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Antimicrobial Efficacy of Nut Oil of *Semecarpus anacardium*: A Marking Nut Tree

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Abstract: The present investigation was undertaken to evaluate *in vitro* antimicrobial activity of *Semecarpus anacardium* L. (Anacardiaceae) nuts oils. Essential oil from nut of *S. anacardium* was extracted by hydro-distillation method in a Clevenger type apparatus. The antimicrobial screening of the isolated essential oil was performed against Gram positive-*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923) Gram negative-*Proteus vulgaris* (ATCC 2027), *Escherichia coli* (ATCC 25922) and fungal strains *Aspergillus niger* (ATCC 16404), *Aspergillus fumigates* (ATCC 26933), *Candida albicans* (ATCC5027) and *Candida glabrata* (ATCC 66032) using disc diffusion method. The essential oil was found to be more or less active against almost all tested pathogenic strains with varied spectrum of inhibition zone (7.0±0.06-13.0±0.01 mm). The significant inhibitory effect was observed against *Staphylococcus aureus* (12.0±0.05 mm) and *A. niger* (13.0±0.01 mm). The significant potential of *Semecarpus anacardium* nut oil concludes that it could serve as a source of antimicrobial agents.

Key words: *Semecarpus anacardium*, anacardiaceae, essential oils, antimicrobial activity

INTRODUCTION

Antibiotics, since their introduction had near-universal effectiveness against serious infections. However, in recent years, the emergence of drug resistant pathogens has become a threat caused by the in discriminate use of modern antibiotics (Mulligan *et al*., 1993; Enne *et al*., 2001). Therefore, medicinal herbs are back into prominence because the demands to investigate phyto-medicines from natural sources with lesser resistance and broad-spectrum activities are increasing. For a long period of time, plants have been used because of their antimicrobial traits, which are due to compounds known by their active substances which may represent new source of anti-microbial with stable, biologically effective components that can establish a scientific base for the use of plants in modern medicine (Kelmanson *et al*., 2000; Ahmad and Beg, 2001).

Semecarpus anacardium (Anacardiaceae) is a deciduous tree, distributed in Himalayan and sub-Himalayan region of India. Commonly known as marking nut tree and bhallataka, is used as an herbal drug in Ayurvedic and in Unani medicines (Chopra, 1982; Khare, 1982; Nardkarni, 1993). It is reported to be caustic, astringent, counterirritant, vesicant and used in cough, asthma, ulcers, piles and various nervous diseases. Major focus remains on its anti cancerous and anti-arthritis activity (Vijayalakshmi *et al*., 1996; Premalatha *et al*., 1997). Biologically active compounds like bhilawanols (Rao *et al*., 1973; Lamture *et al*., 1982), biflavonoids (Murthy, 1983, 1985, 1986) sterols, glycosides and anacardic acid has been isolated from *S. anacardium*. Biological activities such as anti-helmintic and antispermatogenic properties of the milk extract have also been reported (Sharma *et al*., 2003; Sharma and Chaturvedi, 1964).

Essential oils are the vast reservoir of secondary metabolites produced by higher plants evolved in defense against herbivores and pathogens (Duke *et al*., 1991). They are complex mixture of monoterpenes and ses-quiterpenes which are hydrocarbons with the general formula $(C_5H_8)_n$. More than 1000 monoterpenes and 3000 sesquiterpenes have been isolated from a number of aromatic and medicinal plants. The essential oils from aromatic plants are of particular interest to industrial markets due to potent biological activities (Isman, 2000; Ibrahim, 2001; Skold *et al*., 2006). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Seazzocchio *et al*., 2001). In the present investigation, the antimicrobial potential of nut essential oil of *S. anacardium* has been evaluated.

MATERIALS AND METHODS

Plant material: The nuts of *Semecarpus anacardium* were collected from the plants growing in the adjoining

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region of Midnapore, West Bengal, India, during the month of April 2009. The plant was authenticated from National Institute of Ayurveda (NIA), Jaipur, Rajasthan and voucher specimen was conserved under the reference number (RUBL NO:- 20625) to the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India.

Extraction of essential oils: The air dried nuts (250 g) were subjected to hydro-distillation in a Clevenger-type apparatus for 4 h. Following this procedure, 10 mL of essential oil was extracted and dehydrated with anhydrous sodium sulphate and stored at 4°C in a clean sealed glass vials until used for further analysis.

Antimicrobial screening

Test microorganisms: *In vitro* antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive-*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923) Gram negative-*Proteus vulgaris* (ATCC 2027), *Escherichia coli* (ATCC 25922) and fungal strains *Aspergillus niger* (ATCC 16404), *Aspergillus fumigates* (ATCC 26933), *Candida albicans* (ATCC5027) and *Candida glabrata* (ATCC 66032). All the tested microorganisms were obtained from Batra Hospital and Medical Research Centre (BHMRC), New Delhi. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37°C for 24 h while the fungal cultures were maintained on Potato Dextrose Agar slants and incubated at 27°C for 48 h.

Antimicrobial activity: Antimicrobial assay of the isolated essential oil was performed against eight tested pathogenic strains by disc diffusion method (Gould and Bowie, 1952). The nutrient agar plates and potato dextrose agar plates were seeded with suspension (10^6 cfu mL^{-1}) of the bacterial and fungal strains vice-versa. The empty sterilized Whatmann No.1 filter paper disc (6 mm) were impregnated with 10 μL of oil diluted with two volumes of DMSO, dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 min (Pre diffusion time). The standard discs (6 mm) impregnated with antibiotics streptomycin (2 μg mL^{-1}) and fluconazole (2 μg mL^{-1}) was used as positive control while with DMSO (10 μL) as negative control. The plates were incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi, respectively. The diameter of the inhibition zone (mm) was measured. The experiment was done in triplicate and the mean values (±SD) calculated for conclusion.

RESULTS AND DISCUSSION

Officinal plants are potential source of bioactive compounds. Essential oils are natural bioactive compounds which accumulate in specialized structures such as oil cells, glandular trichomes and oil or resin ducts. The formation and accumulation of essential oils in plants have been thoroughly reviewed by Croteau (1986), Guenther (1972) and Runeckles and Mabry (1973). Chemically, the essential oils are primarily composed of mono-and sesquiterpenes and aromatic polypropanoids synthesized via the mevalonic pathway for terpenes and the shikmic acid pathway for aromatic poly-propanoids (Runeckles and Mabry, 1973). Essential oils are well known to have a range of useful biological properties against insects, pests, fungal, bacterial and viral diseases (Ibrahim, 2001). In addition, they are more readily degraded in the environment than synthetic compounds.

Herbal medicines have been used as an exemplary source for treating infectious diseases as they are efficient, non-narcotic and having no side-effects (Ahmad and Beg, 2001). Thus, there is renewing interest to screen medicinal plants for bioactive compounds as a basis for pharmacological studies. Several reports have shown that essential oils from plants have control on the growth of pathogenic strains (Ruberto *et al*., 2000; Singh *et al*., 2002; Abed, 2007).

In the present investigation, essential oil of *S. anacardium* was obtained by hydro-distillation method using a Clevenger type apparatus. The yield of essential oils obtained from leaves was quite high (2.4 % w/w) and the oil was light yellow in color. *In vitro* antimicrobial activity were determined by using agar disc diffusion method and summarized in Table 1. The essential oil was found to be more or less active against almost all tested pathogenic strains with varied spectrum of inhibition zone (9.0±0.0-13.0±0.01 mm). However, essential oil isolated from *S. anacardium* nut showed significant antifungal inhibitory effect. Among bacterial pathogens, gram positive bacterial strains were found to be more

Table 1: Antimicrobial activity of nut oil of *Semecarpus anacardium*

	Diameter of inhibition zone (mm)			
Tested microorganisms	Nut oil	Streptomycin	Flucanozole	DMSO
Bacillus subtilis	9.3±0.22	10.0±0.01	-	-
Staphylococcus aureus	12.0±0.05	17.7±0.33	-	-
Proteus vulgaris	7.0±0.06	16.7±0.80	-	-
Escherichia coli	8.0±0.00	11.0±0.02	-	-
Aspergillus niger	13.0±0.01	-	12.0±0.05	-
Aspergillus fumigatus	10.3±0.6	-	11.3±0.4	-
Candida albicans	9.3±0.20	-	11.1±0.23	-
Candida glabrata	08.0±0.0	-	10.0±0.01	-

Control: Streptomycin and flucanozole at 2 μg $disc^{-1}$, Diameter of inhibition zone (mm) including the diameter of disc (6 mm), Values are means of three replicates (±SD)

susceptible than gram negative bacterial strains. This may attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas, gram negative cell wall is multilayered structure bounded by an outer cell membrane (Yao and Moellering, 1995).

The essential oil extracted from nut oil of *S. anacardium*, at 1:2 dilutions in dimethyl sulphoxide showed potential antimicrobial activity against *B. subtilis*, *S. aureus*, *P. vulgaris* and *E. coli*, *A. niger*, *A. fumigates*, *C. albicans* and *C. glabrata* However, essential oils from nut showed a remarkable inhibitory effect against *S. aureus* (12.0±0.05 mm) a gram positive bacterium which is known to play significant role in skin diseases. Among fungal strains, oils showed a pronounced inhibitory zone in *Aspergillus niger* (13.0±0.01 mm) in comparison with standard drug flucanozole (12.0±0.05 mm). Control treatment (DMSO) did not show an inhibitory effect on any of the tested strains. The findings of the present investigation suggest that essential oil of *Semecarpus anacardium* nut is source of biologically active compounds which may potentially prove to be efficient natural antimicrobial agents. Further studies on chemical characterization of bioactive compounds of *Semecarpus anacardium* nut oil through GC-MS are carried on and reported later.

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