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Full Length Research Paper

Antimicrobial activity of the water extracts of the leaves and fruits of *Carissa edulis* Vahl (Apocynaceae)

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***Carissa edulis* Vahl (Apocynaceae) is used commonly in Northern Nigeria for the treatment of various microbial infections such as venereal, upper respiratory, skin and gastro-intestinal. The antimicrobial activity of the water extracts of the leaves and fruits were screened using *Staphylococcus aureus*, NCTC 6571, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 6750 and *Bacillus subtilis* NCT 10342. The gram positive organisms, *B. subtilis* and *S. aureus* were more susceptible than the gram negative organisms, *E. coli* and *P. aeruginosa*. The leaves extract was more active than that of the fruits extract. The minimum inhibitory concentration for *E. coli* and *B. subtilis* was 4.0% w/v of the fruits aqueous extract while that of the leaves was 3.0% w/v for the same organisms. *B. subtilis* was killed at a faster rate than *E. coli.* A percentage kill of 99.52% was obtained for *B. subtilis* and 91.36% for *E. coli* with the leaves extract as compared to that of the fruits extract where a percentage kill of 93.6% was obtained for *B. subtilis* and 56.4% for *E. coli* at 120 min. The results of this work justify the use of the plant in the treatment of microbial infections in ethno medicine.**

Key words: *Carissa edulis*, water extract, fruits, leaves, antimicrobial.

INTRODUCTION

The plant *Carissa edulis* has been used in ethno medicine for the treatment of many microbial infections such as venereal, respiratory and gastrointestinal infections (Burkill, 1985; Irvine, 1961; Sofowora, 1980; Omino and kokwaro, 1993; Oliver, 1994). The plant is a spiny shrub up to 5 m in height. It is widely distributed throughout tropical Africa extending southwards to Zambia and Zimbabwe. This is also found in Madagascar, Arabia, India and Indochina (Burkill, 1985; Sofowora, 1980; Hutchinson and Dalziel, 1963). In Nigeria, it is commonly known as 'cizaki' in Hausa language (Gbile, 1980). In Malawi, it is known as Mpambala and 'Mkokolo' (Sofowora, 1980). Economically the fruits are made into jam or vinegar. (Irvine, 1961; Omino and Kokwaro, 1993; Banker and Verma, 1987). The leaves and fruits were reported to contain carbohydrates, tannins, flavonoids, saponins, cardiac glycosides, terpenes and steroids (Ibrahim and Bolaji, 2002; Ibrahim, 1997; Ibrahim et al., 2005). The dried leaf (Sudan) ethanolic 95% extract was reported to possess week mulluscicidal activity 40% (mortality) using *Biomphalaria pfeifferi* and *Truncatus* animal species (Abel et al., 1990).

This study aims at determining and comparing the minimum inhibitory concentrations (MIC) and the rate of kill of the aqueous extracts of the leaves and fruits of *C. edulis*.

MATERIALS AND METHODS

Collection of plant materials

The plant samples, leaves and fruits were collected in June, 2001 at Jama'a bushes Zaria. The plant was identified on the field using description given in the literature (Irvine, 1961; Hutchinson and Dalziel, 1963). The plant was authenticated at the Herbarium Biological Sciences, Ahmadu Bello University (ABU) Zaria. Herbarium number was given as 900182.

Preparation of samples

The leaves and fruits were removed from the branches and dried

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separately. When dried the seeds were removed from the fruits. The fruits and leaves were powdered separately and stored in air tight containers.

Preparation of extracts

The powdered leaves and fruits were extracted with distilled water. 50 g each were immersed in 250 ml of water and allowed to macerate for 24 h, the suspension was shaken on an electric shaker for the first 6 h. The suspensions were filtered and the filtrates were freeze dried. The extracts were weighed and stored separately in air tight containers.

Antimicrobial activity studies

Preparation of nutrient media

The nutrient media used were nutrient agar (Biotec Limited) and nutrient broth (Oxoid Limited). The media were prepared according to the manufacturer instructions.

Test organisms

Standard cultures of *Staphylococcus aureus* NCTC 6571, *Bacillus subtillis*, NCTC 10342, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 6750 were obtained from the stock of the Department of Pharmaceutics and Pharmaceutical Microbiology, ABU, Zaria.

Susceptibility studies

The susceptibility of all the organisms to the water extracts were tested using cup plate method (Salle, 1991). The Petri dishes were sterilized at 150°C for one hour in the oven and allowed to cool. One milliliter of standard organism was added to 19 ml of melted prepared nutrient agar and mixed properly. This amount was poured into each Petri dish and allowed to set. Using sterile size 4-cork borers (8 mm), holes were made in the agar medium and the cut agar removed with sterile forceps. 3 holes were made on each plate, the bottom of each hole was covered with molten agar.

Thirty percent leaves aqueous extract and 4.0% fruits aqueous extract were used for this study. Serial dilution, obtaining 1/10, 1/100, 1/1000, 1/5000 and 1/10,000 of these extracts were prepared. 0.13 ml of each was poured into each hole on different plates. Two hours diffusion time was allowed and then the plates were incubated at 37°C for 24 h. The procedure was carried out under aseptic technique. Zones of inhibition were recorded in millimeters.

Determination of minimum inhibitory concentration (MIC)

The MIC of the extracts against the standard cultures was determined using the broth dilution method (Hugo and Russell, 2000). Double strength nutrient broth was prepared and 5 ml each dispensed into test tubes. The concentrations of the extract used were 6.0%w/v of leaves aqueous extract double diluted serially to obtain 3.0, 1.5, 0.75, 0.375, 0.18 and 0.09% w/v extracts and 8.0% of fruits aqueous extract double diluted serially to obtain 4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.063% w/v extracts as the leaves are more active than the fruits. 5 ml of each extract was mixed with 5 ml of the prepared double strength nutrient broth. A drop of the standardized organisms was dropped into each test tube. The inoculums were periodically standardized to obtain 10^4 cfu/ml for *B. subtillis* and 10^3 cfu/ml for *E. coli*. The test tubes were then incubated at 37°C for 24 h. Then the presence or absence of growth was observed. (CFU = colony forming units).

Determination of rate of kill

Three percent w/v of the leaves aqueous extract and 4%w/v of the fruits aqueous extract were used for this study. 0.1 ml of the standard culture was added to 5 ml of the extracts. After a contact time of 30, 60, 90 and 120 min, 0.1 ml of the mixture was withdrawn and added to sterile agar and mixed gently.

The agar was poured into plates and allowed to solidify. The plates were then incubated at 37°C for 24 h. The observed colonies were counted using the Gallenhamp Colony Counting Machine (Hugo and Russell, 2000). The percentage viability and percentage kill were also determined taking into account all dilutions.

RESULTS

Susceptibility results

The zones of inhibitions obtained for the various extracts used against the tested organisms are given on Tables 1 and 2. The zones of inhibitions of the leaves aqueous extract are more than that of the fruits aqueous extracts for 1/10, 1/100 and 1/1000 but that is not the case with 1/5000 and 1/10,000.

Determination of minimum inhibitory concentration

The results of the MIC determination are given on Tables 3 and 4.

Determination pf rate of kill

The results of the rate of kill of the extracts against the various organisms are given on Figures 1 and 2 and the percentage viability and percentage kill are given on Table 5.

DISCUSSION

The susceptibility test showed the extracts to be more active on the gram +ve organisms (*B. subtilis* and *S. aureus*) compared to gram -ve organisms (*E. coli* and *P. aeruginosa*) (Tables 1 and 2). The activity of the leaves extract is more than that of the fruits extract. The activity of both extracts is quite high even at 1/10,000 dilution (Table 1 and 2). This is further indicated from the MIC, 3%w/v for leaves extract and 4%w/v for the fruits extract (Tables 3 and 4). The rate of kill *of B. subtilis* was found to be more than that of *E. coli*. A percentage kill of 99.52% was obtained for *B. subtilis* and 91.36% for *E. coli* with the leaves as compared to that of the fruits, 93.6% was obtained for *B. subtilis* and 56.4% for *E. coli* at 120 min (Figures 1 and 2).

Table 1. Result of the diameter of zones of inhibition for 3% w/v leave aqueous extract of *C. edulis,*

Dilution	Organisms	Diameter of zones of inhibition (mm)
1/10	*E. coli*	23
	P. aeruginosa	22
	B. subtilis	27
	Staph. aureus	22
1/100	*E. coli*	20
	P. aeruginosa	17
	B. subtilis	25
	Staph. aureus	18
1/1000	*E. coli*	14
	P. aeruginosa	12
	B. subtilis	19
	Staph. aureus	16
1/5000	*E. coli*	13
	P. aeruginosa	10
1/10,000	*B. subtilis*	15.7
	Staph. aureus	14

Table 2. Result of the diameter of zone of inhibition for; 4% fruit aqueous extract.

Dilution	Organisms	Diameter of zones of inhibition (mm)
1/10	*E. coli*	22
	P. aeruginosa	20
	B. subtilis	25
	Staph. aureus	24.6
1/100	*E. coli*	18
	P. aeruginosa	14
	B. subtilis	22
	Staph. aureus	22
1/1000	*E. coli*	14
	P. aeruginosa	11
	B. subtilis	21
	Staph. aureus	21
1/5000	*E. coli*	13.6
	P. aeruginosa	11
1/10,000	*B. subtilis*	16
	Staph. aureus	18

The antimicrobial activities of the extracts is attributed to the secondary metabolites present *viz* tannins, saponins and flavonoids (Ibrahim and Bolaji, 2002; Ibrahim, 1997; Ibrahim et al., 2005). The difference in the extent of activity between gram positive and gram negative could be due to the structural differences between these

Table 3. Result of MIC for leave aqueous extract of *C. edulis.*

Organisms	%w/w	Test-tube 1	Test-tube 2
	6.0	-	-
	3.0	-	-
	1.5	+	+
E. coli	0.75	+	+
	0.375	+	+
	0.188	+	+
	0.094	+	+
	6.0	-	-
	3.0	-	-
	1.5	+	+
B. subtilis	0.75	+	+
	0.375	+	+
	0.188	+	+
	0.094	+	+

+: Growth of microbial organism, -: No growth of microbial organism.

Table 4. Result of MIC for fruit aqueous extract of *C. edulis*.

Organisms	%w/w	Test-tube 1	Test-tube 2
	8.0	-	-
	4.0	-	-
	2.0	+	+
E. coli	1.0	+	+
	0.5	+	+
	0.25	+	+
	0.125	+	+
	0.063	+	+
	8.0	-	-
	4.0	-	-
	2.0	+	+
B. subtilis	1.0	+	+
	0.5	+	+
	0.25	+	+
	0.125	+	+
	0.063	+	+

+:Growth, -:No growth.

organisms and the mode of antibacterial activity (Hugo and Russell, 2000).

Conclusion

The results obtained justify the use of the leaves and fruits of *Carissa edulis* in traditional medicine for the treatment of infections; venereal, respiratory and gastrointestinal diseases. This could also serve as a potential source of new drug.

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Table 5. Result for percentage viability and percentage kill.

Water extract	Test organisms	Percentage viability contact time (min)					Percentage kill contact time (min)				
		0	30	60	90	120	0	30	60	90	120
Fruits	*B. subtilis*	100	35	7.68	3.2	6.4	0	65	92.32	96.8	93.6
	E. coli	100	83.4	78	61.4	43.4	0	61.6	22	38.6	56.4
Leaves	*B. subtilis*	100	15	6.72	1.9	0.48	0	85	93.28	98.1	99.52
	E. coli	100	35	18.72	13.44	8.64	0	62	81.28	86.56	91.36

$$\text{Percentage Viability} = \frac{\text{No of surviving organism at time (t)}}{\text{Initial inoculum level}} \times 100$$

Percentage kill = 100 - % Viability

Initial inoculum level (that is, count at zero minutes) for rate of kill experiment for *B. subtilis* and *E. coli* are 10 and 10 CFU, respectively.

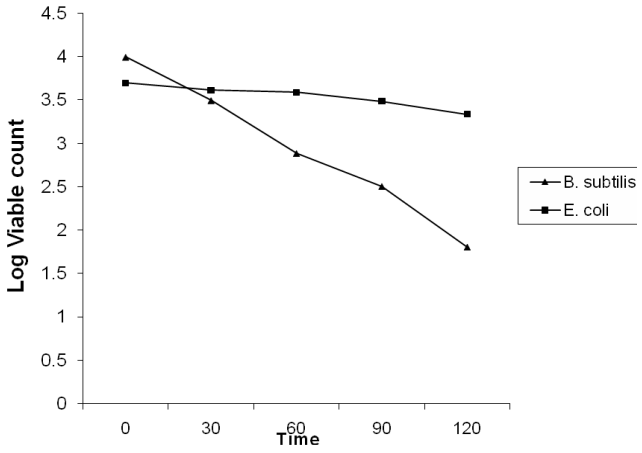

Figure 1. Graph of rate of kill of 4.0%w/v fruits water extracts of *C. edulis.*

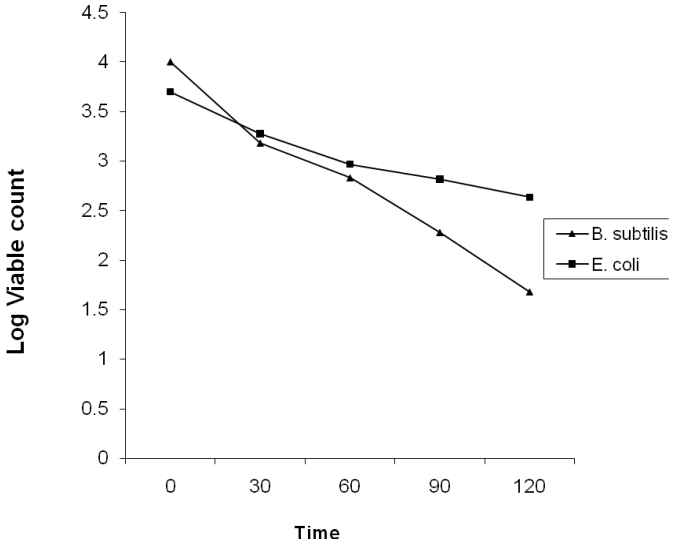

Figure 2. Graph of rate of kill of 3.0%w/v leaves water extract of *C. edulis.*

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