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Screening of antibacterial activity of leaf and root extracts of *Carissa carandas* L. - An important traditional medicinal plant

A.N. Sundar[1] and M. Jawahar[2*]

[1]Department of Botany, J.J. College of Arts and Science, Pudukkotai, Tamil Nadu, India,
[2]Department of Botany, Rajah Serfoji Govt. College, Thanjavur, Tamil Nadu, India

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The present studies deals with the antibacterial activity of the hexanes, dichloromethane and methanol extracts of leaf and root of *Carissa carandas* L. which were tested against selected bacterial species of *Escherichia coli*, *Pseudomonas auriginosa*, *Bacillus substilis*, and *Staphalococous aureus*. Agar well diffusion assay was used to evaluate their antibacterial potential. The antibacterial activities in terms of percentage relative inhibition zone diameter (RIZD) were calculated and minimum inhibitory concentrations (MIC) were determined. In conclusion the methanol extracts of the leaf and all the extracts of root were found more effective against all tested bacteria. This result indicated that this plant is a potential candidate to be used as an antibacterial compound. These promising results open the possibility of leaf, root finding new clinically effective antibacterial compounds.

Key words: *Carissa carandas* L., Leaf, Root, Extracts, Antibacterial activity, Agar diffusion.

Introduction

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Millions of rural house holds use medicinal plants in a self – help mode. Over one and a half million practitioners of the Indian system of medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world (Ahmedulla and Nayar, 1999). In recent years, secondary plant metabolites, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*.,

* Corresponding author: M. Jawahar ; e-mail: somajainn@yahoo.co.in

2005). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini *et al*., 2004). The systemic screening of antimicrobial plant extracts represents a continuous effect to find new compounds with potential to act against multi – resistant pathogenic bacteria and fungi.

A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. The so – called secondary metabolites (Evans *et al*., 1986), which are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Purohit and Mathur, 1999).

The antimicrobial activity of *Apocyanaceae* members were well documented in the literature. These include *Alstonia scholaris* (Khan *et al*., 2003), *Nerium oleander* (Hussain and Gorsi, 2004), *Rauvolfia tetraphylla* (Nayeemulla sheriff *et al*., 2006 and Suresh *et al*., 2008), *Alstonia scholaris*, *Carissa carandas* and *Catharanthus roseus* (Singh and Sangwar, 2011).

Carissa carandas L. belong to the family *Apocyanaceae*, an important traditional medicinal plant, has been used to treat antidiarrhoeal, anthelmintic properties, bitter, skininfections, remittent fevers, earache, soreness, syphilitic pain of the mouth, stomachic and blood pressure. The Paste of aerial parts obtained by crushing is given orally for rinderpest in cattle (Ali, 1999). Root juice is used to treat diarrhea and dysentery (Taylor *et al*., 1996). Whole plant is powdered and mixed with cow's milk and taken orally to treat diabetes. Only a few reports are available for the antimicrobial properties of this plant (Omino and Kokwaro, 1993; Taylor *et at*., 1996; Rajasekaran and Murugesan 2005; Singh and Sangwan, 2011).

The present study was to investigate the antibacterial activity of hexane dichloromethane, and methanol extracts of leaf and root of *Carissa carands* L. against selected bacterial species. The selected bacteria were antibiotic resistant or multi resistant human pathogens. The extracts with the highest antibacterial effectiveness were chosen for subsequent use in pharmaceutical formulations.

Materials and methods

Plant material

Disease free fresh plant materials were collected from college garden, Rajah Serfoji Govt. College, Thanjavur. The collected plant materials were thoroughly cleaned and dried at room temperature. The dried materials were then homogenized to fine powder with the help of a mixer grinder.

Preparation of crude extracts

Three different solvents namely hexanes, dichloromethane and methanol were used for extraction from the fine powder using the method of Quiroga *et al.* (2001) with some modifications. 25g of powder were extracted with 150ml of solvent for 24hr by using soxhlet apparatus. The extract was dried in a flash evaporator of 30 min and the left over powder was considered 100%. Different concentrations 50, 75,100 and 125 mg/ml were prepared by redissolving the extracted powder in the same solvent which was used in the extractions.

Antibacterial assay

Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria were obtained from MTCC, Chandigarh. Bacteria were grown in nutrient agar slants for sub culturing. From these slants, inoculums were inoculated in test tubes containing about 10ml of LB broth and after inoculation these test tubes were kept at 37°C for overnight or more up to desired growth of the bacteria. This bacterial culture was used for antibacterial assay of various extracts by the agar well diffusion method of Perez *et al*. (1990) and Rojas *et al*. (2006).

About 20 ml of nutrient agar media was poured into the petriplates. Once the agar was solidified culture of bacteria was spread after mixing with small amount of LB broth. It was them punched with a six millimeter diameter cork borer to prepare wells. These wells were then filled with about 50il of the plant extract of desired concentration level. Simultaneously, streptomycin was used as positive control. Similarly a negative control was also tested using the different solvents. The test was carried out in triplicates. The plates were incubated at 35°C for 24 hrs. Zone of inhibition was then measured using a scale. The antibacterial activity in terms of percentage relative inhibition zone diameter (RIZD) was also calculated.

$$\%\ RIZD \quad = \frac{IZD\ sample - IZD\ negative\ control}{IZD\ antibiotic\ standard}\ x\ 100$$

Where RIZD is the relative inhibition zone diameter (mm). The resulting IZD of the samples were either higher than or equal to the IZD blanks. Therefore the obtained percentages were positive. The test was considered negative when the IZD of the sample was equal to the IZD of blank. Streptomycin was used as standard antibacterial drug for the purpose.

The MIC was determined for the various extracts of the leaf and root by an agar well diffusion technique. Serial dilutions were prepared by diluting in respective solvents to achieve a decreasing concentration range of 125 mg/ml to 25mg/ml. A 5o il volume of each dilution was introduced in triplicate wells into nutrient agar plates already seeded with the standardized inoculums of different bacterial cells. All test plates were incubated at 37°C for 24 hours. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

Results and discussions

Exploring the healing power of plants is an ancient concept. For many centuries, people have been trying to alleviate and treat diseases with different plant extracts and formulation. Natural products are the most consistently successful source of drug leads. Little of the world's biodiversity has been tested for biological activity, yet natural products have been the single most productive source of drug leads. As less than 10% of the world's biodiversity has been tested for biological activity, many more useful natural lead compounds are awaiting discovery (Harvey, 2000). The scientific evaluation of the antimicrobial activity of widely distributed plants still remains an area of intensive investigation. In the present study, it had been tried to work out the antibacterial potential of the *Carissa carandas* L. as can be seen in the Tables 1, 2 and 3, our results indicated the goods potential of various extracts of the studied *Carissa carandas.*

Table 1. Antibacterial activity of *Carissa carandas* L.

Microorganism used	**Conc. mg /ml**	**Leaf**			**Root**			**Control**
		Hex	DCM	Me	Hex	DCM	Me	
	50	-	-	2	3	-	2	-
E.coli	75	-	-	5	7	2	6	-
	100	-	-	7	9	5	8	-
	125	-	-	10	12	9	10	-
	50	6	9	3	6	2	5	-
P.auriginosa	75	9	13	6	10	6	8	-
	100	12	15	8	12	10	12	-
	125	15	20	10	16	12	16	-
	50	-	-	4	3	2	2	-
S.aureus	75	-	-	8	6	6	6	-
	100	-	-	12	8	8	9	-
	125	-	-	17	10	10	11	-
	50	3	-	2	9	-	2	-
B.subtilis	75	5	3	5	13	2	5	-
	100	7	4	7	19	4	7	-
	125	10	6	9	3	6	9	-

All the values are mean of triplicates. No inhibition zone is denoted by (-). Hex, DCM and Me the stand for hexane, dichloromethane and methanol respectively.

Leaf and root of *Carissa carandas* L. were evaluated for antibacterial potential. As vivid from the table 1, out of these two, root exhibited more antibacterial potential than leaf. For both leaf and root, among the three solvents, maximum activity was observed in case of methanol extracts. Whereas hexane and dichloromethane extracts showed maximum RIZD value only up 70% and 75% respectively, methanol resulted in RIZD value 95% (Table 2).

Table 2. Percentage of Relative Inhibition Zone Diameter (RIZD) in mm for different solvents

Microorganism used	Conc. mg /ml	Leaf			Root		
		Hex	DCM	Me	Hex	DCM	Me
	50	-	-	25	20	20	25
E.coli	75	-	-	29	30	23	35
	100	-	-	35	40	38	46
	125	-	-	56	60	48	65
	50	24	27	26	26	25	45
P.auriginosa	75	28	45	35	40	30	68
	100	46	56	60	54	46	78
	125	68	75	75	70	60	95
	50	-	-	28	21	20	26
S.aureus	75	-	-	50	32	24	35
	100	-	-	62	58	42	42
	125	-	-	70	65	50	65
	50	25	-	25	23	25	20
B.subtilis	75	30	22	33	36	35	32
	100	52	37	55	50	40	52
	125	65	45	70	68	50	70

The leaf extracts of *Carissa carandas* L. in methanol showed MIC of 10.0 mg / ml against all tested bacteria. Both hexanes and dichloromethane extracts were found to be ineffective or showed poor inhibition of bacterial growth. Hexanes, dichloromethane and methanol extracts of *Carissa carandas* L. root showed maximum activity against all tested bacteria. Growth of all the four bacterial strains were inhibited by the use of all the extracts of root showed MIC of 10.0 mg /ml or higher concentration is shown in Table 3.

The extracts of higher plants can be very good source of antibiotics (Fridous *et al*., 1990) against various fungal and bacterial pathogens. The root extracts showed maximum activity against *E.coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. These data revealed that extracts of *Carissa carandas* L. exhibited significant zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. After literature survey only few reports could be found for this particular plant. Rajasekran and Murugesan (2005), Salar and Dhall (2010), Siddiqui *et al*. (2011) and Singh and Sangwan (2011) have been reported the antibacterial activity in their studies. Siddiqui *et*

al. (2011) reported both antifungal and antibacterial activity in their studies. However, in these studies except Singh and Sangwan (2011), methanol extracts were not used, which in our studies both leaf and root showed maximum inhibition. Singh and Sangwan (2011) reported that aqueous extract and petroleum ether extracts were ineffective against bacterial strains but methanol extracts proved to be useful for restricting growth of bacteria.

Table 3. Minimum inhibitory concentrations (MIC) (mg /ml) for variousextracts used against tested bacterial strains

Microorganism used	**Leaf**			**Root**		
	Hex	DCM	Me	Hex	DCM	Me
E.coli	-	-	20	20	25	35
P.auriginosa	20	10	10	50	10	25
S.aureus	-	-	42	30	35	40
B.subtilis	32	18	15	60	20	38

All values are mean of triplicates. (-) = No inhibition zone.
Hex, DCM and Me the stand for hexane, dichloromethane and methanol respectively.

The antibacterial activity of *Carissa carandas* L. may be attributed to the various phytochemical constituents present in the extract. The purified components may have been expressed more potency with respect to inhibition of microbes. The work was carried a basic approach to find out the antibacterial activity in *Carissa carandas* L. This type of studies forms a good basis for selection of plant species for further phytochemical and pharmacological investigations. In our studies, methanol extract of leaf and all the extracts of root possess potential activity against bacterial strains. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

Conclusion

Successful prediction of phytochemicals is largely on the type of solvent used and portion of plant material used. The solvents and extraction procedure may modify the results. The traditional healers make use of water primarily as a solvent but our studies showed that methanol extracts of the plant materials studied were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvents. Despite of using water as a solvent, lot of practitioners has been able to cure the ailments. This can be explained by the fact that they generally apply whole crushed plant preparations rather than extracts. Moreover, they generally used decotions and other preparations in combinations i.e. preparations of many plants simultaneously.

In our studies, all the extracts of leaf and root possess potential activity against bacterial strains. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

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