpus anacardium* is due to AQE and PEE-extractable compounds. However, the active component (s) responsible for the antibacterial activity can be isolated**.

Key words: *Semicarpus anacardium*, Anacardiaceae, Phytochemicals, Antibacterial activity.

INTRODUCTION

The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drugs constitute a major part in all the traditional system of medicines (Higa et al., 1994). A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity, the so-called secondary metabolites (Castello et al., 2002). Screening of compounds obtained from plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents representing molecular diversity engineered by nature. It is therefore necessary and urgent to fight against emerging and re-emerging infectious diseases with a view to discover and invent new agents of greater therapeutic profile to mitigate frequent outbreaks of diseases which has posed a new threat to global health security. Further, newer strains are being continuously discovered which are refractory to the current arsenal of drugs (Erturk et al., 2006). Plant extracts have been used for a wide variety of purposes for many thousands of years. These purposes vary from the use of rosewood and cedarwood in perfumery, to flavouring drinks with lime, fennel or juniper berry oil and the application of lemongrass oil for preservation of stored food crops (Hammer et al., 1999).

Screening of medicinal plants for antimicrobial agents has gained much importance because lately World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in these countries (Goud et al., 2005).

Semicarpus anacardium L.f. (Anacardiaceae) is a deciduous tree distributed in the sub-Himalayan tract and in tropical parts of India. It is also widely distributed in the forests of Similpal Biosphere Reserve, Orissa. The plant is used for treatment of various ailments like rheumatism, asthma, epilepsy, nervous debility and also tumors (Ambasta, 1986). The tribals of Similpal Biosphere Reserve use it for curing headache, hydrocoel and as antiseptic

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Table 1. Antibacterial activity of petroleum ether nut extracts of *Semicarpus anacardium*.

Microorganisms	Inhibition zone (mm)				DMSO
	10 mg/ml	50 mg/ml	100 mg/ml	10 mg G/ml	
Staphylococcus aureus	_	3	10	26	14
Shigella flexneri	_	-	-	23	11
Bacillus licheniformis	_	-	-	23	11
Bacillus brevis	_	2	7	23	26
Vibrio cholerae	_	-	-	26	15
Pseudomonas aeruginosa	_	-	-	ND	18
Streptococcus aureus	_	-	-	ND	-

G = gentamycin (Standard antibiotic), (-) = No activity, ND = Not detected.

etc. Some studies on immunomodulatory and anti-inflammatory effects of *Sermicarpus anacardium* have been reported by Ramprasath et al., (2006) . Anticancer proper-ties have also been experimentally verified (Ambasta, 1986). So far the antimicrobial effect of this plant has not been studied. This study evaluates the antibacterial acti-vity and phytochemical properties of nuts and oils of *Sermicarpus anacardium*.

MATERIALS AND METHODS

Collection of samples

The nuts of *Semicarpus anacardiumn* L.f. were collected from the hilly area near the village of Baunshpur in Similpal Biosphere Reserve of Mayurbhanj District, Orissa, India during March-April-2006.

Preparation of plant extract

The nuts were shed dried for about 20 days and then pulverized into fine powder using pestle and mortar. The extraction was done by Soxhlet extraction techniques. Different solvents were used successively with gradient polarity (petroleum ether, chloroform, ethanol and water). The extracts were evaporated to complete dryness by vaccuum distillation and stored in refrigerator for further use.

Organisms

Seven bacterial species used were *Staphylococcus aureus, Streptococcus aureus, Bacillus licheniformis, Bacillus brevis, Vibrio cholerae, Shigella flexneri* and *Pseudomonas aeruginosa.* They were clinical isolates obtained from Central Drug Laboratory and Indian Institute of Chemical Biology, Kolkata. Bacterial species were subcultured into nutrient broth and incubated at 37^{o} C for 18 - 24 h.

Disc diffusion method

Nutrient agar plates were inoculated with 0.1 ml each of bacterial organisms (1.5×10^{7} cfu/ml) in triplicates and spread well with sterile swabs. Filter sterilized paper discs were soaked with 10, 50 and 100 mg/ml of PEE and AQE nuts extracts of *S. anacardium* and were aseptically placed apart from each other on each agar plate. Standard drug, Gentamycin a product of HIMEDIA was used as positive control and dimethyl sulfoxide (DMSO) was used as negative control. The plate was incubated at 37^{o}C for 18 - 24 h. The zones of inhibition was measured and expressed in millimeter. Three nutrient agar plates were inoculated with each type of bacterial cultures and the zone diameter expressed in millimeters (Vineela and Elizabeth, 2005; Pal et al., 2006).

Agar well method

The antibacterial activity of the oils from the nuts of *S. anacardium*, was done using agar well diffusion method. Nutrient agar plates were inoculated with 0.1 ml of each bacterial organism in triplicates and spreaded well with sterile swabs. Wells of 6 mm size were made into the agar set plates containing the bacterial culture and the lower portion was sealed with a little molten agar media. Concentration of 200 µl of the crude oil extract was allowed to diffuse for about 2 h. The plates were incubated at wells 37^{o} C for 18 - 24 h. The zone of inhibition was measured and expressed in millimeter. Antibacterial activity was recorded if the zone of inhibition was greater than 6mm (Hammer et al., 1999).

Phytochemical screening

The four solvent extracts and oils were evaluated for the presence of different phytochemicals using procedures of Mukharjee (2002); Parekh and Chanda (2007).

RESULTS AND DISCUSSION

The preliminary antibacterial screening, indicated petroleum ether extract (PEE), aqueous extract (AQE), chloroform extract and ethanol extracts of *Sermicardium anacardium* L.f. to be effective against the test organisms. Results of antibacterial activity of nuts extract of *Sermicardium anacardium* L.f. against seven human pathogens are shown in Table 1, 2, 3 and 4. The solvent extracts of *Sermicardium anacardium* L.f. showed zones of inhibition ranging from 02 – 16 mm (excluding inhibition zone of solvent). The results showed that petroleum ether extract was significantly active against *S. aureus* at 100 mg/ml concentration. It showed less inhibition zone in *B. brevis* and no inhibition zone was seen in S. *flexneri, B. licheniformis, V. cholerae, P. aeruginosa* and *S. aureus*. The aqueous extracts were more effective against S*. flexneri* with 16 mm as zone of inhibition (Table 2). Chloroform extract was effective against *V. cholerae* and *P. aeruginosa* at 100 mg/ml (Table 3) . Whereas the ethanol extract was effective against *P. aeruginosa* and *S. aureus* (Table 4.).The oils of *S. anacardium* did not show any

Table 2. Antibacterial activity of aqueous nut extracts of *Semicarpus anacardium*.

Microorganisms	Inhibition zone (mm)				DMSO
	10 mg/ml	50 mg/ml	100 mg/ml	10 mg G/ml	
Staphylococcus aureus	_	1	2	26	14
Shigella flexneri	_	4	16	23	11
Bacillus licheniformis	_	-	-	23	11
Bacillus brevis	_	-	-	23	26
*Vibrio cholera*e	_	-	-	26	15
Pseudomonas aeruginosa	_	-	-	ND	18
Streptococcus aureus	_	-	-	ND	-

G = gentamycin (Standard antibiotic), (-) = No activity, ND = Not detected.

Table 3. Antibacterial activity of chloroform nut extracts of *Semicarpus anacardium.*

Microorganisms	Inhibition zone (mm)				DMSO
	10 mg/ml	50 mg/ml	100 mg/ml	10 mg G/ml	
Staphylococcus aureus	ND	ND	ND	26	14
Shigella flexneri	_	-	-	23	11
Bacillus licheniformis	_	9	-	23	11
Bacillus brevis	_	_	_	23	26
*Vibrio cholera*e	_	_	10	26	15
Pseudomonas aeruginosa	_	8	10	ND	18
Streptococcus aureus	_	_	_	ND	_

G = gentamycin (Standard antibiotic), (-) = No activity, ND = Not detected.

Table 4. Antibacterial activity of ethanol nut extracts of *Semicarpus anacardium*.

Microorganisms	Inhibition zone (mm)				DMSO
	10 mg/ml	50 mg/ml	100mg/ml	10 mg G/ml	
Staphylococcus aureus	ND	ND	ND	26	14
Shigella flexneri	_	_	_	23	11
Bacillus licheniformis	_	_	_	23	11
Bacillus brevis	_	_	_	23	26
*Vibrio cholera*e	_	_	_	26	15
Pseudomonas aeruginosa	_	_	8	ND	18
Streptococcus aureus	_	8	9	ND	_

G = gentamycin (Standard antibiotic), (-) = No activity, ND = Not detected.

antibacterial activity.

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections diseases and in food spoilage. The active components usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba et al., 2006). Preliminary phytochemical screening showed that the aqueous extracts contain most of the phytochemicals (Table 5 and 6) like alkaloids, tannins, saponins, flavonoids, anthroquinone and ascorbic acid (Table 3). However anthraquinone and ascorbic acid were present in all the four extracts. Fixed oil, fats and triterpenoid were only present in petroleum ether extract with steroid in both petroleum ether and chloroform extract. Phytochemical analysis (Table 6) of oil showed the presence of alkaloid, tannin, flavonoid and anthraquinone. Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties (Aboaba et al., 2001). Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens (Aboaba et al., 2001). Therefore,

Table 5. Phytochemical analysis of nut extracts of *Semicarpus anacardium*.

Test/Reagent Used	Petroleum Ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloid	-	-	-	+
Glycosides	-	-	-	-
Proteins and amino acids	-	-	-	+
Tannin	-	-	-	+
Triterpenoid	+	-	-	-
Saponin	-	-	-	+
Flavonoid	-	-	-	+
Steroid	+	+	-	-
Anthraquinone	+	+	+	+
Gums	+	+	+	+
Ascorbic acid	+	+	+	+
Fixed oil and fats.	+	-	-	-

- = Absence, + = Presence.

Table 6. Phytochemical analysis of oils of *Semicarpus anacardium*.

Phytochemicals	Extract
Alkaloid	+
Carbohydrates	-
Glycosides	-
Proteins and amino acids	+
Tannin	+
Saponin	-
Flavonoid	+
Steroid	-
Anthraquinone	+
Gums	-

- = Absence, + = Presence.

the compounds detected may be responsible for the antibacterial activity of the nuts of *S. anacardium* L.f. Several research works were done on phenolic constituents (Govindachari et al., 1971; Prakasa Rao and Ramachandra Rao, 1973). Besides, the plants were also screened for anticancer activity (Gothoskar and Randaive, 1971) and cytotoxicity studies (Shin et al., 1999; Phatak et al., 1983) and was reported to contain biflavonoids (Murthy, 1983). Attempts were made to isolate the active phytochemical agent from the plant extracts for pharmaceutical applications.

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