International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 1036-1045**
http://www.ijcmas.com

Original Research Article

Antimicrobial effects of C*ombretum paniculatum* (bush willow) on some pathogenic organisms

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A B S T R A C T

The crude extract of Combretum *paniculatum* was investigated with the aim of determining the antimicrobial activity, the best solvent to be used for extraction and the organisms that are most susceptible to the crude extract of C*ombretum paniculatum.* Acetenoe, ethanol and aqueous (cold) were used as solvents for extraction. Although acetone extract has the lowest yield (0.6g) after extraction as compared to ethanol and aqueous (cold) extract (1.7g) respectively, it was still regarded as the best solvent for extraction. The susceptible of three microbial isolates (E*scherichia coli, Staphylococcus aureus and Candida albicans)* to the crude extracts of Combretum *paniculatum* was determined by using the disc diffusion method. The crude extracts of C*ombretum paniculatum* were prepared into antimicrobial disc standard. Gentamycin was used as positive control while Dimethylsulphoxide (DMSO) was used as a negative control. The antimicrobial activity of the crude extract of C*ombretum paniculatum* was susceptible to the microbial isolates. The antimicrobial activity showed that microbial growth was inhibited by acetone extract, ethanol extract, ethanol extract and aqueous (cold) extract with acetone extract having the highest zone of inhibition (9.0,7.0 and 4.0mm) and the aqueous (cold) extract having the lowest zone of inhibition (6.0, 4.0 and 3.0mm) on *Escherichia coli, staphylococcus aureus and Candida albicans respectively.* The acetone extract gave a better Minimum inhibitory concentration (MIC) result (12.5,12.5 and 25mg/ml) than ethanol and aqueous (cold) extract (12.5,25 and 50mg/ml) and (25,25 and 50mg/ml) on *Escherichia coli, Staphylococcus aureus, and Candida albicans respectively.* The study revealed that the acetone, ethanol and aqueous (cold) extract of C*ombretum paniculatum* was susceptible to the three pathogens and also lend more weight to general acceptability of these crude extract for culinary and therapeutic purposes.

K e y w o r d s

Antimicrobial; C*ombretum paniculatum* (Bush Willow); Minimum Inhibitory Concentration (MIC); Some Pathogenic Organisms and Zones of Inhibition

Introduction

The use of plants for medicinal purposes is an important part of the culture and tradition in Africa. Thus, up to 80% of the population depends directly on the

traditional medicine for the primary health care (Anita, 2001).

With the increasing incidence of diseases caused by bacterial and other pathogens as well as the development of drug resistance, there is an urgent need to search for alternatives from plants and other sources to combat these pathogens. The ease of national and international travels means that resistant organisms can be transported easily making it a global problem. Despite all efforts by health bodies the treath of bacterial and other infectious diseases persists, making the search for more effective and efficient drugs ever more pressing (Morman, 1996). Considerable attention has been given to screening of plant extracts for possible antimicrobial activity. Such endeavors have been undertaken with the aim of isolating bioactive compounds as an alternative source to chemical synthesis (Robbers *et al;* 1996). In Brazil, around 80,000 species of higher plants were described which offer enormous prospects for discovery of new compounds with therapeutic properties (Lewis, 2001). Though most of the clinically used antibiotics are produced by soil micro-organisms or fungi, higher plants have also been a source of antibiotics.

Plants based antimicrobials have enormous therapeutic potentials. They are effective in the treatment of infectious diseases. C*ombretum paniculatum* (combretaceae), has been used widely in ethnomedicine in the treatment of chronic diarrhea and dysentery, flatulence, vomiting, colic, and enlarge spleen and liver (Cheng *et al;* 2003). C*ombretum paniculatum* has been noticed to inhibits the growth of enteric bacteria other good example are *Hydratics Canadensis,* not only does it has antimicrobial activity but also increases blood supply to the spleen promoting optimal activity of the spleen to mediating compounds (Murray *et al;* 1995). *Xytopia aethiopica* has an attractive aroma and has been applied in ethnomedicine in the treatment of cough, bronchitis, dysentery and female sterilization (Iwu, 1986). It is believed to aid uterine ecotraction and applied as and abortificient (Smith et al; 1996).

Many plants extracts have shown to acquire antibacterial properties active against many micro-organisms inside the body (in vivo) or outside the body (in vitro). For example, *Combretum erythrophyllum* (combretaccae) have been found to be active against a wide variety of microorganisms such as *Staphylooccus aureus, Enterococcus fascalis, Escherichia coli,* and *Pseudomonas aeruginosa (*Martini *et al;* 2004). The root of *Nauclea latifolia smith* Rubiacea) has antimicrobial activities against gram 0positive and gram negative bacterial and antifungal activity (Iwu, 1993). It is most effective against *Corynebacterium diphtheria, streptobacillus spp, streptococcus spp.* (Deeni and Hussan 1991).

An avenue for research was suggested and carried out to screen medicinal plants for side effects, toxicity and most especially in the form in which they exhibit certain antimicrobial activities. Many plants were found in the form in which they exhibit certain antimicrobial activities. Many plants were found to contain substances such as alkaloid, glycosides, tannins, steroids, phenol and hosts of other that are responsible for the medicinal value (Baker and Breach, 1980).

In 1997, it was reported that out of the three hundred thousand (300,000) different plant species identified to be medicinal,

only about five thousand (5,000) have been studied so far for their possible medicinal usefulness.

This therefore, necessitate for continuous search for new antimicrobial agents especially from the root, stem and leaves of plants since the synthetic drugs have retarded in many respects. Research on plants with antimicrobial activities should indeed be a continuous one so as to reveal all hidden aspects of traditional medicine (Hammer *et al;* 1990). This work therefore, examines the antimicrobial activities of *Combretum paniculatum* on some human pathogens such as *Escherichia coli, Staphyloccus aureus* and *Cadida albicans.* The main aim of this study was determine the antimicrobial activity of *Combretum paniculatum.* And to determine the best solvent used for extraction. Also the determination of organisms that is most sensitive to the plant extract.

Materials and Methods

Collection and identification of plants materials

The leaves of *Combretum paniculatum* was collected from Olokoro in Umuahia South Governmen area of Abia state in eastern part of Nigeria. A herbarian in the department of forestry and environmental management in Michael Okpara university of Agriculture, Umudike, Mr. IBE KALU identified the plant taxonomically. The plant was then dried at room temperature for two weeks.

It was then grinded into powder using disinfected hand manual grinder at biological science laboratory before transporting it to microbiology laboratory for extraction and analysis.

Acetone preparation

Twenty (20.0g) of the grounded plant material was weighed using and electronic weighing balance (OHAUS) and the weighed sample were soaked in a clean 250ml conical flask containing 150ml of acetone the mixture were vigorously stirred with a stirrer and left for 72 hrs the mixture were filtered using Whatman Number 1 filter paper into a clean beaker, the filtrate was transferred into the sample holder of the rotary vacuum evaporator when the acetone solvent was evaporated at it's boiling temperature the standard extract s obtained were weighed and stored in the refrigerator at 4^0C until require for use.

Ethanol extract preparation

Twenty (20.0g) of the grounded plant material was weighed using and electronic weighing balance (OHAUS) and the weighed sample were soaked in a clean 250ml conical flasks containing 150mls of ethanol.

The mixture were vigorously stirred with a stirrer and left for 71 hours, the mixture were filtered using Whatman number filter paper into a clean beaker, the filtrate were transferred into the sample holder of the rotary vacuum evaporator when the ethanol solvent was evaporated out at boiling temperature the standard extract obtain were weighed and stored in the refrigerator at 4^0C until required for use

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Aqueous (cold) extract preparation

Twenty 20.0g of the grounded plant material was weighed using an electronic weighing balance (OHAUS) and the weighed sample were soaked in 140ml of water. The mixture were vigorously stirred

with a stirrer and left for 71 hours the mixture was then filtered using Whatman number 1filter paper, into a clean beaker, the filtrate were transferred into the sample holder of rotary vacuum evaporator when the cold water (Aqueous solution) was evaporated out at it's boiling temperature the standard extract obtain were weighed and stored in the refrigerator at 4^0C until required for use. The filtrate had the following colr after filtration.

Acetone extract was dark brown.
Ethanol extract was brown.
Aqueous extract was dark green.
After evaporation with the extractor, the extract were recovered and weighed.
Acetone extracts 1.6g.
Ethanol extracts 1.7g.
Aqueous extract 2.7g.

Methods of laboratory analysis

Preparation of media

The media used were Mueller Hinton Agar, Nutrient Agar and Sabouroud Dextrose Agar; the required amount was measured and prepared according to manufacturer's instruction and poured into a conical flask Stoppard with no-absorbent cotton-wool and wrapped with aluminum fool, sterilized by autoclaving at 121^0C for 15 minutes.

Test for purity and sterilization of materials.

The dried extract was exposed to ultraviolet rays for 24hours and checked for sterility by streaking on a freshly prepared sterile nutrient agar which was incubated for 24 hours at 37^0C (Baker and Palllister 1998). The materials used were sterilized. All the materials used were washed with detergent and rinsed with water, they were drain dried, and the glass wares were foiled and carefully packaged into the autoclave for sterilization at 12^0C for 5minutes (Cheesbrough, 2000).

Bacterial species confirmation

Clinical strains of micro-organisms used are *Escherichia coli Staphylococcus aurerus and candida albicans* were obtain from the microbiology laboratory of Federal Medical Centre (FMC) Umuahia , Abia state. *Escherichia coli* was subcultured on nutrient agar (Baker and Pallister, 1998). All the micro-organisms were maintained at 4^0C on their respective slants.

Preparations of plant extract concentration

The aqueous, ethanol and acetone extracts were reconstituted by weighing 0.2g of each extract and was dissolved in 2mls of distilled water and 50% dimethylsulphoxide (DMSO) respectively. Each dilution gave a concentration of 100g/ml.

Disc diffusion assay

The disc diffusion method as reported by barker and Pallister (1998) was adopted by the determination of the antimicrobial activity of plant extracts. Whatman number 1 filter paper was used. The filter paper was cut into circular disc using a perforator giving a diameter of 6mm. The disc was treated by boiling for 30 minutes so as to denature and destroy completely the entire chemical used in its preservation and also to prevent the inactivation of the extract imbedded into the disc. After it was boiled, the disc was transferred into a glass Petri dish and kept in the oven until

it became dry. After drying it was stored in a sterile bottle and autoclaved for 15minutes at 121^0C and 115 atmospheric temperatures it was stored in the refrigerator for use.

Media preparation and antimicrobial activity

Mueller Hinton Agar was prepared by weighing 38 grams of the powered agar into 1000mls of distilled water in a clean conical flask. It was covered with a foil, mixed properly until it became a mixture and was autoclaved at 121^0C for 15minutes. The medium was cooled at 47^0C and 20mls of the molten medium was poured into a sterile glass Petri dish and allowed to solidify the sterility of the medium was tested by incubation for eight (8) hours looking out for contaminants (Baker *et al;* 1975). A sterile wire loop was used to pick a colony of the test organisms and placed into 2mls of peptone water. A sterile swab was dipped into the test tube containing the organism and it was used to seed the organisms on the solidified Mueler Hinton Agar in an inoculating chamber already set aseptically. The prepared disc was carefully transferred into the inoculated culture plates using sterile forceps the placed disc included 100mg/ml Acetone extract, ethanol extract, aqueous extract, and gentamycin 2mg/ml. The plates were incubated for 24 hours at 37^0C.

Control experiment using gentamycin and dimethyl sulphoxide (DMSO)

Gentamycin was used as the positive control in order to compare the diameter of zone of inhibition or clearance from the extracts and already standardized antibiotic (Gentamycin) and it was carried out aseptically (Oyagede *et al;* 1993). This is to ensure the prescription of either antibiotics or plant herbs for antimicrobial activities. Gentanycin (280mg/ml) bottle with solution 80mg/2ml was used by diluting 1ml of gentamycin in 19mls of distilled water that is 1.20 dilution (1ml + 19mls) giving a final concentration of 2mglml. Dimethlsulphoxide (DMSO) was used as a negative control in pregnating the disc in 50% ethanol. Aqueous (cold) extract and gentamycin.

Determination of the minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) is the concentration giving the least inhibitory activity and below which there is no further inhibition. It is therefore .It is therefore regarded as the concentration giving the lowest possible zone of inhibition.

The Minimum Inhibitory Concentration (MIC) of the extracts were determined by incorporating constant volume (0.2ml) of each the dilution of the extract into the punch hole on a pre-seeded appropriate agar medium as described in the antimicrobial susceptibility test section. 0.2g of the extract was dissolved in various ml of peptone water (2ml, 4ml, 8ml, 16ml and 32ml) to obtain 100mg/ml, 50mg/ml, 25mg/ml 6.5mg/ml respectively. 0.2ml of the respective obtained dilution (100mg/ml to 6. 25mg/ml were incorporated into the punch hole on a pre-seeded appropriate agar. After 16-18 hours of incubation the results were taken.

Results and Discussion

Preparation of the plant extract

The sample was dried for 7 days, after which it was grinded to get 152g as shown

in figure 1a and 1c. The colour of the sample was green and smooth in texture.

Twenty grams (20.0g) of the grounded plant material were weighed and soaked into 150ml of each of the solvent respectively (Acetone, Ethanol and Aqueous (cold) solvent in a separate conical flask with rubber corks and left for 72 hours. They were filtered off with sterile filter paper (Whatman number 1 filter paper) into a clean conical flasks and the filtrate was obtained weighed 1.6g. the standard ethanol extracts obtained weighed 1.7g and standard cold water extract obtained weighed 2.7g.

Determination of antimicrobial activity of *Combretum paniculatum*

The antimicrobial activity of *Combretum panicaulatum* extract was assayed in-vitro by agar disc diffusion against three pathogenic micro-organisms. A Gram positive organism, Gram negative organism and fungal. (*Staphylococcus aureus, Escherichia coli* and *Candida albicans)* respectively as shown in plate 1,2 and 3.

The microbial, growth inhibition of Acetone, Ethanol and Aqueous (cold) extracts of the screened plant was summarized in Table 3 and 4. All the extract of *Combbretum paniculatum* had conspicuous zone of inhibition on *Esherichia coli, Staphylocccusaruerus* with little zone of inhibition of *Candida albicans.,* With the acetone extract having the highest zone of inhibition 9.0, 7.0 and 4.0 in diameter respectively and the aqueous (cold) extract having the lowest zone of inhibition 6.0, 4.0 and 3.0 as shown in table 4.

On the contrary, 3mg/ml of gentamycin (positive control shows wide zone of inhibirion on all the test organisms which is comparable to the 100mg/ml concentration of plant extracts. Dimethysulphoxide (DMSO) negative control shows no zone inhibition.

Determination of the minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) result of the plant leaves extract which is the concentration giving the least inhibitory activity and below which there is no further inhibition. The *Combretum panicaulatum* Minimum Inhibitory Concentration (MIC) of acetone on *Echerichia coli, Staphylococcus aureus* and *Candida albicans* was 12.5, 12.5 and 25, respectively as shown in table 5. The test result of ethanol extract of *Combretum* of ethanol extract of *Combretum paniculatum* Minimum Inhibitory Concentration (MIC) of cold water extract on *Esherichia coli, staphylococcus aureus* and *Candida albicans* 12.5, 25 and 50 as shown in table 6. And the *Combretum paniculatum* Minimum Inhibitory Concentration (MIC) of cold water extract on *Escherichia coli, Staphylococcus aureus* and *Candida albicans* was 25, 25 and 50 respectively as shown in table 7.

According to Carr, (1998) it has been reported by several investigators that flowering plants contain antimicrobial substances. The result of the present study agrees with the reports of these investigators.

The result shows that the crude extract of *Combretum paniculatum* inhibited the growth of both gram positive and gram negatives organisms and also had little inhibition on fungi. This is presumed to be due to the active compound present in the leave of this plant.

Table.2 Percentage yield of the crud extract of *Combretum paniculatum*

Plant species	Extract Type	Weight pulverized sample used (g)	Weight of Extract (g)	Percentage yield of Extract (g)
Combretum	Acetone	20.0	1.6	8
Paniculatum	Ethanol	20.0	1.7	8.5
	Aqueous	20.0	2.7	13.5

Table.3 Antimicrobial activity of *Combretum paniculatum*

Bacterial species	Acetone	Ethanol	Aqueous
Escherichia coli	+	+	+
Staphylococcus aureus	+	+	+
Candida albicans	+	+	+

Key: + Inhibition (>3.00mm).

Table.4 Diameter of zones of inhibition of various extract as well as control in millimeter of *combretm panicaualatum*

Bacterial species	Acetone Extract	Ethanol Extract	Aqueous Extract	Gent	DMSO
Escherichia coli	9.0	6.0	6.0	13.0	0.00
Staphylococcus aureus	7.0	5.0	4.0	12.0	0.00
Candida albicans	4.0	4.0	3.0	9.0	0.00

Key: Gent = Gentanycin ; DMSO= Dimethylsulphoxide.

Table.5 Minimum inhibitory concentrations (MIC) of Combretum paniculatum extract *on bacteria isolates*

Bacteria species	(Concentration in mglml)					
	100	50	25	12.5	6.25	MIC
Escheruchu coli	+	+	+	+	-	12.5
Staphylococcus	+	+	+	-	-	25
Candida albicus	+	+	+	-	-	50

Key: + = Inhibition— = No inhibition

Table 6 Minimum inhibitory Concentrati9on (MIC) of Combretum Paniculatum of ethanol extract on bacterial isolates

Bacterial species		(Concentration in mg1m1).				
	100	50	25	12.5	6.25	MIC
Escherichi coli	+	+	+	+	-	12.5
Staphylococcus aureus	+	+	+	-	-	25
Candida albicans	+	+	-	-	-	50

Key:+ = Inhibition; — = No inhibition

Table.7 Minimum Inhibitory Concentration (MIC) of Concentration paniculatum of aqueous (cold) extract on bacterial isolates

Bacterial species		(Concentration in mg1m1).				
	100	50	25	12.5	6.25	MIC
Escherichi coli	+	+	+	—	—	25
Staphylococcus aureus	+	+	+	—	—	25
Candida albicans	+	+	—	—	—	50

Key: + = Inhibition; — = No inhibition

The crude extract of Combretum paniculatum showed conspicuous degree of antibacterial activity. All bacteria agents were susceptible to the crude extract of *Combretum paniculatum*. However, *Escherichia* coli were observed to be most susceptible organism. This conforms to the result of (Cheng et al, 2003). The acetone, ethanol and aqueous (cold) extract of *Combretum paniculatum* were least effective against *Candidas albicans* (fungi) than a bacteria strain but this result was not surprising since the leave of this plant is used traditionally in the treatment of chronic diarrhea, dysentery and vomiting Carr, (998). The result obtained with the crude extract of *Combretum Paniculatumcontnues* the numerous searches for more effective (Calixto, 2000). In most cases the liquour (aqueous or ethanol) of the leaf of *Combretum Paniculatum* is used in cooking or drunk as remedy for dysentery and chronic diarrhea (Oyagede et al, 1993).

The plant extract were more susceptible to Escherichia coli (Gram Negative) followed by *Staphylococcus aureus* (Gram positive) and then Candida albican (Fungi). Alade *et al*., (1993) and Hammer et al., (1999) reported that plant extract shows stronger retardation effects on the fungi test strains than on gram negative bacterial. This therefore, indicates higher drugs of plant origin which are less toxic and available for low socio economic population in the treatment of disease caused by pathogenic organism.

The result presented above shows that the Acetone, Ethanol and Aqueous (cold) extracts of *Combretum paniculatum* leave possess appreciable antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and low activity o n *Candida albican*. One of the test organisms used Staphylococcus *aureus* a gram positive organism has been found in very large number in wound infection and in food poisoning (Gorinsten et al, 2003). Also a few stains of Escherichia coli may be associated with an acute gastroenteritis

antibacterial activities of the extract on gram negative and gram positive organisms. The Minimum Inhibitory Concentration MIC of the active substance in the extract might be reducing if the extract is purified further. The result from this research has high lighted the inhibitory properties of *Combretum paniculatum* on the test organisms, the Minimum inhibitory concentration MIC of each extract of the plant and revealed the best solvent for extraction.

The result obtained with the crude extracts of *Combretum paniculatum* opens perspectives to find more effective drugs

of vegetal origin, which is less toxic and available for low social economic population in the treatment of infections diseases caused by pathogenic microbes. In this study *Combretum paniculatum* produce narrow inhibitory effects on *Escherichia coli Staphylococcus aureus* and with little on Candida albican invitro.

The study also revealed that *Combretum paniculatum* crude extract possessed antimicrobial property. The ability of the crude extract to inhibit the three pathogen (*Escherichia coli Staphylococcus aureua and Candida albican*). Justifies the use of this plant by traditional practioners in Nigeria in curing diseases arising from the effect of these human pathogens. Further research on the antimicrobial activity of plant extract of *Combretum panniculatum* should be carried out on other pathogenic organisms using stem and root of this plant and other forms of extraction should be used and also the best solvent to be used can be conducted further to know the best form of use of this plant extract of *Combretum paniculatum.*

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