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Research Article

Antimicrobial Studies of Selected Species of Carissa Roots

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Abstract

The present study aimed to identify the effect of Carissa roots on its antibacterial potential. Six medically important bacterial strains were selected to identify the antibacterial potential by measuring their zone of inhibitions using agar well diffusion method. Antimicrobial activity of roots has been evaluated against various diseases causing organisms (*Escherichia coli, Salmonella typhi, Staphylococcus aureus, Agrobacterium tumefaciens, Erwinia carotovora, Entarococcus faecalis*) has shown positive activity against some of the tested extracts. Ethanol and methanol extracts of roots of all the four species have shown very promising activity againt *Escherichia coli* and *Entarococcus faecalis*. For ethanol extract the *Carissa carandas L*. has shown the highest activity against *Escherichia coli* and Carissa congesta Wight and *Carissa spinarum L*. has shown the highest activity against *Entarococcus faecalis*. In methanol extract *Carissa* carandas L. and *Carissa opaca* Stapf ex Haines have shown very promising activity againt *Staphylococcus aureus*. Thus, the study proves significant difference between the antibacterial activities of roots of *Carissa* species.

Keywords: Antibacterial activity, Carissa L., Roots, Underutilized.

INTRODUCTION

'*Kasamarda*' had been included in ten drugs which are cordials (Charak Samhita, Su. 4/10). The same had been mentioned in Amalakagana of Sushrut Samhita (Su. 10/26-26). Usage of roots leaves and fruits of *Carissa* congesta Wight, *Carissa* carandas L., *Carissa* opaca Stapf ex Haines and *Carissa* spinarum L. for number of ailments had been reported in ethnobotany literature (Jain, 1991). Roots are anthelminitic, stomachic and antiscorbutic, and are useful in stomach disorders, intestinal worms, scabies and pruritus (Anonymous, 1993). Roots of *Carissa* spinarum L. pounded and mixed with country liquor made from Madhuca longifolia flowers are applied on sore for removing the worm, Body pains, cuts, injuries. Paste with cold water is administered orally in fever (Kirtikar and Basu, 1935). Roots of *Carissa* opaca Stapf ex Haines are purgative, also used in worm infested sores of animals; juice of roots in wounds of cattle (Anonymous, 1976). This review reveals that roots of four species viz. *Carissa* carandas L., *Carissa* congesta Wight, *Carissa* opaca Stapf ex Haines and *Carissa* spinarum L. are used as medicine. These ethno-medico-botany claims however, needs validations.

MATERIALS AND METHOD

Plant Extract

The root material collected was cleaned, dried in shade and grounded in powder form. The powder was stored in air tight bottle. This powder was used for further extraction, cold extraction was done for 12 hours at room temperature and then the extract was filtered through Whatman filter paper. Different solvents, viz. petroleum ether, ethanol 38 and Methanol were used for extraction. The extracts were dissolved in Dimethyl Sulfoxide (DMSO) except for the petroleum ether extract as it is not soluble in DMSO, and therefore dissolved in Hexane. These extracts were used for the antimicrobial assay.

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Preparation of Inoculum

Microorganisms were grown in Muller Hinton broth at 37°C to a cell density of approximately 105–106 CFU/ml and used for the antimicrobial activity assay.

Test Organism

For the antimicrobial activity screening, bacterial strains used in this study was *Escherichia coli* (MCMB-813), *Salmonella typhi* (MCMB-814), *Staphylococcus aureus* (MCMB-818), *Agrobacterium tumefaciens* (MTCC-413), *Erwinia carotovora* (MTCC-1428) and *Entarococcus faecalis* (MCMB-812). All these stains were acquired from MACS collection of Microorganisms (MCM) at ARI:

Agar well Diffusion Method

This method was adopted to determine the antibacterial activity of root extract against the test organisms.The Muller Hinton Agar (3.8 g/100 ml) weighed and dissolved in 100 ml of distilled water in a sterile conical flask. The medium was sterilized by autoclaving and allowed to cool at 50oC temperature. The medium was poured into the sterile petri plate. The antimicrobial activities of methonal, ethanol and Petroleum ether extracts were determined by agar well diffusion method (Ojala et al., 2000) with slight modification to evaluate the antibacterial activity. Minimum Inhibitory Concentrations (MICs) of the extract and of *Carissa* was determined using the agar-well diffusion method. All the extracts were diluted in Dimethyl Sulfoxide (DMSO) and hexane to obtain series of concentrations of 100, 40, 20 µg /500µl. The MIC was taken as the lowest concentration of extract that caused a clear to semi-clear inhibition zone around the well after 24 h incubation at 37°C. Sterile petri dishes were prepared with a base layer of Muller-Hinton agar. Bacterial cultures at density of 106-108 cfu (100 µl) were inoculated on solid agar. Wells (6mm) were made in the agar with a sterile cork borer and filled with 50 µl of different dilutions of the extract and fractions. Petri dishes were incubated at 37°C for 24 h. The diameters of the circular inhibition zones obtained were measured. Commercial antibiotics namely Gentamicin and tetracycline were used as positive controls. DMSO 25% and hexane were used as a negative control. The whole experiment was carried out under aseptic conditions in the laminar flow.

RESULTS AND DISCUSSION

The Gram negative bacterial strain *S. typhi* was found to be one of the high resistant bacterial strains, since only ethanolic extracts of *Carissa carandas L*. and *Carissa spinarum L*. at their highest concentration (50 µl) showed an inhibitory zone of 4.5 and 3.3 mm respectively, while *S. typhi* was highly resistant to *Carissa congesta* Wight and *Carissa opaca* Stapf ex Haines which showed no inhibitory zone at other concentrations. In contrast, both *Carissa congesta* Wight and *Carissa opaca* Stapf ex Haines exhibited higher zone of inhibiton (4.8 and 4.3 mm respectively) with methanolic extracts. In addition *Carissa spinarum L*. also showed inhibitory zone of 4.5 mm with a higher concentration of 50 µl, while *S. typhi* proved to be highly resistant and showed no inhibitory zone with lower concentrations of 10 and 20 µl methanolic extracts of all *Carissa sp* (Table 1).

Table 1: The results of antimicrobial activity of different plant extracts.

Extract	Strains used	*C. car*			*C. con*			*C. opa*			*C. spi*		
		Concentrations			Concentrations			Concentrations			Concentrations		
		10 µl	20 µl	50 µl	10 µl	20 µl	50 µl	10 µl	20 µl	50 µl	10 µl	20 µl	50 µl
		Zone of Inhibition (mm)			Zone of Inhibition (mm)			Zone of Inhibition (mm)			Zone of Inhibition (mm)		
CRE	*E. coli*	3.75	4.75	9.5	-	6	7	2	5.25	8.5	3.75	4.25	8.25
	S. typhi	-	-	4.5	-	-	-	-	-	-	-	-	3.75
	S. aureus	-	4.5	6.75	5.25	5.25	4.75	6.5	6.25	4.25	-	4.75	3.75
	A. tumefaciens	3.5	5.25	6.75	-	5.75	5.5	5.75	5.25	6.25	5	4.75	4
	E. carotovora	-	4.5	5	3.25	4.5	4.5	3.75	-	-	3.5	5.25	-
	E. faecalis	-	3.5	5.25	5	5.25	7.75	4.75	4.5	6.25	6.5	-	7.25
CRM	*E. coli*	-	5.5	5.25	-	-	4	-	-	4	-	-	5.75
	S. typhi	-	-	-	-	-	4.75	-	-	4.25	-	-	4.5
	S. aureus	4	9	7	4.75	7	6.25	-	8.5	9	3.75	6.75	5.5
	A. tumefaciens	3.5	4.25	5.75	-	3.25	4.75	4.5	5	6	4	4.25	5.25
	E. carotovora	7	5.75	5.25	-	4.25	4.75	5	3.5	4.75	-	3.5	4.75
	E. faecalis	-	-	5.25	-	-	5.75	-	-	4.5	-	-	4.75
Standard Tetra cyclin 30 µg	*E. coli*	8.5											
	S. typhi	15											
	S. aureus	15											
	A. tumefaciens	12											
	E. carotovora	10											
	E. faecalis	10											

S. aureus, Gram positive bacterial strain proved to be highly susceptible against alcoholic extracts of all *Carissa sp*. except for *Carissa carandas L*. and *Carissa spinarum L*., each at 10 µl. Besides, the highest zone of inhibition (6.8 µl) was observed with *Carissa carandas L*. extract at 50 µl. Similarly, all methanolic extracts showed higher zone of inhibition against *S. aureus* except for *Carissa opaca* Stapf ex Haines at 10 µl concentration. Also highest inhibitory zone was observed (9 mm) with *Carissa carandas L*. and *Carissa opaca* Stapf ex Haines extract at 20 and 50 µl respectively.

Among all the alcoholic extracts tested against *Agrobacterium tumefaciens*, the results showed greater susceptibility with higher zone of inhibition using 50 µl extract of *Carissa carandas L*., while no inhibitory zone was observed using *Carissa congesta* Wight at 10 µl. Similarly, methanolic extract of *Carissa* congesta Wight at 10 µl showed no inhibitory zone, while *Carissa opaca* Stapf ex Haines at 50 µl showed a inhibition zone of 6 mm.

Gram negative bacterial strain *Erwinia carotovora* was recorded to be highly susceptible against most of the alcoholic extracts tested, except for extracts of *Carissa carandas L*. (10 µl), *Carissa opaca* Stapf ex Haines (20 and 50 µl) and *Carissa spinarum L*. (50 µl), while extract of *Carissa spinarum L*. at 20 µl showed highest inhibitory zone of 5.3 mm. However methanolic extract of *Carissa carandas L*. at 10 µl demonstrated highest inhibitory zone of 7 mm, while extracts at other concentrations of *Carissa sp*. exhibited some inhibition zone except for *Carissa congesta* Wight and *Carissa spinarum L*. at 10 µl each.

Enterococcus faecalis, the Gram positive bacterial strain showed remarkable difference between the alcoholic and methanolic extracts used. Alcoholic extract of *Carissa sp*. showed highest zone of inhibition of 7.8 mm with 50 µl extract of *Carissa congesta* Wight, while no inhibitory zone was observed with 10 and 20 µl extract *Carissa carandas L*. and *Carissa spinarum L*. respectively. In contrast, the methanolic extract at 50 µl concentration inhibited the growth of *Enterococcus faecalis* at the highest concentration tested (50 µl) using all *Carissa sp*. tested, while other extracts of all species at lower concentrations showed no activity.

When compared to the different polar system extracts used, alcoholic extracts proved to be the more effective than methanolic extracts. Among all the *Carissa sp*. used, *Carissa carandas L*. showed higher inhibition against five bacterial strains, while *Carissa congesta* Wight, *Carissa opaca* Stapf ex Haines and *Carissa spinarum L*. showed higher inhibition against only two bacterial strains used. Bacterial strains, *E. coli* and *S. aureus* both were highly susceptible, while bacterial strain *S. typhi* was the most resistant bacterial strain. The lowest concentration at which the only bacterial strain that showed highest zone of inhibition (7 mm) was *Erwinia carotovora* with 10 µl methanolic extract of *Carissa carandas L*.

For ethanol extract the *Carissa carandas L*. shows the highest activity against *Escherichia coli* and *Carissa congesta* Wight and *Carissa spinarum L*. shows the highest activity against *Entarococcus faecalis*. In methanol extract *Carissa carandas L*. and *Carissa opaca* Stapf ex Haines showed very promising activity againt *Staphylococcus aureus*.

Dhanushka (2006) demonstrated activity of compounds volatile 2'-hydroxy acetopheneone, lignan carinol and eudesmane sesquitepene carissone from *Carissa lanceolata* root against Gram positive and Gram negative organisms viz. *E. coli*, *S. aurues*, *B. substilis* and *P. aeruiginosa*. The results obtained in present investigation are more or less similar to above mentioned reference. Presence of carissone and carinol have been reported in roots of *Carissa congesta* Wight (Joshi and Boyce, 1957; Morton and Miami, 1987); in *Carissa carandas L*. (Rastogi et al., 1966, 1967; Satyavati (1987); Bhaduri et al., 1968; Hettiarachchi (2006); Jagadeeshwar Rao (2005); Naim et al., 1988; Wangteeraprasert & Likhitwitayawuid, 2009); Warrier (1993), in *Carissa spinarum L*. (Rao et al., 2005). The antimicrobial activity therefore, may be due to presence of compounds carissone, carinol in respective species.

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