PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTIES OF *Carissa edulis* EXTRACTS OBTAINED FROM KAIMOSI FOREST, VIHIGA COUNTY (KENYA).

ABSTRACT

Medicinal plants still play an important role in human and animal healthcare. About 60% of the world's population and 80% of Africa's population depend on herbal medicine for their primary healthcare. In Vihiga county Kenya, C. *edulis* has been reported to manage several human disease conditions such as epilepsy, headache, toothache, cough, chest complaints, sickle cell anemia, gonorrhea, syphilis and rabies among others. Its therapeutic efficacy is attributed to numerous phytochemicals present in its extracts. In this study, a phytochemical analysis was carried out in the roots, leaves and bark extracts, antimicrobial effects on *Escherichia coli* and *staphylococcus aureus* laboratory cultures determined. The extracts were modified by grinding and ethanol used as solvent in the extraction. Different concentrations of 25%, 50%, 75% and 100% of plant extracts were used in the analysis and sterile distilled water used as the control. Data on zone growth inhibition was subjected to analysis of variance and the means were separated and compared using least significant differences at $p \leq 0.05$ by use of SAS statistical package. The results showed the presence of an array of phytochemicals in the leaves, bark and roots of the plant. The bark for example tested positive for tannins, saponins, flavanoids, terpenoids, glycosides and sterols while the leaves and roots tested negative for saponins and flavanoids respectively. The leaves exhibited the greatest inhibition against *Escherichia coli* with a mean diameter of 7.57mm and all the three parts exhibited a significant difference. The different concentrations also showed that inhibition increased with increase in concentration. The mean inhibition diameter at 100% was 8.96 while at 25% it was 7.21.

Keywords : Medicinal plants, phytochemicals. Antimicrobial, cultures, inhibition

1.0 INTRODUCTION.

1.1 Background

Carissa edulis is used as a medicinal plant in Kenya, where it is usually found on termite

Plate 1. *Carissa edulis* plant growing in Kaimosi forest

mounds in wooded grassland, especially in low-lying areas associated with *Grewia similis* and in thickets in woodlands, forest edges and secondary scrub with dry conditions. It is a tree found in deciduous forest and coastal thickets, extending across drier parts of tropical

Africa (Plate 1) and across Asia to Indo-China. It has been reported as a parasite of the henna plant in northern Nigeria [1]. It is found in the Arabian sub continent and its spread is reported throughout the tropics in Africa; E. Africa, South Africa in the Transvaal, Botswana and northeastern Namibia where there is warm bushes and scrub.
It has been used among different Kenyan communities for centuries to manage disease conditions such as epilepsy, headache, toothache, cough, chest complaints, fever, sickle cell anemia, sexually transmitted diseases such as syphilis and rabies [2]. The plant belongs to the family Apocynaceae.

Medicinal plants are the most common sources of remedies in traditional medicine in Africa; it is the most ancient way of curing diseases. These plants are also the sources of many conventional drugs [3] Despite advances in health care delivery, medicinal plants still play an important role in human and animal healthcare and about 60% of the world's population and 80% of Africa's population depend on herbal medicine for their primary healthcare [4] Many of these plants have been screened for their phytochemical constituents and antimicrobial properties with the view to authenticate their folkloric uses and safety used as a medicinal [2]

Phytochemicals are chemical compounds that occur naturally in plants. Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. This term is generally used to refer to those chemicals that may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients [5].

Although various studies have been carried out in India, Nigeria and other parts of the world, fewer studies have been done in Kenya to shed light into this area of the unknown thus creating a great gap of knowledge and interest because chemical constituents and biological activities of a plant have been found to be affected by seasonal variation, state of maturity and location among other factors [6]. Documented information on *Carissa edulis* in the communities of Kenya is limited and thus very little knowledge on medicinal efficacy of its plant parts [7]. Thus this study was initiated to determine the antimicrobial effects of plant parts on two selected human pathogenic microorganisms and determine phytochemical constituents of *Carissa edulis* .

2.0 MATERIALS AND METHODS

2.1 Study area.

The experiment was done in the Biology laboratory in Kaimosi Friends University, Kaimosi Kenya. It is located in western Kenya adjacent to the Kapsabet- Chavakali road at geographical coordinates are 0.1256° N, 34.8445° E

2.2 Sample collection

Complete plant samples of *Carissa edulis* were obtained from Kaimosi forest extending into the Kaimosi Friends University compound, then positively identified before the leaves, stem and back were washed.

2.3 Sample extraction

Samples were dried under room temperature in the laboratory and grinded using a blender to a fine powder to obtain 100g each of the leaves, bark and root. The powder were then stored in separate containers and labelled.100g of each powdered extracts samples were weighed and dissolved in 100ml of ethanol in sterile conical flasks. The samples were then left to stand for 48 hours. After 48 hours the mixture was filtered using a filter paper. The filtrate

was then heated to vapor using a mantle heater to concentrate the extracts. The concentrate was then collected in sterile flasks and used for analysis.

2.4 Qualitative phytochemical analysis

The extract phyto-constituents analysis for the identification of bioactive chemical constituents was done as earlier described by [8], [9], [10] [11].

2.4.1 Test for alkaloids

Two grams of the plant material were extracted by warming it for 2 minutes with 20ml of 1% H2SO4 acid in a 50ml conical flask on a water bath, with intermittent shaking. It was then centrifuged and the supernatant was pipetted off into a small conical flask. One drop of Meyer's reagent was added to 0.1ml supernatant in a semi-micro tube and a cream precipitate formed indicating the presence of alkaloids [10].

2.4.2 Test for flavonoids

Five millilitres of dilute ammonia solution was added to a portion of aqueous filtrate of the extract followed by addition of concentrated H2SO4. A yellow coloration was formed indicating the presence of flavonoids [10].

2.4.3 Test for saponins

Two grams of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. Ten millilitres of the filtrate was mixed with 5 ml of distilled water and shaken vigorously and a stable persistent froth formed. The froth was mixed with 3 drops of olive oil and shaken vigorously and an emulsion formed indicating the presence of saponins [8] [12]

2.4.4 Test for steroids.

One milliliter of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by side of test tube. The upper layer did not turned red and the sulphuric acid layer did not show a yellow with green fluorescence. This indicated the absence of steroids [8] [12]

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2.4.5 Test for tannins

Tannin presence was determined by the Folin-Denis colorimetric method described by Harborne, (1998). One gram of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered through Whatman No. 42 filter paper. A few drops of 0.1% ferric chloride were added. A blue-black coloration formed indicated the presence of tannins [11] [12]

2.4.6 Test for phenols

One milliliter of extract was dissolved in 2ml of distilled water and a few drops of 2% FeCl3 were added, appearance of a bluish green coloration indicated the presence of phenols [11] [12]

2.4.7 Test for Cardiac glycosides

Five millilitres of each plant extract was treated with 2ml of glacial acetic acid containing a drop of FeCl3 solution. This was then underplayed with 1ml conc. H2SO4. A brown ring at the interface indicated a deoxy-sugar characteristic of cardenolides thereby confirming the presence of cardiac glycosides [11] [12]

2.4.8 Test for terpenoids

Five millilitres of each plant extract was mixed with 2 ml of chloroform, and concentrated sulphuric acid was carefully added by side to form a layer. A reddish brown coloration that formed at the interface indicated the presence of terpenoids [9] [12]

2.5 Media preparation

The nutrient agar was reconstituted using distilled water according to the manufacturer's instructions, sterilized by autoclaving at 121 degrees for 15 minutes. It was then dispensed aseptically into petri dishes and left to solidify [8].

2.5.1 Preparation of discs

Filter papers disc of 6mm diameter were made by punching the paper. The sterile extracts of ethanol were serially diluted using ethanol as a diluent. The discs were then dipped into the dilutions and placed on a glass plate to allow the solvents to evaporate and the test chemicals to be adsorbed in the discs. Discs in water were used as control and the other concentrations were 25%, 50%, 75% and 100% of the root, leaves and barks.

2.6 Determination of antimicrobial activity

2.6.1 Test organisms

The bacterial strains chosen based on their availability and pathogenicity were *Escherichia coli* and *staphylococcus aureus.* There organisms were cultured in the Kaimosi Friends University biology laboratory [8].

2.6.2 Disc diffusion method

The disc diffusion method was used to evaluate antimicrobial activities [12]. To determine susceptibility, four concentrations were used (25%, 50%, 75% and 100%). Ethanol was used for organic extraction. The plates were incubated at 37 degrees for 24 hours. Inhibition zones were recorded as the diameter of growth zones [8].

2.7 Data Analysis

Data on zone growth inhibition was subjected to analysis of variance and the means were separated and compared using least significant differences at $p \leq 0.05$ by use of SAS statistical package.

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical screening

Phytochemical screening of leaves extracts tested positive for tannins, flavonoids, terpenoids and cardiac glycosides and negative for saponins. The roots tested positive for tannins, saponins, sterols and cardiac glycosides and negative for flavanoids and the barks tested positive for all of the phytochemicals. This is as shown in table 1 below.

Table 1*:* Phytochemical screening of secondary metabolites

Tests	leaves	Bark	Roots

Tannins	+	+	+
Saponins	_	+	+
Flavanoids	+	+	_
Terpenoids	+	+	+
Glycosides	+	+	+
Steroids	+	+	+

Absent (-); present (+)

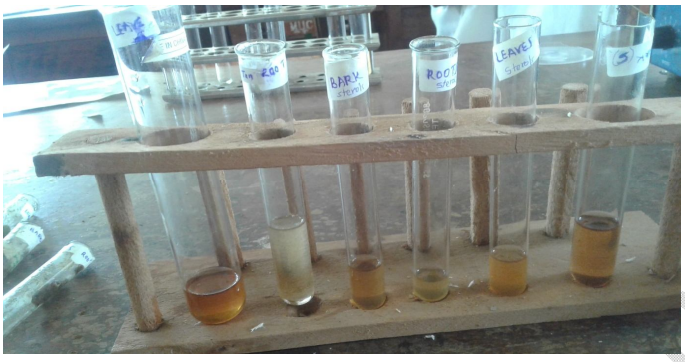

Plate 2: Showing phytochemical analysis of *Carissa edulis* extracts

3.2 Antimicrobial Analyses

The antimicrobial activities varied among the three ethanol extracts tested, among the extract concentrations, the isolates used and within the two days that the experiment was carried out. The ethanol extract had a greater inhibition against *Escherichia coli* than *staphylococcus aureus*.

Table 2: Antimicrobial activity of *Carissa edulis* root, leave and bark ethanol extracts, the different concentrations used on the two microbes within two days.

Variables	Growth inhibition zone diameter (mm)
Microbe	
Staphylococcus aureus	6.40a
Escherichia coli	6.00b
LSD	0.19
Plant Extract	
Leaves	7.57a
Roots	5.72c
Bark	6.13b
LSD	0.23
Concentration	
0	0.00e
25	7.21d
50	7.74c
75	8.44b
100	8.96a
LSD	0.28
Days	
1	6.23b
2	6.71a
LSD	0.18

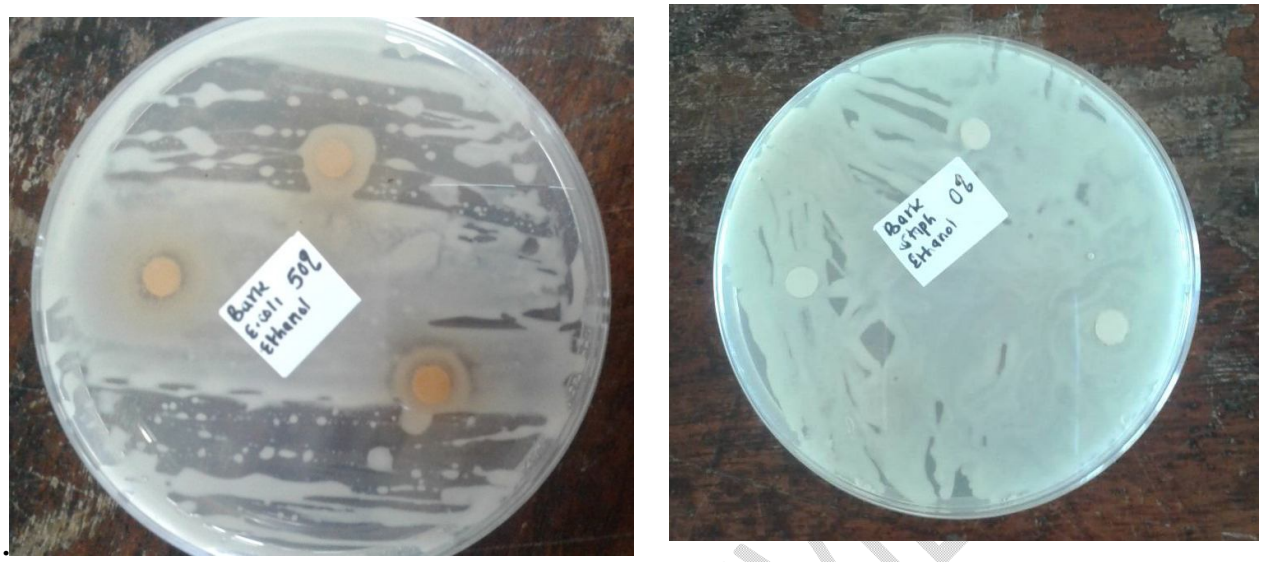

Plate 3: Showing inhibition zones of ethanol bark extract on *S .aureus* at 0% and *E.coli* at 50% respectively

Phytochemical screening of *C. edulis* showed the presence of some active phytochemicals which previous research has reported to produce a definite physiological action on the human body [8]. As can be seen from the obtained results, the roots, barks and leaves contained Tannins and cardiac glycosides. The roots and barks tested positive for saponins, steroids and terpenoids. The leaves and barks tested positive for flavonoids. The leaves also tested positive for terpenoid and sterols. The antibacterial activity of *C. edulis* could be as a result of this phytochemicals present. For instance saponins have been reported to inhibit bacterial colonization, lowering surface tension of extracellular medium or by lysing bacterial membrane [14] tannins act by complexing baterial proteins, interfering with bacterial adhesion, inactivating enzymes and disrupting material membrane [15] Results on antimicrobial activity of the roots, leaves and barks of *C. edulis* at different concentrations showed that inhibition increased with increase in extract concentration. For example, the leave extracts on both *Escherichia coli* and *staphylococcus aureus* showed an increased inhibition from 25% to 100% concentrations. The means of the concentrations at 100% was 8.96 while at 25% it was 7.21 hence there was a significant difference in the concentrations used. Hence, the more concentrated an extract the greater the activity against an organism (table 3). The leaf extracts however showed the greatest inhibition zones against the two test microorganisms with its mean being 7.57. *Escherichia coli*, however, was the most inhibited and it was significantly different from *staphylococcus aureus*. The inhibition zones increased significantly from the first day to the second day, and showed different changes in millimeters as shown in table 3 below.

The phytochemicals present in the roots, barks and leaves of *Carissa edulis* were compatible with those of [16]**.** On the bark and roots of *Carissa edulis* who found phytochemicals such as terpenoids and tannins to be present. The susceptibility of *Escherichia coli* and *staphylococcus aureus* to the extracts of *Carissa edulis* is an indication that the plant is suitable for use as an antibacterial compound. This also explains the various

Table 3: Showing plant parts, solvent and microbe interactions at each concentration and their inhibition zones.

Plant extract	Solvent	isolate	Concentration (%)	Inhibition Zones (mm)
Leaves	Ethanol	*E. coli*	0	0.00
			25	8.00± 0.29
			50	8.17± 0.33
			75	10.33±0.44
			100	9.67±0.17
		Staphylococcus aureus	0	0.00
			25	6.83±0.93
			50	8.67±0.17
			75	10.00±0.50
			100	10.50±0.58
Barks		*E. coli*	0	0.00
			25	6.58±0.20
			50	7.10±0.24
			75	7.42±0.20
			100	8.17±0.17
Roots		*E. coli*	0	0.00
			25	6.83±0.44
			50	6.83±0.33
			75	7.17±0.17
			100	8.50±0.29
		Staphylococcus aureus	0	0.00
			25	6.67±0.17
			50	6.83±0.44
			75	6.67±0.33
			100	6.67±0.17

traditional medicinal uses of the plant in treating diseases such as gonorrhea, syphilis, rabies, epilepsy, chronic joint pain and as a diuretic [17]. The aqueous extracts of different parts of the plant have also been reported to show almost equivalent and at times better analgesic activity in comparison with aspirin [16]

4.0 CONCLUSIONS AND RECOMMENDATIONS

The bark of *Carissa edulis* tested positive for all the phytochemicals tested that is the tannins, saponins, flavonoids, terpenoids, sterols and cardiac glycosides which is an indication of greater medicinal value while the roots and the leaves varied in the composition of the phytochemicals. Some tested positive while others negative. It is now clear that the roots, barks and leaves of *C. edulis* have antimicrobial activities against *Escherichia coli* and *staphylococcus aureus* at higher concentrations. Ethanol showed a greater inhibition zone against *Escherichia coli* than *staphylococcus aureus*.

4.1 RECOMMENDATIONS

1. The barks of *C. edulis* seamed to contain more phytochemicals than the roots and the leaves and hence there is need for trials to be initiated by pharmaceutical industries in the manufacture of drugs developed from its extracted products.
2. *C. edulis* can be used to treat various infections that are caused by *S. aureus* and *E. coli* for example treatment of diarrhea because it has shown potency in the inhibition of E. coli growth in the laboratory.
3. Ethanol provides an effective solvent to water during the extraction of the various plant chemicals though there need to experiment with other alcoholic solvents.

4.2 SUGGESTIONS FOR FUTURE RESERCH

1. A further research is required to know the exact phytochemical compounds that have an inhibitory effect on the test microorganisms.
2. Further extractions using other solvents such as methanol should be carried out so as to be able to establish the best solvent to be used while working with *C. edulis*.
3. The toxicity of ethanol as an extract in the extraction process should be carried out in vivo and in vitro to be able to determine the safest dosage or concentrations to be used for therapeutic purposes in humans.

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