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Bioactivity of crude extracts of Carissa carandas Linn. extracted in polar and non polar solvents

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Abstract: ***Evaluation of the antibacterial activity of crude extracts from different parts (root, stem, leaf) of Carissa carandas (Apocynaceae) Linn. was carried out in the present investigation. Extraction was done by using polar (Water and Methanol) and Non-polar (Petroleum ether) solvents and were screened by 'Disc Diffusion Assay' against 3 Gram negative (E.coli, K.pneumoniae and A.tumifaciens) and 2 Gram positive (S.aureus and B.subtilis) bacteria. Minimum inhibitory concentration, Minimum bactericidal concentration and Total activity were recorded. Mean and Standard deviation also has been calculated. B.subtilis and A.tumifaciens found to be most susceptible organism followed by E.coli. Leaf and stem crude extracts, showed the best activity against B.subtilis [pet. ether (IZ=23mm), methanolic (IZ =22.5) & water (IZ =22.5)] and [pet ether (IZ =20mm), methanolic (IZ=18mm) & water (IZ =18mm)]. The range of MIC & MBC was found to be 1.25-0.078 mg/ml & 0.625-0.039 mg/ml respectively. Results indicate that almost all the tested crude extracts have potent antibacterial activity.***

Keywords: **Crude extract, Minimum inhibitory concentration, Minimum bactericidal concentration, Total activity.**

I. INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs in microorganisms has gradually increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized frequently as therapeutic agents [5]. This fact is the cause for concern, as numbers of patients are not responding positively towards existing antibiotics. Further emerging new multi-resistant bacterial strains, aggravate the problem. The problem of microbial resistance is continuously growing hence the use of existing antimicrobial drugs in future is still uncertain. Therefore, immediate action is required to combat the problem, by encouraging research to develop new drugs; more so of herbal origin as synthetic drugs are known to cause side effects. The ultimate goal is to offer appropriate and efficient antimicrobial and present study is an effort in this direction.

Carissa carandas (common name Karaunda) is a perennial shrub belongs to family Apocynaceae. It grows naturally in the Himalayas at elevations of 300 to 1800 meters, in the Siwalik Hills, the Western Ghats and in Nepal and Afghanistan. It flourishes well on lands with high temperatures. At present it is grown on a limited scale in Rajasthan, Gujarat, Bihar and Uttar Pradesh regions of India. Various medicinal properties viz. Stomachic, Anthelmintic, Cardio tonic, Lowering blood pressure are attributed to this plant. Other properties attributed are strengthening tendons, effective against remittent fever, earache and syphilitic pain. The present investigation was undertaken to find out the antibacterial potential of crude extracts of different parts of *C.carandas* against some Gram positive and Gram negative bacteria.

Antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Aspergillus niger, Candida albicans* was seen in aqueous, ethanol, methanol, chloroform and acetone extract of *C. carandas* [12]. Unripe root and fruit of *C. carandas* exhibited antimicrobial activity in their methanol and petroleum ether extract [11]. Antimicrobial activity of ethanolic extract of fruits of *C. carandas* have been reported against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Bacillus subtilis, Escherichia coli, Proteus vulgaris* and *Proteus mirabilis* [8].

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant crude extracts for their antimicrobial activity may provide new antimicrobial substances. Review of the current literature reveals that no work has been carried out for extraction and screening of selected plant in such a way. Hence, in the present work an extraction and screening for antibacterial activity of the crude extracts of different parts of *C.carandas* has been undertaken.

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II. MATERIAL AND METHODS

Different parts of *C.carandas* (leaf, stem and root) were collected in the month of April to June from the western parts of India (Jaipur, Rajasthan). Plants were identified by senior taxonomist at Department of Botany, University of Rajasthan and voucher specimen no: RUBL 21130 was submitted to the Herbarium, Botany Department, and University of Rajasthan.

A. Preparation of Extracts

Powder of all the three plant parts (Leaf, Stem & Root) were taken in different round bottom flasks in different solvents. 20 g powder was taken in each flask and water, methanol and petroleum ether were used as solvent. Dried material and solvents were taken in 1:10 ratio. Those were kept at soxhlet unit for 24 hours. Then extracts were filtered. The filtrates were subjected to evaporation to obtain dried extract. The percentage yield of each dried plant extract was calculated.

B. Selected Test Microorganisms

Five pathogenic bacteria were screened, viz., *Escherichia coli* (MTCC no.46), *Bacillus subtilis* (MTCC no. 121), *Staphylococcus aureus* (MTCC no. 3160), *Klebsiella pneumoniae* (MTCC no.4030) and *Agrobacterium tumifaciens* (MTCC no. 431). The pathogens were procured from IMTECH (Chandigarh, Punjab, India). Bacterial strains were grown and maintained on Muller-Hinton Agar medium.

C. Antimicrobial assay

Disc diffusion assay was performed for screening [3]. MH agar base plates were seeded with the bacterial inoculums (inoculum size 1×10^8 CFU/ml). Sterile filter paper discs of Whatmann no.1 (6mm in diameter) were impregnated with 100µl of each of the extract of concentration 10mg/ml to give a final concentration of 1 mg/disc. Discs were left to dry in vaccuo so as to remove residual solvent, which might interfere with the determination. Discs with extract were then placed on the corresponding seeded agar plates. Each extract was tested in triplicate along with streptomycin or Gentamycin (1mg/disc) as standard drug for bacteria. The plates were kept at 4°C for diffusion of extract, thereafter were incubated at 37°C for 24h. Activity index for each extracts was calculated [Table I] by the standard formula viz.

Activity index = IZ produced by the extract/ IZ produced by standard

Where, IZ = inhibition zone.

D. Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal (MBC)/ fungicidal (MFC) concentration

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against the test pathogens. 'Broth micro dilution' method was followed for determination of MIC values [4]. Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10 mg/ml final concentration. Two fold serially diluted extracts were added to broth media of 96-wells of micro titer plates. Thereafter 100µl inoculum (1×10^8 CFU/ ml) was added to each well. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control. Micro titer plates were then incubated at 37°C for 24 h. Each extract was assayed in duplicate and each time two sets of micro plates were prepared, one was kept for incubation while another was kept at 4°C for comparing the turbidity in the wells of micro plate. The MIC values were taken as the lowest concentration of the extracts in the well of the micro titer plate that showed no turbidity after incubation. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The minimum bactericidal concentration (MBC) was determined by sub culturing 50 µl from each well showing no apparent growth [Table II]. Least concentration of extract showing no visible growth on sub culturing was taken as MBC.

E. Total activity (TA) determination

Total activity is the volume up to which test extract can be diluted without losing the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [7] [Table III].

III. RESULTS

Petroleum ether, methanolic and water extract were assessed for their antimicrobial potency by IZ, AI (Table-I), MIC & MBC (Table-II).Quantity of extracts per gram of plant material was also calculated (Table-III). In the

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Volume 3, Issue 5, September 2014

present investigation total 9 extracts were tested and all were found active against at least one of the tested pathogens. *B.subtilis* & *A.tumifaciens* were observed to be the most susceptible organism in the investigation followed by *E.coli*. Best activity observed in pet ether extract of leaf (IZ=23mm, MIC=0.078 mg/ml, AI=0.77±0.04), methanolic extract of leaf (IZ=22.5mm, MIC=0.078 mg/ml, AI=0.75±0.07) & water extract of leaf (IZ=22.5mm, MIC=0.078 mg/ml, AI=0.75±0.01) against *B.subtilis.* All the three crude extracts of Stem showed best activities, Pet ether extract (IZ=20mm, MIC=0.156 mg/ml, AI=0.67±0.01), methanolic extract (IZ=18mm, MIC=0.156 mg/ml, AI=0.60±0.12) & water extract (IZ=18mm, MIC=0.156 mg/ml, AI=0.60±0.12) against *B.subtilis*. Pet ether (IZ=13mm, MIC=0.312 mg/ml, AI=0.59±0.01) & methanolic extract of leaf (IZ=12mm, MIC=0.312 mg/ml, AI=0.55±0.06) and Pet ether (IZ=19.5mm, MIC=0.156 mg/ml, AI=0.65±0.01) & methanolic extract of root (IZ=13.5mm, MIC=0.312 mg/ml, AI=0.45±0.01) showed very good activities against *A.tumifaciens*. Pet ether extract of stem (IZ=11mm, MIC=0.312 mg/ml, AI=0.50±0.01) & water extract of root (IZ=10.5mm, MIC=0.625 mg/ml, AI=0.42±0.01) showed good activity against *Klebsiella pneumoniae*. Among all the three extracts pet ether and methanolic extracts of leaf & stem found to be most bioactive crude metabolite of the plant. MIC & MBC values (Table-II) were evaluated for plant crude extracts which had shown activity in diffusion assay. The range of MIC & MBC of extracts recorded was 1.25-0.078 mg/ml & 0.625-0.039 mg/ml respectively.

In present investigation, lowest MIC value 0.078 mg/ml was recorded against *B.subtilis*, indicating significant antimicrobial potential of test extracts. Quantity of extract obtained per gram from plant parts & TA calculated was recorded (Table-III). TA indicates the volume up to which extract can be diluted without losing ability to kill microorganisms. High values of TA observed against *B.subtilis* (1506.41ml/g) followed by *A.tumifaciens* (376.60ml/g), K. *pneumoniae* (105.6ml/g) & *E.coli* (188ml/g), which proves the potential to inhibit the growth of the test microorganisms, even at low concentration.

IV. DISCUSSION

Crude extracts from different parts of *C.carandas* have previously been studied for their antibacterial activity [2], [6], [10] but, still meager work has been carried out as far as the antimicrobial activity of crude extracts is concerned. Most of the research has been restricted on determination of IZ of crude extracts without calculating AI, MIC, MBC/MFC and TA. Determination of MIC and MBC/MFC has now become an inevitable step in antimicrobial studies in order to establish their antimicrobial activity so as to explore them at industrial level for production of drugs, which could replace the existing ones. Hence, most of the studies carried out so far could only reveal their antimicrobial activities, but are not helpful for establishing them as antibiotic. Result of the present study indicated that the crude extracts of all the three parts of *C.carandas* have activity against both gram-positive and gram-negative bacteria indicative of the presence of broad spectrum antibiotic compounds. The results of the antibacterial activity of the study were in agreement with the findings of previous studies [1], [9]. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and provide biochemical tools for the study of infectious diseases.

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Table I: Antimicrobial activity of extracts of Carissa carandas against some pathogenic bacteria

Plant part	Extract	Microorganisms									
		E.coli		*B.subtilis*		*S.aureus*		*K. pneumoniae*		*A.tumifaciens*	
		IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Leaf	P1	8	0.23±0.01	23	0.77±0.04	-	-	-	-	13	0.59±0.01
	M1	9	0.26±0.01	22.5	0.75±0.07	7	0.64±0.01	-	-	12	0.55±0.06
	W1	-	-	22.5	0.75±0.01	-	-	-	-	-	-
Stem	P2	8	0.28±0.01	20	0.67±0.01	-	-	11	0.5±0.01	11	0.37±0.01
	M2	7	0.25±0.01	18	0.60±0.12	-	-	9	0.41±0.01	9.5	0.30±0.01
	W2	9	0.32±0.01	18	0.60±0.12	-	-	-	-	8	0.27±0.04
Root	P3	-	-	7	0.20±0.01	-	-	-	-	19.5	0.65±0.01
	M3	-	-	7	0.20±0.01	-	-	-	-	-	-
	W3	-	-	-	-	-	-	10.5	0.42±0.01	13.5	0.45±0.01

IZ=Inhibition zone in mm (value: including 6mm diameter of disc),

AI= Activity index (IZ developed by extract/IZ developed by standard),

P1, P2, P3=Pet ether extracts of respective plant parts,

M1, M2, M3=Methanolic extracts of respective plant parts

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Volume 3, Issue 5, September 2014

W1, W2, W3=Water extracts of respective plant parts,

(-)= no activity, ±=SEM.

Table II: MIC and MBC of active extracts of Carissa carandas against different pathogens

Plant parts		**Leaf**			**Stem**					**Root**
Microorganism		**P1**	**M1**	**W1**	**P2**	**M2**	**W2**	**P3**	**M3**	**W3**
E.coli	MIC	1.25	0.625	-	1.25	1.25	0.625	-	-	-
	MBC	0.625	0.312	-	0.625	0.625	0.312	-	-	-
B.subtilis	MIC	0.078	0.078	0.078	0.156	0.156	0.156	1.25	1.25	-
	MBC	0.039	0.039	0.039	0.078	0.078	0.078	0.625	0.625	-
S.aureus	MIC	-	1.25	-	-	-	-	-	-	-
	MBC	-	0.625	-	-	-	-	-	-	-
K. pneumoniae	MIC	-	-	-	0.312	0.625	-	-	-	0.625
	MBC	-	-	-	0.156	0.312	-	-	-	0.312
A.tumifaciens	MIC	0.312	0.312	-	0.312	0.625	1.25	0.156	-	0.312
	MBC	0.156	0.156	-	0.156	0.312	0.625	0.078	-	0.156

P1, P2, P3=Pet ether extracts of respective plant parts,

M1, M2, M3=Methanolic extracts of respective plant parts,

W1, W2, W3=Water extracts of respective plant parts,

MIC= Minimum inhibitory concentration,

MBC=Minimum bactericidal concentration,

(-)= no activity.

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ISO 9001:2008 Certified
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Volume 3, Issue 5, September 2014

Table III: Quantity & Total activity of extracts of Carissa carandas

Plant part	Extract	Quantity of extract mg/g dwt	Total Activity(ml/g)				
			E.coli	*B.subtilis*	*S.aureus*	*K. pneumoniae*	*A.tumifaciens*
Leaf	**P1**	24.17	19.34	309.87	-	-	77.46
	M1	117.5	188	1506.41	94	-	376.60
	W1	68.75	-	881.41	-	-	-
Stem	**P2**	12.67	10.14	81.21	-	40.60	40.60
	M2	66	52.8	423.07	-	105.6	105.6
	W2	28	44.8	179.48	-	-	22.4
Root	**P3**	12.67	-	10.136	-	-	81.21
	M3	77	-	61.6	-	-	-
	W3	25	-	-	-	40	80.12

P1, P2, P3=Pet ether extracts of respective plant parts,

M1, M2, M3=Methanolic extracts of respective plant parts,

W1, W2, W3=Water extracts of respective plant parts,

TA= total activity (extract per gm dried plant part/MIC of extract).