The Activities of the Aqueous Crude Extracts of the Leaf, Root and Bark of *Combretum Molle* Against Selected Test Organisms

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Abstract

Combretum molle has been used in many traditional medicines for treatment of microbial infections (diarrhea, dysentery, fever) and several inflammatory conditions (abdominal pain, headache, and toothache). This work was carried out with the aim of determining the phytochemical compounds present in the methanol extracts of the leaves; stem-bark and roots of *C. molle* and their biological activities in some selected microorganisms. Phytochemical screening also revealed the presence of Tannins, Flavonoids, Glycosides (in leaves only), Terpenes and Saponins, whereas Alkaloids, Anthraquinones and Steroids were absent in both extracts. In a qualitative antimicrobial study, six microorganisms were tested (*Bacillus subtilis, Staphylococcus aureus, Shigella dysenteriae, Salmonella* Typhi, *Escherichia coli* and *Candida krusei*) using Ciprofloxacin and Fulcin as positive controls. *B. subtilis, S. dysenteriae* and *S. typhi* proved to be the most sensitive bacterial species with MIC values of as low as 1.5mg/ml, 3.125mg/ml and 6.25mg/ml respectively, whereas *S. aureus, E. coli* and *C. krusei* were resistant to the plant extract. The minimum bactericidal concentration also had low values such as 1.5mg/ml of the stem methanolic extract against *S. dysenteriae,* and 1.5mg/ml of the leaf methanolic extract against *B. subtilis.* There was no inhibition of the extract on *S. aureus, E. coli* and *C. krusei* for the MIC and MBC. The analysis of variance at $p < 0.05$ indicated that there was significant difference in the performance of the extracts (stem methanol, root methanol and leaf methanol) on the microorganisms (*B. subtilis, S. aureus, S. dysenteriae, S. typhi, E. coli* and *C. krusei*). Therefore the study above indicates that *C. molle* contains phytochemical compounds which makes it a good inhibitor of microbial growth and could be exploited through in-depth studies to determine the active compounds that could be utilized in the treatment of common ailments.

Key words: *Combretum molle,* Traditional medicine, phytochemical compounds, Aqueous extracts

INTRODUCTION

People have used plants for millennia and vast information of the medicinal uses of plants has therefore accumulated especially in the tropical parts of the world. According to the World Health Organization (WHO) in 1995, about 80 % of the people in developing countries rely primarily on medicinal plants for their primary health care (Wood *et al*., 1997). In many remote areas in African countries people consult the traditional healer of the village in case of illness. Western hospitals and medicines are many times too expensive for the people to afford. Although combinatorial techniques have been used for the optimization of a number of recently approved agents, these methods have not been able to identify a *de novo* combinatorial compound (Newman *et al*., 2003). Examples

of successful medicines derived from natural product leads include most antibiotics, the acethylcholine esterase (ACE) inhibitors, many anticancer agents, the immuno suppressants, cyclosporine and rapamycin and the antiparasitic avermectins (Harvey and Waterman, 2004).

MATERIALS AND METHODS

SOURCE AND PREPARATION OF PLANT MATERIALS

The plant materials were collected from neighboring communities near ABU dam, in Samaru, Zaria (latitude 11.07^{o} N, longitude 7.73^{o} E and altitude 613meters), Nigeria. These were brought and identified with voucher number 900191 at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. The plant parts were dried for two weeks at room temperature in the laboratory and then ground to powder.

EXTRACTION PROCEDURES

The ground plant parts were extracted at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, following the methods of Sofowora (2006). Separation funnels were taken and 200g of each ground plant parts (stem, leaf and roots) were placed in three separation funnels. One litre of distilled water was added to the plant parts in the funnel. The mixture was allowed to stand for an hour and a conical flask was placed below the funnel which was then opened for the aqueous extract to flow in. The extract was heated at 100^{o} C in the water bath so as to obtain the dry extract.

PHYTOCHEMICAL ANALYSIS

The method of Sofowora, (2006) was employed for the test of the presence of the phytochemical properties.

SOURCE AND PREPARATION OF TEST MICROORGANISMS

The stock cultures of the test microorganisms were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. Their validity was determined by sub culturing onto nutrient agar and confirmed by standard cultural, morphological and biochemical techniques as described by Cowan and Steel (2004). The inocula of the test organisms were standardized by the method of Barry and Thormsberry (1991). This was done by suspending each test organism in 5ml of nutrient broth and the turbidity was

compared with that of 0.5 McFarland standard. McFarland standard was prepared by adding 0.6ml of 1% barium chloride ($BaCl_2$) to 99.4ml of 1% sulphuric acid (H_2S0_4) solution. The turbidity of the 0.5 McFarland standards was used for estimation of the number of bacteria in broth culture (culture for 24 hours at 37^0C) to pour into 5ml of distilled water in order to obtain a standard bacterial suspension of 1 x 10^5 cfu/ml (Bauer *et al.*, 2003).

PREPARATION OF CONCENTRATION OF EXTRACTS

Approximately 1g of each extract was dissolved in 5mls of distilled water to yield 200mg/ml. 1ml of the 200mg/ml was taken and added to 1ml of distilled water to give a concentration of 100mg/ml. 1ml of the 100mg/ml fractions concentration was also taken and added to 1ml of distilled water to get a concentration of 50mg/ml. The procedure was repeated twice to give concentrations of 25mg/ml and 12.5mg/ml.

ANTIBACTERIAL SUSCEPTIBILITY TESTING

The antibacterial activity of the crude extracts of *C. molle* was determined using the well method (Kirby-Bauer Methods) as described by Abalaka *et al*. (2011). Standard aseptic Microbiological methods were followed throughout this antibacterial study.

WELL METHOD FOR ANTIBACTERIAL ACTIVITY

The well method was employed to assay the plant aqueous extracts for antibacterial activity. Petri dishes were poured with nutrient agar and allowed for 30 minutes to solidify (This was done in duplicate for each fraction and test organism). The test organisms were then inoculated by spreading on the inocula on the surface of the medium using a sterile swab stick. A sterile Cork borer (size 3) was used to bore 4 wells in the medium. The different concentration of the plant extracts were placed in the wells using a sterile syringe and needle (different for each sample and test organism). These were then allowed a diffusion time of 1 hour after which the plates were incubated at 37 ^{0}C for 24 hours. The positive control was ciprofloxacin (100mg/ml). The potency of the extracts was determined by the clear zones of inhibition around the wells and was respectively measured as the diameter zones of inhibition. MIC was determined using the method of Doughari *et al.* (2007), while MBC was determined using the method of Rotimi *et al.* (1988).

Results And Discussion

Table 1. Qualitative Phytochemical Screening of crude aqueous extracts of *Combretum molle*

	Leaves	Stem	Root
	Aqueous	Aqueous	Aqueous

Tannins	+	+	+
Flavonoids	+	+	+
Glycoside	-	+	-
Alkaloids	-	-	-
Anthraquinones	-	-	-
Steroids	-	-	-
Triterpenes	-	+	+
Saponins	+	+	+

Key: + = Present, - = Absent.

Table 2. Analysis of inhibition zones by aqueous extracts

Source	Df	Leaf Aqueous	Stem aqueous	Root aqueous
Bacteria	5	293.99*	542.95*	390.20*
Concentration	4	920.60	761.18*	929.50
Interaction	20	59.22	83.48	50.90
Error	30	1.63	1.35	1.00

* =Significantly different at 95% ($p \geq 0.05$)

Table 3. The Mean of the Sensitivity Test of the Microorganisms to the aqueous stem extract of *C. molle*

Microorganism	Zone of inhibition (mm) (Mean±SE)				
	25mg/ml	12.5mg/ml	control	100mg/ml	50mg/ml

B. subtilis	17±1.0	15±1.0	14±1.0	12±1.0	42±1.0
S. typhi	18±1.0	15.5±0.5	13.5±0.5	12±1.0	43±1.0
S. dysenteriae	11±1.0	9±1.0	8±1.0	8±1.25	28±1.0
S. aureus	-	-	-	-	33±1.0
E.coli	-	-	-	-	-
C. krusei	-	-	-	-	-

- = No activity, Control: Ciprofloxacin for bacteria, Greseofulvin for fungi

Table 4 The Mean of the Sensitivity Test of the Microorganisms to the aqueous root extract of *C. molle*

Microorganism	Zone of inhibition (mm) (Mean±SE)				
	25mg/ml	12.5mg/ml	control	100mg/ml	50mg/ml
S. Typhi	20±1.0	16±2.0	11±1.0	8.5±0.5	43±0
B. subtilis	12±2.0	11±1.0	9±1.0	5±1.0	40±0
S. dysenteriae	13±1.0	12±1.0	10±1.0	8.5±0.5	30±1.0
S. aureus	-	-	-	-	28±4.0
E.coli	-	-	-	-	23±1.0
C. krusei	11±0	-	-	-	-

- = No activity, Control: Ciprofloxacin for bacteria, Greseofulvin for fungi

Table 5 The Mean of the Sensitivity Test of the Microorganisms to the aqueous leaf extract of *C. molle*

Microorganism	Zone of inhibition (mm) (Mean±SE)				
	25mg/ml	12.5mg/ml	control	100mg/ml	50mg/ml
B. subtilis	18±1.0	15±1.0	12±1.0	11±1.0	40±2.0
S. typhi	10±1.0	8±1.0	6±1.0	5±1.0	40±1.0
S. dysenteriae	12±1.0	10±1.0	7.5±0.5	5.5±0.5	32±1.0
S. aureus	-	-	-	-	31±2.0
E.coli	-	-	-	-	23±1.0
C. krusei	-	-	-	-	-

- = No activity, Control: Ciprofloxacin for bacteria, Greseofulvin for fungi

Table 6 Minimum inhibitory concentration **(**MIC) for microorganism of different extracts of *C. molle in* mg/ml

	Leaf Aqueous	Stem aqueous	Root aqueous
S. aureus	-	-	-
E.coli	-	-	-
B. subtilis	12.50	12.50	12.50
S. typhi	6.250	12.50	12.50
S. dysenteriae	3.10	12.50	3.10
C. krusei	-	-	-

- = no activity

Table 6 Minimum bactericidal concentration (MBC) for microorganism at different extracts of *C. molle in* mg/ml

	Leaf Aqueous	Stem aqueous	Root aqueous
S. aureus	-	-	-
E.coli	-	-	-
B. subtilis	3.10	12.50	12.50
S. typhi	6.25	6.25	25.00
S .dysenteriae	3.10	12.50	3.10
C. krusei	-	-	-

- = no activity

The qualitative screening of the extracts of *C. molle* (table 1) shows that it contains tannins, flavonoids, terpenes and saponins. Glycosides were present in the leaves only. This confirms the report by many researchers that *C. molle* contains phytochemical compounds (this also agrees with the work of Fyhrquist *et al*. (2002) who reported the presence of tannins, flavonoids and saponins in the roots and leaves of extracts of *C. molle*). Harborne (1999) found tannins and anthraquinones (the largest group of quinones) to posses antibacterial effects by inhibiting nucleic acid synthesis. Bajaj (1988) reported that tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhage, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds. Schuier *et al*. (2005) reported that epicatechin, quercetin and luteolin (all flavonoids) can inhibit the development of fluids that result in diarrhea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl– transport inhibiting cAMP-stimulated Cl– secretion in the intestine. This study confirmed the presence of phytochemicals in the extracts of *C. molle* which is a possible reason why traditional healers use it for healing diarrhoea. Glycosides were found in the aqueous stem only. Alkaloids, anthraquinones and steroids were completely absent.

From the results, *B. subtilis*, *S. dysenteriae* and *S. typhi* showed activity against the extracts of *C. molle* even at lower concentration as 1.5mg/ml against *S. dysenteriae*, 3.125mg/ml against *B. subtilis* and 3.125mg/ml against *S. typhi*. This is in line with findings of Haerdi (2001) that the juice of the leaves and roots of *C. molle* are used as antidiarrhoic remedy and with Kokwaro, (2000) and Chhabra *et al*. (1999) on its use to treat typhoid fever. This also confirms Fyhrquist *et al*. (2002) that the extracts of *C. molle* gave

good antibacterial effects. There was evidence of activity of the extract against *C. krusei* at 100mg/ml. Fyhrquist *et al*, (2002) reported that the leaf extracts of *C. molle* showed no activity against *Candida* species. Arses *et al*. (2001) reported that the extract inhibited completely the growth of *Candida* at a concentration of 400mg/ml. This may not be fully explained as the highest concentration used during the test was100mg/ml. Therefore it could be that higher concentrations of the extract would inhibit *Candida* species or that it was simply due to the extraction solvent which might have extracted other metabolites.

The extracts did not inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* as can be seen in table 3, table 4 and table 5. Fyhrquist *et al.* (2002) reported that the extracts of *C. molle* showed no activity against *E. coli* which was contrary to Eloff (1999) who showed that extracts of *C. molle* are active against *E. coli.* This could be possibly explained since Eloff (1999) used a different strain of *E. coli* from the one used in this study which might be more susceptible to the extracts of *C. molle*. Arses *et al*. (2001) using acetone extract of the stem, were able to show inhibition of the growth of *E. coli* and *Shigella* species with an MIC value of 50mg/ml. This could be that there were some compounds that were not extracted by the aqueous extracts.

The three crude extracts of *C. molle* showed excellent antibacterial activities both against *S. typhi* (gram-negative), *B. subtilis* (gram-positive) *and S. dysenteriae* (gram-negative). The extracts, however, gave no effects against the gram-negative *E. coli* and gram-positive *S. aureus*. The leaf aqueous showed bactericidal effects against the gram-positive *B. subtilis* and gave a low MIC value 1.5mg/ml. The aqueous root extract of *C. molle* were effective also against the gram-negative *S. dysenteriae* with MIC value of 1.5mg/ml.

The analysis of variance at $p < 0.05$ (table 2) showed that there was significant difference in the activities of the extract (stem aqueous, root aqueous and leaf aqueous) on the microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli* and *Candida krusei*) whereas there was no significant difference aqueous stem extracts.

CONCLUSION

The use of *C. molle* by many traditional cultures in folk medicine prompted the investigation the antibacterial and antifungal properties of the plant. Traditional healers use mainly aqueous extracts. This is because water is not harmful to humans and domestic animals and also it is the only cheap extraction solvent available. The study shows that *C. molle* contains secondary metabolites such as tannins, flavonoids, triterpenes and saponins, where as compounds such as anthraquinones, alkaloids and steroids where absent. The leaf, stem and roots of *C. molle* contain several phytochemical compounds. These compounds possess antibacterial effects against both gram-positive and gram-negative bacteria as they were active against *S. typhi* (gram-negative), *B. subtilis* (gram-positive) *and S. dysentriae* (gram-negative). These results validate the ethno botanical use of *C. molle* for ailments that may be of bacterial aetiology. The extract, however did not inhibit the growth of *S. aureus* and *E. coli*.

The extracts of *C. molle* are not very good antifungal agents. This is evident in the poor zones of inhibition of the fungi *C. krusei*.

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