



# Assessment of Antimicrobial Activity of Velvet Bush Willow (*Combretum molle*) Crude Bark Extracts on Selected Bacteria Species

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**Abstract:** Treatment and control of infectious diseases in humans and animals play a vital role in prevention of illness and death. Conventional drugs treatment has been providing effective therapy for treatment of infections caused by pathogenic microorganisms; However, some do not respond to conventional therapy. Conventional therapy are expensive and have more adverse side effects. Plant based medicinal products has been used as an alternative curative for infections caused by resistant pathogenic microorganisms, moreover, plant medicinal products have less adverse side effects. This study was conducted to assess the crude *Combretum molle* bark extracts antimicrobial activity against three selected bacteria species. Three solvents, distilled water; ethanol and acetone were used for crude *combretum molle* bark extraction. The agar well diffusion method was used to assess antimicrobial activity against *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*. A minimum inhibition concentration (MIC) of the most active extracts was determined by the broth dilution technique. Extracts from three solvents tested demonstrated antimicrobial activity with zone of inhibition diameters ranging from 14 to 24 mm. Acetone extract was the most potent with its minimum inhibitory concentration (MIC) ranging from 1.25 to 2.50 mg/ml. There was no statistically significant difference ( $P > 0.05$ ) in the potency of the three extracts and standard antibiotic ciprofloxacin on the bacteria species tested. The study showed that the crude bark extract of *C. molle* has antimicrobial activity against all the test microorganisms.

**Keywords:** Combretum Molle Crude Bark Extract, Streptococcus Pyogenes, Pseudomonas Aeruginosa Escherichia Coli, Antimicrobial Activity

## 1. Introduction

Plant based medicinal plants has been used globally for many decades before and after discovery of conventional drugs. According to WHO (World Health Organization), nearly, 80% of the population, mainly from developing countries, depend on plant-based medicinal products for basic health care [6]. Plant based medicinal products are made from different parts of the plant including leaves, root, bark, seeds, tubers and exudates [7]. In African countries the ethno-pharmacological and botanical knowledge on the uses of medicinal plants is not passed down from generation to generation formally, instead

informal ways like narration and learning from herbalists is used. However, this abundance of knowledge is in danger of disappearing since it is often kept secret to herbalists, and not documented formally. A large number of higher plants are not yet investigated as alternative source of new curative drugs [5]. There is more than one way in selecting plant materials when screening for new medicinal plants/active compounds. The first way is Ethno-pharmacological information on medicinal plants is often of substantial importance for the finding of new potential medicinal plants, in this way knowledge on the already known plant is used. It has been estimated that over 70 % of the pharmacologically active plant-derived components were

discovered after the ethno-medical uses of the plants been investigated [4]. Second good way of exploring new medicinal plants is the phylogenetic approach in which a highly related plant species are considered to have related chemical compounds (chemotaxonomy), can be screened for their biological effects [2].

There is over 1340 species of plant known to be vital source of antimicrobial activity compounds, while only few of them have been systematically investigated in a scientific approach [13]. Among the plants species used by heberlists as a source of traditional medicines is *Combretum molle* (velvet bush willow). It is a plant from the family *combretaceae*, found in tropical countries including Tanzania. Leaves, roots, seeds and barks extracts are used to treat bacterial, parasitic and fungal infections. Also parts of *C. molle* are used for treatments of various conditions including, stomach pains, snake bite, leprosy, fever, dysentery, general body swellings, wound dressing, gargling, abdominal oedema, sterility and constipation. It is a shrub with a straight regular bole, widely distributed in tropical Africa. Its wood is brownish or yellowish green, hard, strong and durable. In some African countries such as Sudan a leafy and stem bark decotin is taken to treat fever, jaundice and bacterial infection. Although bark is used for tanning, the stem bark extract together with leaves have significant antimicrobial activity against chloroquine sensitive plasmodium falciparum strain NF54 [13].

This study was carried out to assess the antimicrobial activity of *Combretum molle* crude bark extract by using three different solvents in, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*.

## 2. Materials and Methods

### 2.1. Study Area

This study was carried out in Morogoro region. The fresh stem barks of *Combretum molle* were collected from the bush at Doma village; in Mvomero district.

### 2.2. Study Design

The study was experimental, where by the pure cultures of selected bacteria were subjected to the crude extracts of *C. molle* stem barks for antimicrobial activity test.

### 2.3. Plant Material Preparation and Extraction

The stem barks of *Combretum molle* were harvested from bush at Doma village in Mvomero district. The barks were cleaned and allowed to dry at normal room temperature, until they were completely dry. They were ground to powder by mechanical grinding. Crude extracts were obtained by using two technical grade extraction solvents which are acetone and ethanol; water was used as universal solvent. 50g of powder were immersed in 150ml extraction solvents, for 72 hours with intermittent shaking. Concentration of the product by evaporating the solvents using a water bath at 80°C for 1-2 hours was done.

### 2.4. Bacteria Strains

Pure cultures of selected microorganisms were obtained from preserved bacterial strains in microbiology laboratory. *Escherichia coli* and *Pseudomonas aeruginosa* were sub-cultured on McConkey agar (MCA) and *Streptococcus pyogenes* on blood agar (BA). They were all incubated at 37°C for 24 hours. The bacterial species were identified macroscopically and microscopically. Biochemical tests were performed to confirm their identities.

### 2.5. Antibacterial Sensitivity Test

Agar well diffusion method was used for sensitivity test along with ciprofloxacin as a positive control. Inocula of pure bacterial strains were subcultured; in which four to five colonies of the isolates were dipped in sterile normal saline and turbidity adjusted to  $1 \times 10^9$  CFU/ml (0.5 McFarland standard). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the Mueller Hinton agar plates. 6mm wells made on the inoculated Mueller Hinton agar by sterile cork borer. The wells were filled with 50µl of 50 mg/ml; incubation of plates was done for 30 minutes to allow the products to diffuse. After innoculation, incubation was done at 37°C for 24 hours. The antimicrobial activity of the plant extracts were observed by measuring the inhibition zones in mm.

### 2.6. Determination of Minimum Inhibitory Concentration (MIC)

Nine (9) test tubes containing nutrient broth were used for the determination of minimum inhibitory concentration of the crude extractproduct. A 2-fold serial dilution of the extract was made with 160 mg/ml in test tube 1 and 1.25 mg/ml in test tube 8 while in test tube 9 sterile 95% acetone was used as a control for negative test. The test tubes inoculated with the standardized bacterial suspensions incubated at 37°C for 24 hours. MIC was determined from lowest concentration showing no bacterial growth. After 24 hours, inhibition for bacteria growth was confirmed by transferring a loopful of bacterial suspension from each test tube and inoculating into separate solid culture media. Incubation for inoculated culture plates were at 37°C was done for 24 hours.

### 2.7. Data Processing

Data processing was done by using Microsoft excel® spread sheet for descriptive statistics, statistical significance in the mean differences of each extract based on the zone of inhibition was determined by using Chi-square test.

## 3. Results

### 3.1. Antimicrobial Activity

Results revealed that crude bark extracts of *C. Molle* have antimicrobial activity against all bacterial species tested. *S. pyogenes* was the most sensitive to all extracts with mean zone of inhibition of  $22.22 \pm 4.02$  mm ranging from

18.00±2.00 to 26.00±2.00 mm. While, *E. coli* was least sensitive by all extracts with mean zone of inhibition of 19.00±3.00 mm ranging from 15.67±2.52 to 20.00±2.00 mm. The most effective against all the test microorganisms with mean zone of inhibition of 23.11±1.33 mm ranging from 21.33±3.10 to 26.00±4.02 mm was an extract product from acetone solvent, and aqueous extract being the least effective with mean zone of inhibition of 16.56±2.52 mm ranging from 15.67±2.52 to 18.00±2.00 mm. However, there was no statistical significance difference among the antimicrobial activity of the extract products and the control for positive

antibiotics at  $p < 0.05$ .

### 3.2. Minimum Inhibitory Concentration (MIC)

Plates inoculated by bacterial suspensions from test tube 1 to 6 showed no bacterial growth for all test bacterial species. *Escherichia coli* showed growth in test tube 7, 8 and 9 while *Streptococcus pyogenes* and *Pseudomonas aeruginosa* showed growth in test tube 8 and 9 indicating that the MIC of the extract was 2.5 mg/ml for *E. coli* and 1.25 mg/ml for both *P. aeruginosa* and *S. pyogenes*.

**Table 1.** Average inhibition zones (in mm) of the three extracts against the test organisms.

Test organisms	Positive control	Aqueous extract	Acetone extract	Ethanol extract
<i>P. aeruginosa</i>	20.33±1.53	16.00±1.00	22.00±1.00	21.00±1.15
<i>E. coli</i>	32.67±2.08	15.67±2.52	21.33±2.52	20.00±2.00
<i>S. pyogenes</i>	23.67±1.15	18.00±2.00	26.00±2.00	22.22±3.10

**Table 2.** Average inhibition zones (in mm) of each test organism to show mean sensitivity of each test organism to all extracts.

Test organism	Average inhibition
<i>P. aeruginosa</i>	19.78±3.29
<i>E. coli</i>	19.00±3.00
<i>S. pyogenes</i>	22.22±4.02

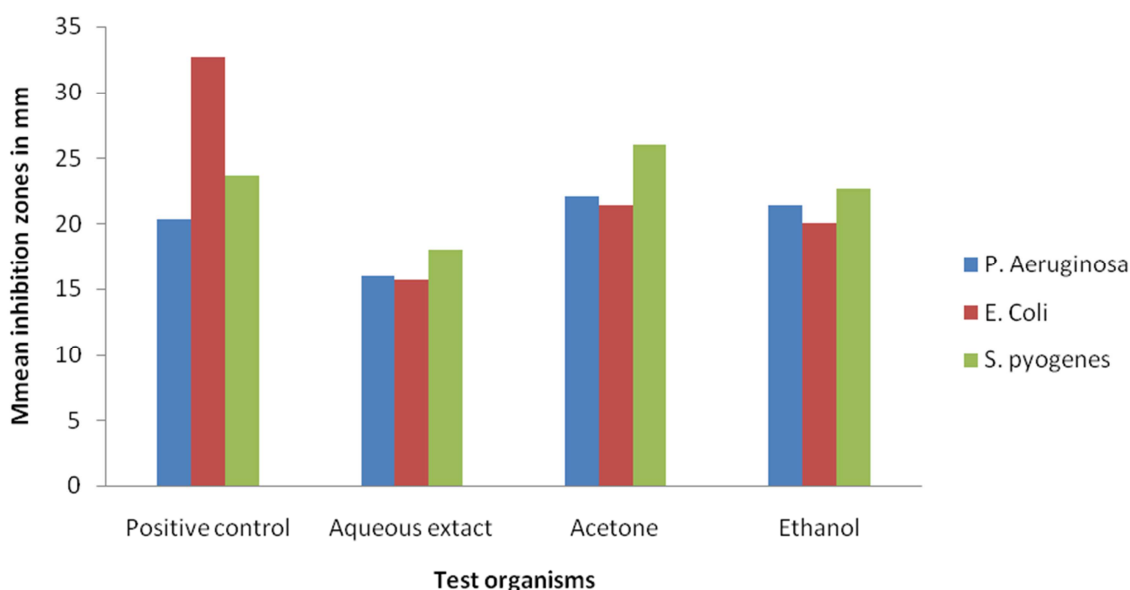
**Table 3.** Average inhibition zones (in mm) of each extract to show effectiveness of each extraction solvent.

Extraction solvents	Average inhibition zones
Distilled Water	16.56±2.52
Acetone	23.11±1.33
Ethanol	21.33±1.68

**Table 4.** Minimum inhibitory concentration of acetone extract against the test organisms.

Test organisms	Extract concentration in mg/ml in test tubes 1-8 and 95% acetone in test tube 9								
	160	80	40	20	10	5	2.5	1.25	95% acetone
	1	2	3	4	5	6	7	8	9
<i>p. aeruginosa</i>	-	-	-	-	-	-	-	+	+
<i>E. coli</i>	-	-	-	-	-	-	+	+	+
<i>P. pyogenes</i>	-	-	-	-	-	-	-	+	+

Key: - = no bacterial growth, + = bacterial growth.



**Figure 1.** Average inhibition zone of the three extracts against the test organisms.

## 4. Discussion

Conventional antibiotics have been used to treat infections caused by virulent microorganisms, however they are expensive with more side effects. Also a good number of virulent microorganisms are developing resistant, this has led to search for alternative way to treat infectious diseases which are efficient and cost effective [12, 8]. Past researches have reported the antimicrobial activity of different plants including *C. molle* against bacteria, fungi and helminthes [1, 3, 11]. Moreover, the phytochemical constituents of the stem bark of *C. Molle* are known, the bioactivity against these test organisms has not yet been established. This study was carried out to assess the antimicrobial activity of crude bark extracts of *C. Molle* against selected bacteria species. It was observed that acetone was good extractant. The findings correlate with previous studies [1, 3]. The three extracts products tested revealed different degree of antibacterial activity against the test bacterial species. The antimicrobial activities of acetone and ethanol extracts were in line to that of antibiotic (ciprofloxacin) at  $p < 0.05$  which was a standard. Acetone extract showed minimum inhibitory concentration (MIC) against the test organisms ranging from 1.25 to 2.5 mg/ml. This finding are comparable to the earlier study which found that acetone solvent extract had minimum inhibitory concentration (MIC) ranging from 0.078 to 2.5 mg/ml [10].

Some extracts demonstrated weak activity *in vitro*, this does not necessarily mean that they would demonstrate the same weak activities *in vivo* due to immuno-modulation of chemical compounds from medicinal plants which has been proven to be inactive or weakly active *in vitro* but showed marked activity *in vivo*. Metabolic transformation of these plants extracts can occur *in vivo* and make them more active intermediates [9, 8].

## 5. Conclusion

From this study, therefore it can be conclude that *C. molle* bark extracts have potential antimicrobial activity against the bacterial species tested. Acetone crude extract product of the stem bark of *C. molle* demonstrated good antimicrobial activity against selected bacteria species. Furthermore, active compounds from *C. molle* are non volatile.

## Conflict of Interest

The authors declare that there is no conflict of interests.

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