Antimicrobial Study of Oil and Stem Bark Extracts of Pentaclethra Macrophylla Benth on Six Selected Pathogens

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ABSTRACT
The antimicrobial activity of oil and stem bark extracts of pentaclethra macrophylla benth was determined against staphylococcus aureus, streptococcus pneumonia, Eschericia coli, Haemophilus influenza, α-Haemolytic Streptococcus and Yeast, using hole diffusion method. The results revealed that the most inhibitory zone (25.0mm) was recorded in hexane oil extract against staphylococcus aureus and α-Haemolytic Streptococcus. The minimum inhibitory concentration (MIC) of the oil extract ranges between 7.8125-62.5mg/ml on all the test organisms and aqueous stem bark extract inhibited only Eschericia coli with minimum, inhibitory concentration (MIC) of 62.5mg/ml. However, the oil extract possessed more antimicrobial activity than the stem bark extract. These findings lend more knowledge to the use of pentaclethra macrophylla benth for medicinal purposes.

Key words: Pentaclethra macrophylla benth, antimicrobial, clinical isolates.

INTRODUCTION
Enthropharmacological study has shown that some medicinal plants in the forest and some medicinal plant used by local human population for various parasite infections, inflammatory, pains and related illness (Cousins et al., 2002). Medicinal uses of these plants range from the stem, leaves and seeds to the use of extracts from whole plant. The use of antimicrobial agents for control of infection is almost entirely a development of this century. The need for new antimicrobial agents is closely associated with the problem of emergence of strains that are resistance to most present day antibiotics (Ibekwe, et al., 2000, Olaitan, et al., 2009).

Pentaclethra macrophylla benth is one of the plants in Africa used in traditional herbal practice for the treatment of disorders of both domestic and wild animals and human disease (Akah, et al., 1999) Pentaclethra macrophylla benth commonly called African oil bean is a large leguminous woody plant that belongs to the family Mimosoïdæ (Keay, 1989). They are trees which can be found in tropical African countries especially Cameroon, Cote D’ivore, Decorative Republic of Congo, Ghana, Niger, Nigeria and Togo (Ladipo, et al., 1993). Antimicrobial property and the fixed oil extracted from the seeds are used in the preparation of formulation against pruritus, worms and dysentery (Kamanzi, et al., 2002 & Olaitan, et al., 2009)

The use of Pentaclethra Macrophylla oil extract as antimicrobial substance is new and no folkloric information has been handed down in this regard. However, it has been shown that pentaclethra macrophylla seed is rich in protein and minerals and serve as a cheap source of protein thus increasing the haematological parameters (red blood cells, platelets and white blood cells) (Odoemelam, 2005). Study also shown that pentaclethra macrophylla seed extract has a spectrum of efficacy and does not damage both the internal and external environments of organs. It is new age antibiotic, antifungal, antinoceptive, anti-inflammatory (Okorie, et al., 2006).
The fact that microorganisms develop resistance to many drugs has created a situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Montetore, et al., 1989). It is therefore important that common diseases causing microorganisms be tested against the efficacy of the extracts of *Pentaclethra macrophylla benth* with a view to examine the traditionally acclaimed antimicrobial properties of the plant and possible further investigations of the extracts to find its pharmacological uses. Based on the affirmation of the fresh pod seed consumed by people and the stem bark used for antimicrobial treatment of wounds, the oil and stem bark extracts were compared for their antimicrobial properties.

**Materials and Methods**

Fresh pods and stem bark of *Pentaclethra macrophylla benth* were collected at the farm opposite the Bells University of Technology, Ota, Ogun State, Nigeria (Voucher No: FH1108453). Pure isolate of *Staphylococcus aureus*, *streptococcus*, *Escherichia coli* and *α-Haemophilus influenza* were obtained from University College Hospital (UCH) at Ibadan Oyo State Nigeria while *α-haemolytic streptococcus* and yeast were obtained from Medical Laboratory of State hospital Ijebu-Ode Ogun state and all were maintained in stock culture medium. The bacteria were sub cultured onto nutrient broth and incubated overnight at 37°C while Yeast isolates were sub-cultured onto yeast extract and incubated at 25°C for 72 hours.

**Preparation of the Extract:** The seeds of *Pentaclethra macrophylla* were sun dried for 5-7 days. The seeds weighed one and half kilogrammes were ground into smaller pieces with blender. One kilogramme (1.5kg) of the blended form was macerated in 3 litres of hexane for 72 hours. It was filtered and liquid extract concentrated using evaporator at 35°C to obtain the oil. The oil was labeled accordingly and stored at room temperature. Stem bark weighed half kilogram (0.5kg) was cut into pieces, dried at ambient temperature for one week and ground into powder using blender. Two hundred grammes (200g) of powdered stem bark was macerated in 150ml distilled water for 24 hours, filtered and filtrate concentrated using water bath. It was labeled and stored at room temperature. The laboratory processes used for the test was hole diffusion method.

**Hole Diffusion Method**

- Pour plate of the test organisms were prepared adding 1ml to 25ml of molten nutrient agar.
- A sterilized cork borer of 8mm diameter was used to make wells on the inoculated plate and each well was filled with 0.05ml of each extract.
- The plates were inoculated at 37°C for 24 hours and examined for zones of inhibition around the wells.
- The zones of inhibition were measured from the edge of the wells to the end of inhibition zone and results were recorded.

**Minimal Inhibitory Concentration**

Different concentrations of the extract were prepared using nutrient broth as diluents. Hexane was used as broth for the oil extract to obtain the following concentrations: 1000mg/ml, 500mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.8125mg/ml, 3.906 mg/ml and 1.95mg/ml. Three drops of over might broth culture of the test organisms were inoculated at 37°C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimal inhibitory concentration (MIC) Yeast extract broth was used to culture yeast.

**Minimal Bactericidal Concentration**

The tubes that showed no visible growth from the MIC test was sub cultured onto nutrient agar at 37°C for 24 hours for bacteria and potato dextrose agar (PDA) was used to cultured yeast at 30°C for 72 hours. The lowest concentration of the extracts yielding no growth was recorded as the minimal bactericidal concentration (MBC).
RESULTS AND DISCUSSION

Table 1: Antimicrobial activities of Pentaclethra Macrophylla benth extracts using Hole diffusion method.

| Extract       | Zone of Inhibition (mm) |
|---------------|-------------------------|
|                | S. aureus   | Streptococcus P. | E.coli | Haemophilus I. | α-Haemolytic S. | Yeast |
| Oil           | 25.0        | 21.0            | 21.0   | 18.0           | 25.0            | 15.0  |
| Stem bark     | -           | -               | 20.0   | -              | -               | -     |

Table 2: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of oil extracts of Pentaclethra macrophylla against the test organism

| Concentration | S.aureus | Streptococcus P. | E.coli | Haemophilus I. | α-Haemolytic S. | Yeast |
|---------------|----------|------------------|-------|----------------|----------------|------|
| MIC (Mg/ml)   | 62.5     | 62.5             | 7.8125| 62.5           | 62.5           | 31.25|
| MBC (mg/ml)   | 125      | 125              | 62.5  | 250            | 250            | 125  |

Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of stem bark extracts of P. Macrophylla against the test organisms

| Concentration | S.aureus | Streptococcus P. | E.coli | Haemophilus I. | α-Haemolytic S. | Yeast |
|---------------|----------|------------------|-------|----------------|----------------|------|
| MIC (Mg/ml)   | ND       | ND               | 62.5  | ND             | ND             | ND   |
| MBC (mg/ml)   | ND       | ND               | 125   | ND             | ND             | ND   |

The diameter of zones of inhibition of the test organism using oil and stem bark extracts in hole diffusion method of the seed and stem bark of *pentaclethra macrophylla* are presented in table 1. The highest zone of inhibition was recorded in oil extracts against *S. aureus* (25.0mm) and *α-Haemolytic S.* (25.0mm) and stem bark extracts against *E.Coli* with zone of inhibition diameter of 20.0mm.

Tables 2 and 3 show the minimum inhibitory concentration of oil and stem bark extracts. The oil extracts shown activities against the six test organisms with highest activity against *E.coli* (MIC=7.8125mg/ml, MBC=62.5mg/ml) and lowest activities against *S. aureus*, *Streptococcus P*, *Haemophilus I* and *α-Haemolytic S*. MIC=62.5mg/ml, MBC=125-250mg/ml.

Aqueous extract of stem bark had no activity against *S. aureus*, *Streptococcus P*, *Haemophilus I*, *α-Haemolytic S.* and *Yeast*. It showed highest activity against *E.coli* (MIC= 62.5 mg/ml, MBC= 125 mg/ml).

From the results obtained, it could be observed that oil extract is more sensitive to the test organisms than the stem bark extract. Therefore the oil extract possess more antimicrobial activities than the stem bark extracts.

The activity of the aqueous stem bark extract on *E.coli* agree favourably with the study of Olaitan, et al., (2009) on antimicrobial potency of *pentaclethra macrophylla* seed extract on seven selected pathogen. The MIC of the oil extract against yeast was 31.25mg/ml and MBC was 125 mg/ml. the stem bark extract had no activity against the yeast. This suggests that the oil extract is a promising antifungal agents and this agree with the study of Kamanzi et al., (2002) that the fixed oil extracted from the seeds of *pentaclethra macrophylla* are used in the preparation of formulation against purities, worms and dysentery.

CONCLUSION

Antimicrobial activities of oil extract carried out revealed in-vitro antimicrobial effects of the oil on local clinical isolates increased with increased concentration. The stem bark extract was only sensitive to *E. Coli*. The oil extract possesses more antibacterial and antifungal activities than the stem bark extracts.

This suggests that oil of the *pentaclethra macrophylla benth* could be used for management and disinfection of wounds. The people of eastern and western Nigeria have been using the oil as skin treatment and cure upper respiratory tract infections (cough) since prehistoric time shows scientific evidence for its antimicrobial properties.

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