# ANTIBACTERIAL EFFECT OF ROOT FRACTIONS OF *COMBRETUM MOLLE* (R.Br. Ex.G.Don) AGAINST SELECTED PATHOGENS

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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). The aqueous, n-butanol and the ethyl-acetate fractions of the roots of *Combretum molle* were analyzed for their phytochemical content and their antimicrobial potential against some selected microorganisms. Ethanol was used as solvent for extraction, after which differential fractionization was carried out using distilled water, ethyl acetate and *n*-butanol. Aqueous, ethyl-acetate and *n*-butanol fractions of the roots of *C. molle* were screened for secondary chemicals. The results revealed the presence of; Tannins, Flavonoids, Saponins, Triterpenes and Steroids. Glycosides were found in all the fractions except the root aqueous and root n-butanol fractions. Alkaloids were also present in all except the root ethyl-acetate and root n-butanol fractions. Anthraquinones were present in the root aqueous and root n-butanol fractions only. Based on the results *X. axonopodis* and *S.* Typhi proved to be the most sensitive bacterial species with minimum inhibitory concentration (MIC) values of as low as 1.5mg/ml, and 6.25mg/ml respectively, whereas *K. pneumoniae* was resistant to the ethyl-acetate root fractions of the plant. The minimum bactericidal concentration (MBC) had low values such as 3.06mg/ml of the ethyl-acetate and aqueous root fraction against *S.* Typhi. The results therefore indicate that the root fractions of *Combretum molle* contain secondary metabolites that are bactericidal against the test pathogens.

**Key words:** *Combretum molle,* Phytochemical Screening, Anti-microbial, Plant fractions

## INTRODUCTION

Thousands of secondary plant products have been identified and it is estimated that thousands of these compounds still exist (Koehn and Carter, 2005). Since secondary metabolites from plants have been elaborated within living systems, they are often perceived as showing more "potentials and are biologically non-toxic than totally synthetic molecules" making most of them, good candidates for further drug development (Cox, 2005). According to Alaribe (2008) majority of Nigerian homes, maintain some sort of private family traditional medicine practitioner. Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities and local therapy is the only means of medical treatment for such communities (Yinger and Yewhalaw, 2007). ). *Combretum molle* is a small, graceful, deciduous tree 3-13 m high; trunk crooked or leaning,

occasionally swollen at the base, up to 30 cm in diameter. Bark grey and smooth when young, grey-brown to almost black, rough and flaking when older, twigs often with reddish hairs. It is deciduous and it yields a gum. Leaves opposite, simple, leathery, 5-17 cm long, 2.5-9 cm wide, narrowly elliptic, broadly ovate-elliptic to almost circular, with dense, grey, velvety hairs on both sides (Palmer, 1972). *Combretum molle* has been used in many traditional medicines for treatment of microbial infections (diarrhea, dysentery, fever) and several inflammatory conditions such as abdominal pain, headache, and toothache (Newman *et al*., 2003).

## MATERIALS AND METHODS

### Collection of Plant Materials

The plant materials were collected from Dogon awu community near ABU dam, in Samaru, Zaria (latitude 11.07º N, longitude 7.73º E and altitude 613meters), Nigeria. These were brought and identified by a Taxonomist with voucher number 900191 at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. The plant parts were air-dried for two weeks at room temperature (25ºC) in the laboratory and then ground to powder.

### Extraction of *Combretum molle* Extracts

Extraction of the plant materials was carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, following the methods of Sofowora (2006).

### Preparation of Ethanol Extraction of *C. molle*

800 g of the dried leaves and roots of *C. molle* were extracted with 10 litres of 80% (v/v) ethanol by maceration at (25ºC) for 3days. The mixture was strained and filtered. The filtrate was concentrated to dryness on a water bath at 100º so as to obtain the dry extract after which was stored at -20ºC for further studies.

### Differential Fractionation of the Ethanol Extract of *C. molle* in Different Solvents

The dried ethanol extract obtained from both the roots and leaf of *C. molle* (50 g) were each suspended in 1 litre of distilled water and partitioned in sequence with ethyl acetate (1 litre), and *n*-butanol (1 litre). The different solvent fractions were concentrated on a water bath at 100º C so as to obtain the dry extract after which was stored at -20ºC in a refrigerator.

### Phytochemical Analysis

The method of Sofowora, (2006) was employed for the test of the presence of the phytocemical 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 $37^0$C) 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 Fractions**

1g of each fraction 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 fractions 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.

**Determination of Antibacterial Activity**

The well method was employed to assay the plant fractions 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 fractions was 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 fractions 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).

**Data Analysis**

One-way Analysis of Variance (ANOVA) of was used to assess the efficacy of the plant parts in terms of the activity as was shown by the zones of inhibition (table 7). Since there was no significant difference, Duncan's multiple range test (DMRT) and Student t-test was not carried.

## RESULTS

The preliminary phytochemical screening of aqueous, n-butanol and ethyl-acetate fractions of the root *Combretum molle* are presented in (Table 1). All the solvents revealed the presence of; Tannins, Flavonoids, Saponins, Triterpenes, and Steroids. Glycosides were found in all the fractions except the root aqueous and root n-butanol fractions.

**Table 1:** Qualitative Phytochemical Screening of *Combretum molle* root fractions

| Phytochemical | Root Aqueous | Root Ethyl-acetate | Root N-butanol |
|---|---|---|---|
| Tannins | + | + | + |
| Flavonoids | + | + | + |
| Glycoside | - | + | - |
| Alkaloids | + | - | - |
| Anthraquinones | + | - | + |
| Steroids | + | + | + |
| Triterpenes | + | + | + |
| Saponins | + | + | + |

Key: + = Present, - = Absent.

The Ethyl-acetate root fraction showed significant zone of inhibition on *E. chrysanthemi, S.* Typhi and *X. axonopodis* at all concentrations, whereas *P. syringae* had zones of inhibition at 100ml/ml and 50mg/ml only. The positive control (ciprofloxacin) performed better than the extracts in terms of zones of inhibition. The Ethyl-acetate root fraction, however, did not inhibit the growth of *K. pneumoniae*. Results also showed that the activities of the Ethyl-acetate root fraction on *X. axonopodis* and *E. chrysanthemi* was better than that of the other microorganisms tested (Table 2).

**Table 2:** Antibacterial activity of Ethyl-acetate root fraction of *Combretum molle* against selected test organisms.

| Microorganism | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.125mg/ml | Control |
| *E.chrysanthemi* | 24±1.0 | 22±1.0 | 20±1.0 | 16±1.0 | 15±1.0 | 21±1.0 |
| *S.* Typhi | 18±0.5 | 16±1.0 | 14±1.0 | 12±1.0 | 11±1.0 | 31±1.0 |
| *K. pneumoniae* | - | - | - | - | - | 28±1.0 |
| *P. syringae* | 19±1.0 | 17±1.0 | - | - | - | 29±1.0 |
| *X. axonopodis* | 26±1.0 | 24±1.0 | 20±1.0 | 18±1.0 | 16±1.0 | 26±1.0 |

- = No activity, positive Control is Ciprofloxacin (100mg/ml) for bacteria. Values above 19 are susceptible, 15-19 is intermediate, while values less than 15 are resistant (CLSI,2014)

The Aqueous root fraction showed high activity against *X. axonopodis* at all concentrations, whereas *E. chrysanthemi, S.* Typhi had zones of inhibition at concentrations of 100mg/ml, 50mg/ml and 25mg/ml. *Klebsiella pneumoniae* and *P. syringae* had zones of inhibition at concentrations of 100mg/ml and 50mg/ml only. Results also showed that the activities of the Aqueous root fraction against *X. axonopodis* was the best with a high zone of inhibition of 27mm (Table 3).

**Table 3:** Antibacterial activity of Aqueous root fraction of *Combretum molle* against selected test organisms.

| **Microorganism** | **Zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|
| | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.125mg/ml | Control |
| *E. chrysanthemi* | 15±1.0 | 13±1.0 | 10±1.0 | - | - | 21±1.0 |
| *S.* Typhi | 20±0.5 | 17±1.0 | 10±1.0 | - | - | 31±1.0 |
| *K. pneumoniae* | 10±1.0 | 9±1.0 | - | - | - | 28±1.0 |
| *P. syringae* | 19±1.0 | 17±1.0 | - | - | - | 29±1.0 |
| *X. axonopodis* | 27±1.0 | 24±1.0 | 20±1.0 | 18±1.0 | 15±1.0 | 26±1.0 |

- = No activity, positive Control is Ciprofloxacin (100mg/ml) for bacteria. Values above 19 are susceptible, 15-19 is intermediate, while values less than 15 are resistant (CLSI,2014)

The N-butanol root fraction showed high activity against *E. chrysanthemi*, *S.* Typhi and *P. syringae* at all concentrations, whereas *X. axonopodis* and *K. pneumoniae* had zones of inhibition at concentrations of 100mg/ml and 50mg/ml. The positive control performed better than the fractions. Results also showed that the activities of the N-butanol root fraction on *E. chrysanthemi*, *S.* Typhi was the same, but the performance was better than that observed on the other microorganisms (Table 4). As well, the minimum inhibitory concentration **(**MIC) and minimum bactericidal concentration (MBC) for the test microorganism at different fractions of *C. molle in* mg/ml were presented in Table 5 and 6 respectively. The statistical analysis of the different variance for inhibition zones by the extracts was tabulaized in Table 7.

**Table 4:** Antibacterial activity of n-butanol root fraction of *Combretum molle* against selected test organisms.

| **Microorganism** | **Zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|
| | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.25mg/ml | |
| *E. chrysanthemi* | 18±1.0 | 16±1.0 | 14±1.0 | 12±1.0 | - | 21±1.0 |
| *S.* Typhi | 18±0.5 | 16±1.0 | 14±1.0 | 13±1.0 | 10±1.0 | 31±1.0 |
| *K. pneumonia* | 14±1.0 | 12±1.0 | - | - | - | 28±1.0 |
| *P. syringae* | 18±1.0 | 16±1.0 | 13±1.0 | 11±1.0 | 10±1.0 | 29±1.0 |
| *X. axonopodis* | 19±1.0 | 17±1.0 | - | - | - | 26±1.0 |

- = No activity, positive Control is Ciprofloxacin (100mg/ml) for bacteria. Values above 19 are susceptible, 15-19 is intermediate, while values less than 15 are resistant (CLSI,2014)

**Table 5:** Minimum inhibitory concentration **(**MIC) for the test microorganism of different fractions of *C. molle in* mg/ml

| Microorganism | Root ethyl-acetate | Aqueous root | N-butanol root |
|---|---|---|---|
| *E. chrysanthemi* | 12.5 | 25 | 25 |
| *S.* Typhi | 6.125 | 25 | 25 |
| *K. pneumoniae* | - | - | 25 |
| *P. syringae* | 25 | 25 | 12.5 |
| *X. axonopodis* | 3.063 | 12.50 | 1.50 |

- = No activity, values below 9 are inhibitory, values above 9-32 is intermediate, while values above 32 is resistant (CLSI,2014).

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

| Microorganism | Root ethyl-acetate | Aqueous root | N-butanol root |
|---|---|---|---|
| *E. chrysanthemi* | 6.125 | 12 | 6.125 |
| *S.* Typhi | 3.063 | 3.063 | 6.125 |
| *K. pneumoniae* | - | - | 12.50 |
| *P. syringae* | 50.00 | 50 | 25 |
| *X. axonopodis* | 12.50 | 12.50 | 25 |

- = No activity, values below 9 are bactericidal, values above 9-32 is intermediate, while values above 32 is resistant (CLSI,2014).

**Table 7.** Analysis of variance for inhibition zones by Extracts

| Source | Df | Leaf Aqueous | Leaf ethyl-acetate | Leaf n-butanol | Root aqueous | Root ethyl-acetate | Root n-butanol |
|---|---|---|---|---|---|---|---|
| Treatment | 5 | 107.19 | 64.45 | 6.42 | 146.45 | 144.69 | 145.41 |
| Error | 24 | 40.18 | 52.65 | 15.15 | 60.40 | 37.06 | 64.95 |
| **Total** | **29** | **147.37** | **117.10** | **2157** | **206.85** | **182.75** | **211.3** |

No Significant difference at 95% ($p \geq 0.05$)

## DISCUSSION

Alkaloids were also present in all except the root ethyl-acetate and root n-butanol fractions. Anthraquinones were present in the root aqueous and root n-butanol fractions 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 possess antibacterial effects by inhibiting nucleic acid synthesis in gram positive bacteria. Alkaloids and anthraquinones were absent in the ethyl-acetate root fraction.

In a qualitative antibacterial study, five microorganisms were tested (*Erwinia chrysanthemi*, *Salmonella* Typhi, *Xanthomonas axonopodis*, *Pseudomonas syringae* and *Klebsiella pneumoniae*) of which they were all susceptible to the fractions at concentrations of100mg/ml. This also confirms Fyhrquist *et al*. (2002) that the extracts of *C. molle* gave good antibacterial effects. The susceptibility of *Salmonella* Typhi to the extract corroborates with the findings of Kokwaro

(2000) and Chhabra *et al*. (1999) on its use to treat typhoid fever.

## CONCLUSION

From the phytochemical assessment of the fractions of the root and leaf of *C. molle*, secondary metabolites; Tannins, Flavonoids, Glycoside, Alkaloids, Anthraquinones, Triterpenes and saponins were found present. The root of *C. molle* contain several phytochemical compounds. These compounds possess antibacterial effects against *S.* Typhi, *P. syringae, X. axonopodis, K. pneumoniae* and *E. chrysanthemi*. The result of the MBC futher indicates that the fractions of *C. molle* were bactericidal against the test organisms. Studies should be conducted to evaluate the effect of abiotic factors such as temperature and light intensity on the production of secondary metabolite in *C. molle*.

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