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Antimicrobial Activities of Methanol Leaf Extract of *Carissa edulis* Vahl (Apocynaceae)

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ABSTRACT

The research aims at evaluating *in-vitro* antimicrobial effects of methanolic leaf extract of *Carissa edulis*. The leaf of *Carissa edulis* was collected air-dried, ground to powder and extracted with methanol by cold extraction method to give a percentage yield of 28.03% $^{w}/_{w}$. The crude extract was defatted with n-hexane and partitioned with chloroform, n-butanol and water to give a percentage yield of 36.04%, 11.24%, 15.94% and 3.08% respectively. The extract fractions exhibited considerable amount of inhibition against almost all the test micro-organisms except for the n-hexane extract which had no activity. The activity of n-butanol extract (500 mg/ml), chloroform extract (500 mg/ml) and aqueous extract (500 mg/ml) was superior to the standard drug (Gentamacin) used. The MIC and MBC against the tested organisms ranged from 25 mg/ml to 50 mg/ml and 50 mg/ml to 100 mg/ml respectively. The results obtained from this study revealed that the leaf extract of *Carissa edulis* possesses antibacterial activity against some pathogenic microorganisms such as *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Keywords: *Carissa edulis*; Antimicrobial;*in vitro*; Microorganism

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INTRODUCTION

Herbal medicines are globally in great demand in both developed and developing countries as a source of primary health care owing to their attributes of having wide biological and medicinal activities, high safety margins and fewer costs [1]. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell. Even with the

advent of modern or allopathic medicine, it has been noted that a number of important modern drugs have been derived from plants used by indigenous people [2].

Traditional medicine is said to be popular among 70% of the populationin Africa. The country inaugurated a Council for traditional medicine and is said to be working out how to integrate African medicine into its health care delivery system [3][4][5].

Infectious diseases are the world's main cause of mortality, in particular in developing nations. Currently, epidemics of infections caused by drug-resistant and unknown microorganisms have raised major health concerns [6]. This situation has called for renewed strategies on treatment and preventive approaches, one of which is the discovery of new antimicrobials agents [7]. Recently, it has been found that pathogenic bacteria are increasingly growing to become multidrug resistant. This troubling condition originates from the unnecessary and frequently use of antibiotics in human and animal health care to manage and prevent bacterial infections [8].Newer drugs armed with a competent mechanism should be formulated to regulate this condition, the plant-derived antimicrobial agents may be the solution to these problems. Recently, medicinal plants have become the focus of intense study regarding their conservation and potential pharmacological effects. Indeed, the search for new pharmacologically active agents, through the screening of natural sources such as microbial fermentations and plant extracts, has led to the discovery of many clinically useful drugs that now play major roles in the treatment of human diseases [9].

Carissa edulis Vahlis widespread in many parts of Africa. It belongs to the family Apocynaceae and grows at forest edges, in forests and woodlands where Euphorbia, Acacia, and Croton commonly occur, especially on rocky hillsides, clay soils, black cotton soils, in dry and moist low- and midlands of altitude 1500-2500m [10].

Carissa edulis is a well-known African medicinal plant widely used in traditional treatment of headaches, chest pains, rheumatism, gonorrhoea, syphilis and rabies. The plant roots have been used in Africa for a variety of medicinal purposes. The vapour from a hot aqueous root bark infusion is inhaled as treatment for chest congestion and the root powder is applied to toothache to relieve pain. The roots are also used to treat gastric ulcers and the decoction is used to treat malaria [11].

In spite of the use of *Carissa edulis* in traditional medicine it is pertinent therefore to test the methanol extract on some pathogenic micro-organisms given the fact that micro-organisms are becoming increasingly resistant to synthetic agents. Thus the need to scientifically examine this plant in order to justify its use as an antimicrobial agent.

MATERIALS AND METHODS

Materials

Equipment used for sample collection, preparation and chemical analysis (extraction and phytochemical analysis, column and thin chromatographic analysis) were: cellophane bags, wooden mortar and piston, beakers, separating funnels, conical flasks, spatula, steel trays, test tubes, measuring cylinders, Whatmann No. 1 filter papers, Muslin cloth, columns glass tube (90x2.8cm), test tubes, petri dishes, Whatmann's No. 1 filter papers, beakers, spatula, conical flasks, office punch, safety surgical gloves, face mask, autoclave, analytical weighing balance, Oven and innoculator.

Chemicals, Drugs and Reagents

Chemicals and reagents used for chemical analyses: 95% methanol, distilled water, n-butanol, n-hexane, ethylacetate, nutrient agar (Biotec Medical Market, UK), sodium chloride (NaCl) prepared by dissolving 4.25g NaCl analar grade, BDH Lab. Poole, England), sabouraud-2% dextrose agar, ciprofloxacin and gentamicin.

Methods

Sample Collection and Identification

Fresh sample of leaf of *Carissa edulis* was collected from Herbs Garden of Faculty of Pharmacy, University of Maiduguri Borno State, Nigeria and authenticated by a plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Nigeria. The sample was air dried under shade rendered free of foreign material through manual picking, labeled (663C) and ground with a wooden mortar and pestle to a coarse powder.

Plant Extraction

Five hundred gram (500g) of the pulverized dried leaves of *Carissa edulis* was extracted by cold extraction method (maceration) with 2.5L of absolute methanol at room temperature for seventy-two hours (72hr) in a round bottom flask with occasional shaking. The soaked sample was passed through a muslin cloth to remove the vegetative debris and the liquid was filtered through Whatman No. 1 filter paper. The crude extract was concentrated to dryness. The extract was weighed, labelled and subjected to further analysis. Fifty gram (50 g) of the crude extract was subjected to defatting with n-hexane and then partitioned with chloroform, ethylacetate and n-butanol based on their polarities.

Evaluation of Antimicrobial Activity of Crude and Partitioned Fractions

The crude methanol extract, n-hexane, chloroform, ethylacetate and butanol partitioned fractions of the plant were evaluated for its antimicrobial activity.

Preparation of Various Concentrations and Dilutions of the *Carissa edulis* Methanol Leaf Extract

The stock solution of the *Carissa edulis* methanol leaf extract was 1000 mg/ml and it was prepared by adding 10 g to 10 ml distilled water. This was diluted to 800 mg/ml, 600 mg/ml, 400 mg/ml and 200 mg/ml respectively.

Preparation of Test Organisms

One ml each of the 24hr pure broth culture of all the bacteria *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi,Candida albicans* and *Aspergillus niger*was obtained in Veterinary Microbiology Laboratory University of Maiduguri, was added to 9ml sterile sodium chloride (NaCl) solution (prepared by dissolving 4.25g NaCl analar grade, BDH Lab. Poole, England) in 500ml distilled water and sterilized in a portableautoclave at 121ºC for 15 minutes. One ml of this was added to another 9ml NaCl solutionand from this another one ml of the suspension was added to 9ml NaCl solution to give afinal dilution of C x 10^3 organisms. (i.e. serial dilution was carried out to make a tenfold suspension) this was used for the antibacterial work and that of the *Candida albicans* [12].

Preparation of Discs Containing Graded Concentrations of the *Carissa edulis* Methanol Leaf Extract and the Tetracycline Discs

Whatman filter paper No. 1 was punched into circular discs (each 6 mm in diameter), with the aid of an office punch. The discs were then put in a glass petri dish and sterilized in a hot air oven at 60 ºC for 30 min. One ml of each of the different concentrations of the extract was placed in a sterile glass plate and twelve (12) sterile discs were put in the prepared extract using a pair of sterile forceps to soak the extract. They were allowed to dry. The discs were checked to be sure that they did not stick together [13]. The discs containing *Carissa edulis* methanol leaf extract were used for the antibacterial tests. One capsule tetracycline 250 mg powder was dissolved in one ml distilled water in a sterile, glass petri dish to give 250 mg/ml. Thirteen sterile discs were put inside it so as to be soaked with the tetracycline and then left to dry. This gave tetracycline discs of 250mg/ml which is equivalent to $2.5x10^5 \mu g/ml$.

Preparation of Culture Media

The culture media used in this study was nutrient agar (Biotec Medical Market, UK) for the bacteria. The nutrient agar was prepared according to the manufacturer's specifications (by dissolving 18.5 g powder in 500 ml distilled water) and sterilized at 121ºC for 15min. The pH was within the range of 7.1-7.5 after autoclaving. This was poured into 90 mm diameter sterile, disposable plastic petri dishes to a depth of 4mm (about 25ml per plate). Care was taken to pour the plates on a level surface so that the depth of the medium would be uniform. The

plates were dried upside down in an incubator at 37ºC with their lids opened and inverted so that water would not condense back into the agar [12].

Disc Diffusion Antibacterial Selectivity Test and Disc Diffusion Antifungal Selectively Test

One ml each of the C x 10^3 test organisms (Gram positive and Gram negative bacteria) were pipetted into the solidified nutrient agar plates and the excess was removed after allowing it to go round the surface of the medium. The antibiotic discs of tetracycline (2.5 x 105 µg/disc) were placed on the plate that had been uniformly inoculated with the test organism using sterile forceps. The discs of blotting paper that had been previously impregnated with graded concentrations of the methanol leaf extract of *Carissa edulis* were placed on each of the plates. The plates were incubated at 37ºC for 24 hrs for bacteria and examined for antimicrobial diffusion from the discs into the medium to see if the growth of the test organism would be inhibited at a distance from the disc that is related to the sensitivity of the organism [14].

The antibiotic discs: ciprofloxacin (5µg/disc) gentamicin (10µg/disc) were placed on the already prepared sabouraud-2% dextrose agar containing graded concentrations of the methanol leaf extract of *Carissa edulis* (6 in all) and the control (1 plate).

Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth dilution technique [15]. The minimum inhibitory concentration was determined from micro-organisms that were sensitive to the extract under study (leaf extract). Equal volume of nutrient broth was dispensed into tubes (bijou bottles) were known concentrations of the extract diluted at concentrations ranging from the lowest to the highest i.e 12.5 mg/ml to 200 mg/ml were prepared.

Minimum Bactericidal Concentration (MBC)

MBC was determined by using the broth dilution technique as modified by Usman *et al*. [16] by assaying the test tubes resulting from MIC determinations. A loop of the content of each test tube was inoculated by streaking on a solidified nutrients agar plate incubating at 37°C for 18 hours and observed for bacterial growth. The lowest concentration of the subculture with no growth was considered the MBC.

Statistical Analysis

Data obtained from the antimicrobial study were presented as mean ± standard deviation (S.D). One-way analysis of variance (ANOVA) was used to test for significance and mean and $P<0.05$ was considered significant.

RESULTS

Antimicrobial Susceptibility Assay of the Various Leaf Extracts of *Carissa edulis*

The extract exhibited considerable inhibition against all the test organisms except for N-hexane extract (Table 1) which did not show activity at all.

Antimicrobial Susceptibility Effect of Methanol Leaf Extract of *Carissa edulis*

Table 2 shows that the crude methanol leaf extract at the concentration of (500 mg/ml) exhibited greater inhibition on *Staphylococcus aureus*, (12.67±0.33 mg/ml) *Escherichia coli*, (12.00±0.00 mg/ml) *Salmonella typhi* (11.33±0.33 mg/ml) and *Candida albicans* (12.33±0.33 mg/ml), at (400 mg/ml) *Staphylococcus aureus* (10.00±0.00 mg/ml), *Escherichia coli* (8.67±0.33 mg/ml), *Salmonella typhi* (8.33±0.33 mg/ml), *Candida albicans* (8.33±0.33 mg/ml) at (300 mg/ml) *Staphylococcus aureus* (7.33±0.33 mg/ml), *Escherichia coli* (7.00±0.00 mg/ml), *Salmonella typhi* (7.00±0.00 mg/ml) while *Streptococcus pyogenes* and *Aspergillus niger* were resistant.

Antimicrobial Susceptibility Effect of n-Butanol Fraction of *Carissa edulis*

Table 3 shows that, the result of the effect of n-butanol fraction. The fraction at 500 mg/ml had the highest activity against *Streptococcus pyogenes* (27.33±0.33 mg/ml), *Escherichia coli* (9.33±0.33 mg/ml) and *Candida albicans* (18.67±0.66 mg/ml) at 400 mg/ml *Streptococcus pyogenes* (24.33±0.33 mg/ml), *Escherichia coli* (8.00±0.00 mg/ml), *Candida albicans* (16.33±0.33 mg/ml), at 300 mg/ml *Streptococcus pyogenes* (19.67±0.33 mg/ml) and *Candida albicans* (12.67±0.33 mg/ml) at (200 mg/ml) *Streptococcus pyogenes* (14.67±0.33

mg/ml) and *Candida albicans* (9.33±0.33 mg/ml) at 100 mg/ml *Streptococcus pyogenes* (10.67±0.33 mg/ml) while *Staphylococcus aureus, Salmonella typhi* and *Aspergillus niger* were resistant.

Antimicrobial Susceptibility Effect of Chloroform Fraction of *Carissa edulis*

The chloroform fraction at 500 mg/ml was observed to have highest the activity against *Streptococcus pyogenes* (17.67±0.33 mg/ml), *Salmonella typhi* (14.33±0.33 mg/ml) and *Candida albicans* (14.00±0.00 mg/ml) at (400 mg/ml) *Streptococcus pyogenes* (15.00±0.00 mg/ml), *Salmonella typhi* (12.33±0.33 mg/ml), and *Candida albicans* (10.33±0.33 mg/ml), at (300 mg/ml) *Streptococcus pyogenes* (11.33±0.33 mg/ml), *Salmonella typhi* (10.00±0.00 mg/ml) and *Candida albicans* (8.33±0.33 mg/ml) at (200 mg/ml) *Streptococcus pyogenes* (8.33±0.33 mg/ml) and *Salmonella typhi* (7.00±0.00 mg/ml) while *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger* were resistant (Table 4).

Antimicrobial Susceptibility Effect of Aqueous Fraction of *Carissa edulis*

Table 5 shows that the water extract at 500 mg/ml) exhibited greater activity against *Staphylococcus aureus*(10.33±0.33 mg/ml), *Streptococcus pyogenes* (19.67±0.33 mg/ml) and *Candida albicans* (15.67±0.33) at (400 mg/ml) *Staphylococcus aureus* (7.67±0.33 mg/ml), *Streptococcus pyogenes* (16.67±0.33 mg/ml) and *Candida albicans* (11.67±0.33 mg/ml) at (300 mg/ml) *Streptococcus pyogenes* (13.33±0.33 mg/ml) and*Candida albicans* (9.00±0.00 mg/ml) at (200 mg/ml) *Streptococcus pyogenes* (10.00±0.00 mg/ml) and *Candida albicans* (7.00±0.00 mg/ml) at (100 mg/ml) *Streptococcus pyogenes* (7.33±0.33 mg/ml) while *Escheichia coli*, *Salmonella typhi* and *Aspergillus niger* were resistant.

Minimum Inhibitory Concentration (MIC)

The MIC of the n-butanol leaf extract of *Carissa edulis* was 25.00 mg/ml for both *Streptococcus pyogenes* and *Candida albicans* as shown in Table 6.

Minimum Bactericidal Concentration (MBC)

The MBC value for *Streptococcus pyogenes* was 25.00 mg/ml N-butanol that for *Candida albicans* was 50.00 mg/ml (Table 7).

Table 1: Antimicrobial Activity of n-hexane Leaf Extract of *Carissa edulis*

Organisms	Concentration in mg/ml /zones of diameter of inhibition (mm) as Means's							
	500	400	300	200	100	Cip	GN	TE
Staphylococcus aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	28	17	NT
Streptococcus pyogenes	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	32	20	NT
Escherichia coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27	14	19
Salmonella typhi	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	29	22	0.00±0.00
Candida albicans	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	26	13	NT
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NT	NT	NT

Data are mean of triplicate values (n = 3). Values with different alphabetical superscript are significantly ($p<0.05$) different

R= Resistance, CIP= Ciprofloxacin, GN= Gentamycin, TE= Tetracycline, NT- Not tested

Table 2: Antimicrobial Activity of Crude Methanol Leaf Extract of *Carissa edulis*

Organism	Concentration in mg/ml /zones of diameter of inhibition (mm) as Mean±S.D							
	500	400	300	200	100	Cip	GN	TE
Stapphylococcus aureus	12.67±0.33^{a}	10.00±0.00^{b}	7.33±0.33^{c}	0.00±0.00	0.00±0.00	28	17	NT
Streptococcus pyogenes	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	32	20	NT
Escherichia coli	12.00±0.00^{a}	8.67±0.33^{b}	7.00±0.00^{c}	0.00±0.00	0.00±0.00	27	14	19
Salmonella typhi	11.33±0.33^{a}	8.33±0.33^{b}	7.00±0.00^{c}	0.00±0.00	0.00±0.00	29	22	0.00±0.00
Candida albicans	12.33±0.33^{a}	8.33±0.33^{b}	0.00±0.00	0.00±0.00	0.00±0.00	26	13	NT
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NT	NT	NT

Data are mean of triplicate values (n = 3). Values with different alphabetical superscript are significantly ($p<0.05$) different

R= Resistance, CIP= Ciprofloxacin, GN= Gentamycin, TE= Tetracycline, NT- Not tested

Table 3: Antimicrobial of N-butanol Leaf Extract of *Carissa edulis*

Organism	Concentration in mg/ml /zones of diameter of inhibition (mm) as Mean±S.D							
	500	400	300	200	100	Cip	GN	TE
Staphylococcus aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	28	17	NT
Streptococcus pyogenes	27.33±0.33^{a}	24.33±0.33^{b}	19.67±0.33^{c}	14.67±0.33^{d}	10.67±0.33^{e}	32	20	NT
Escherichia coli	9.33±0.33	8.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27	14	19
Salmonella typhi	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	29	22	R
Candida albicans	18.67±0.66^{a}	16.33±0.33^{b}	12.67±0.33^{c}	9.33±0.33^{d}	0.00±0.00^{a}	26	13	NT
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NT	NT	NT

Data are mean of triplicate values (n = 3). Values with different alphabetical superscript are significantly ($p<0.05$) different
R= Resistance, CIP= Ciprofloxacin, GN= Gentamycin, TE= Tetracycline, NT- Not tested

Table 4: Antimicrobial Activity of Chloroform Leaf Extract of *Carissa edulis*

Organism	Concentration in mg/ml /zones of diameter of inhibition (mm) as Mean±S.D							
	500	400	300	200	100	Cip	GN	TE
Staphylococcus aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	28	17	NT
Streptococcus pyogenes	17.67±0.33^{a}	15.00±0.00^{b}	11.33±0.33^{c}	8.33±0.33^{d}	0.00±0.00	32	20	NT
Escherichia coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27	14	19
Salmonella typhi	14.33±0.33^{a}	12.33±0.33^{b}	10.00±0.00^{c}	7.00±0.00^{d}	0.00±0.00	29	22	R
Candida albicans	14.00±0.00^{a}	10.33±0.33^{b}	8.33±0.33^{c}	0.00±0.00	0.00±0.00	26	13	NT
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NT	NT	NT

Data are mean of triplicate values (n = 3). Values with different alphabetical superscript are significantly ($p<0.05$) different
R= Resistance, CIP= Ciprofloxacin, GN= Gentamycin, TE= Tetracycline, NT- Not tested

Table 5: Antimicrobial Activity of Aqueous Leaf Extract of *Carissa edulis*

Organisms	Concentration in mg/ml /zones of diameter of inhibition (mm) as Mean±S.D							
	500	400	300	200	100	Cip	GN	TE
Staphylococcus aureus	10.33±0.33^{a}	7.67±0.33^{b}	0.00±0.000	.00±0.000.00±0.00	28	17	NT	
Streptococcus pyogenes	19.67±0.33^{a}	16.67±0.33^{b}	13.33±0.33^{c}	10.00±0.00^{d}	7.33±0.33^{e}	32	20	NT
Escherichia coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27	14	19
Salmonella typhi	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	29	22	R
Candida albicans	15.67±0.33^{a}	11.67±0.33^{b}	9.00±0.00^{c}	7.00±0.00^{d}	0.00±0.00	26	13	NT
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NT	NT	NT

Data are mean of triplicate values (n = 3).Values with different alphabetical superscript are significantly ($p<0.05$) different
R= Resistance, CIP= Ciprofloxacin, GN= Gentamycin, TE= Tetracycline, NT- Not tested

Table 6: Minimum Inhibitory Concentration (MIC) of n-butanol Leaf Extract of *Carissa edulis*

Test Organism	Extract Concentration (mg/ml)				
	6.25	12.50	25.00	50.00	100.00
Streptococcus pyogenes	+	+	β	-	-
Candida albicans	+	+	β	-	-

Positive (+) means turbidity or cloudy
Beta (β) MIC (-) = No growth
MIC for *Streptococcus pyogenes* = 25.00 mg / ml
MIC for *Candida albicans* = 25.00 mg / ml

Table 7: Minimum Bactericidal Concentration (MBC) of n-butanol Leaf Extract of *Carissa edulis*

Test Organism	Extract Concentration (mg/ml)				
	6.25	12.50	25.00	50.00	100.00
Streptococcus pyogenes	+	+	β	-	-
Candida albicans	+	+	+	β	-

Beta (β) = MBC (-) = No growth
MBC for *Streptococcus pyogenes* = 25.00 mg/ml
MBC for *Candida albicans* = 50.00 mg/ml

DISCUSSION

Carissa edulis exhibited considerable amount of inhibition against almost all the test organisms except for n-hexane fraction which had no activity. It shows that the crude methanol leaf extract at the concentration of 500 mg/ml exhibited greater inhibition on *Staphylococcus aureus* and *Candida albicans*, while *Streptococcus pyogenes* is resistant, n-butanol extract at (500 mg/ml) has the highest activity against *Streptococcus pyogenes* and *Candida albicans*, while *Staphylococcus aureus, Salmonella typhi* and *Aspergillus niger* are resistant to n-butanol portion also chloroform extract at (500 mg/ml) it was observed having the activity against *Streptococcus pyogene* and *Salmonella typhi,* while *Staphylococcus aureus, Escherichia coli* and A*spergillus niger* where resistant.

It was further observed that the water extract at exhibited greater activity against *Streptococcus pyogenes* and *candida albican*s, while *Escherichia coli, Salmonella typhi* and *Aspergillus niger* are resistant to water extract. These organisms used for this study have been implicated in food poisoning and water that result in diarrhoea. *Staphylococcus aureus* which is widely distributed in the environment produces enterotoxin and exerts adverse effect on the intestinal mucosa. This reaction alters the absorption and secretion of fluid and electrolytes in the intestinal tract resulting in diarrhea [17]. The ability of the extract to inhibit bacterial cell wall *invitro* may be due the presence of active principles such as flavonoids, terpenoids, saponins, cardiac glycosides and tannins in the extract.

The MIC and MBC concentration assay of the N-butanol fraction for the microorganism tested shows that *Streptococcus pyogene* and *Candida albicans* were the most sensitive showing susceptibility at lower concentration of 6.25 mg/ml and also at 12.5 mg/ml.

CONCLUSION

On the basis of the results from these investigations, it could be concluded that, the methanol leaf extract of *Carissa edulis* was able to exhibit antibacterial property inhibit the growth of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi* and *Candida albicansin vitro* and these are pathogenic bacteria implicated in causing several deadly diseases and ill-health such as diarrhoea, candidiosis, typhoid fever etc. Thus this research work could serve as a lead-way for the development of novel drugs for the treatment and management of such diseases.

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