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Preliminary phytochemical and antimicrobial studies of the leaves of *Varissa edulis* VAHL

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ABSTRACT

The plant *Carissa edulis* is used in traditional medicinal practice in the treatment of chest, venereal infections and other ailments. The studies were carried out to confirm these claims. The preliminary phytochemical screening of the leaves revealed the presence of carhohydrates, tannins, flavonoids, cardiac glycosides, cyanogenic glycosides, saponins, terpenes and steroids. Anthraquinones, balsams, resins and alkaloids were absent. The antimicrobial studies of the water ad methanolic extracts were carried out on standard organisms, *Staphylococcus aureus,* (ATCC13709), *Streptococcus pyogenes,* (NCTC 8198), *Escherichia coli,* (NCTC 10418), *Pseudomonas aeruginosa,* (NCTC 6570), *Candida albicans* (NCTC 3151A) *and clinical isolates (P, aeruginosa. P. putrefaciens),* using cup-plate method on solid media. 10, 15 and 25% w/v of the extract used showed inhibitory activity on the organisms tested. Zone of inhibition ranged between 11.0-22.5mm. Therefore, this result confirms the use of the leaves in traditional medicinal treatment of venereal and chest infections. The presence of tannins or saponins might be responsible for these activities.

Key words: *Carissa edulis* leaves, phytochemical, antimicrobial studies
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INTRODUCTION

Carissa edulis Vahl belongs to the family Apocynacea and is spiny shrub up to 5m high. It is widely distributed throughout tropical Africa extending southwards to Zambia and Zimbabwe. It is also found in Madagascar, Arabia, India and Indochina [1,2,3]. In Nigeria, it is commomly known by the Hausas of Northern Nigeria as 'cizaki' [4]. In Malawi it is known as 'Mpambala' and 'mkokolo' [5].

Ethnomedicinal uses of the plant include the following: the leaves, stem and root barks are used for the treatment of sickle cell anaemia [6] oedema, toothache, also as abortifacient and as purgative [3,5] The root is used for chest complaints, cough, gastric ulcer and in expelling worms especially *Teania* [1,7]. The leaves are used in the treatment of hernia or cyst and the bark is used as aphrodisiac [8].

Phytochemically, the root was found to contain sesquiterpenes [5,9], phenolic compounds [10,11] and cardiac glycosides [1].

Economically, the root and fruits are edible. The root is used to impart an agreeable flavour to food and drinks. The plant is used as a source of dye [3,12].

This paper aims at determining the chemical constituents and antimicrobial activity of the leaves extract of C. *edulis.*

MATERIALS AND METHODS

Plant materials

The leaves of *C. edulis* were collected in July, 1994 from Jama'a village, a place between Aviation compounds and Ahmadu Bello University (A.B.U) Samaru, Zaria. The plant was identified on the field using descriptions given in the literature [2,3]. The plant was confirmed and authenticated at the Herbarium, Biological Science, A.B.U., Zaria. The voucher specimen number is 700132.

Preparation of plant materials and extraction.

The fresh leaves of *C. edulis* were dried under shade, powdered and sieved using sieve of 20-mesh size. The powdered leaves were extracted with distilled water and methanol using both cold and hot extraction methods. 50g each was placed in 250ml distilled water or methanol and macerated for 24hrs, initially shaken on electric shaker for 6 hours. The extract was filtered and 1yophilised. The hot water extract was prepared by boiling 50g in 250ml distilled water for 30minutes, filtered and lyophilized. The dried extracts were weighed separately and stored in airtight bottles until required for use. Soxhlet extraction method was used for methanol (the hot extraction).

Phytochemical screening

Various phytochemical tests were carried out on the powdered leaves of *C. edulis* to detect the presence or absences of carbohydrates, tannins, glycosides, terpenes, steroids, balsams and resins (Table 1) [13,14,15].

Antimicrebial Studies

Clinical isolate

Pseudomonas aeruginosa and *P. putrefaciens* were used. These were obtained from urine samples from patients of Ahmadu Bello University Teaching Hospital, Zaria.

Standard culture

Standard strains used were *Staphylococcus aureus*, NCTC 6571, *Streptococcus pyogenes* NCTC 8198, *Bacillus subtilis* NTCT 8236, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 6750 and *Candida albicans*. 3151A. The organisms were obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, ABU, Zaria.

Preparation of media/inoculum

The nutrient media used were nutrient broth (Oxoid Ltd) and nutrient agar (Biotec Ltd), prepared according to manufacturer's instruction. Each culture prepared in nutrient broth contained 10^6 cells/ml.

Preparation of the crude drug

Three concentrations (10%w/v, 15%w/v and 25%w/v) of water and methanol extracts were prepared in distilled water. Control showed that no viable organism was detected when sample of plant extract were placed on nutrient agar plate that was free of antimicrobial agent.

Susceptibility test

The sensitivity of all the organisms to the water and methanol extracts were tested using cup-plate method on solid media [16]. All prepared nutrient agar plates containing the various concentrations of the water and methanol extracts were incubated at 37°C for 24 hours for bacteria and 25°C for 5-7 day for fungi/yeast organisms inhibition were then recorded in millimeters.

RESULTS

Phytochemical Screening

The various chemical tests revealed the presence of carbohydrates, tannins, cyanogenic glycosides, flavonoids, saponins, and steroids. Anthraquinones, alkaloids, resins and balsams were absent (Table 1) [17].

Table 1: Phytochemical screening of the leaves of *Carissa edulis*

Constituents	Results
Carbohydrate:	
Monosaccharides	+
Free reducing sugars	+
Combined reducing sugars	+
Ketoses	+
Pentoses	+
Phenolic compound	+
Tannins	
Hydrolysable tannins	+
Condensed tannins	+
Psuedotannins	+
Chlorogenic acid	+
Glycosides	
Flavonoids	+
Flavonoid aglycone	+
Anthraquinones	-
Saponins	+
Cardiac glycosides	+
Digtoxose	+
Cardenolide	+
Cardenolide aglycone	+
Terpenes/sterols	
Terpenes	+
Sterols	+
resins and Balsams:	
Resins	-
Balsams	-
Alkaloids	-

Key: (-) = Absent; (+) = Present

Antimicrobial Activity

The water and methanol extract showed inhibitory activity against the organisms tested *(Staph aureus, Strept pyogenes, B. subtilis, E. coli, P. aeruginosa* and *C. albicans).* The activity was found to be dose dependent. The most susceptible organism was *E. coli* and the least susceptible was *Strept pyogenes,*

Table 2: Susceptibility results to the standard organisms against the leaves extracts

A) Types of Extracts	Mean zone of inhibition (mm)					
	S. aureus	*S. pyogenes*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
10%w/v						
Cold water	11.0	11.5	-	12.0	-	-
Hot water	12.5	-	-	-	-	-
Cold methanol	-	-	-	-	-	-
Hot methanol	-	-	-	-	-	11.5
15%W/V						
Cold water	17.75	14.0	13.25	16.5	13.5	11.0
Hot water	12.0	14.5	12.5	13.0	12.0	-
Cold methanol	11.75	13.25	11.5	11.5	11.5	-
Hot methanol	13.5	13.75	13.0	12.75	11.0	12.5
25%w/v						
Cold water	20.5	14.0	18.5	26.0	17.0	15.0
Hot water	17.0	12.0	25.5	25.0	14.0	15.5
Cold methanol	14.5	11.0	22.5	17.0	13.5	15.0
Hot methanol	16.5	14.0	25.25	18.0	13.25	16.25
B) Standard						
Ampicillin 10µg	18.0	10.0	-	16.0	16.0	-

Table 3: Susceptibility results of the clinical isolates against 25%(w/v) extracts of the Leaf

Types of Extracts	Mean zone of inhibition (mm)				
	P. aeruginosa	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Cold water	16.0	16.0	22.5	22.5	-
Hot water	15.0	12.0	19.0	16.0	-
Cold methanol	15.0	12.0	17.0	15.0	12.0
Hot methanol	13.0	14.0	17.5	17.0	14.0

shown by high and low zones of inhibition respectively.

The cold water extract, and the hot methanol extract were more effective than hot water and cold methanol extract, as all the organism were sensitive at 15%w/v
and 25%w/v concentrations (Table 2 and 3) but the cold water extract higher zones of inhibition. Even at 10% w/v concentration, some organisms were sensitive to the cold water and hot methanol extracts.

DISCUSSION

The results have shown that the leaves of *C. edulis* to possess a wide range of antimicrobial activity against both Gram positive (*Staph. aureus, Srept. Pyogenes*, *B. subtilis*) or Gram negative (*E. colis Ps. Arruginosa*) and yeast (*C. albicans*). Also the leaves have wider spetrum of activity than that of Ampicillin which was not effective against *B. subtilis and C. albicans*. (Cold water seems to be a better solvent for extracting the active constituents responsible for the inhibitory activity of the leaves of *C. edulis* since it gave higher zones of inhibition and the organisms were more sensitive to the extracts obtained using cold water the cold water used by the herbalist is justified. Tannins and saponnins are reported to have antimicrobial activity [14], these chemical constituents might be responsible for the antimicrobial activity of the leaves of *C. edulis*. The organisms tested are known to cause chest or venereal infections and other ailments. Therefore the results justify the use of the leaves in ethnomedicine.

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