In vitro Antibacterial activity of ethanolic extract of *Myrsine africana* against laboratory strains of pathogenic organisms

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**Abstract:** The determination of the antibacterial activity (zone of inhibition) and minimum inhibitory concentration of medicinal plants is a crucial step in drug development. In this study, the antibacterial activity and minimum inhibitory concentration of the ethanol extract of *Myrsine africana* were determined for *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Streptococcus pneumoniae*. The zones of inhibition (mm±S.E) of 500mg/ml of *M. africana* ethanol extract were 22.00± 0.00 for *E. coli*, 20.33±0.33 for *B. cereus*, 25.00± 0.00 for *S. epidermidis* and 18.17±0.17 for *S. pneumoniae*. The minimum inhibitory concentration (MIC) is the minimum dose required to inhibit growth a microorganism. Upon further double dilution of the 500mg/ml of *M. africana* extract, MIC was obtained for each organism. The MIC for *E. coli*, *B. cereus*, *S. epidermidis* and *S. pneumoniae* were 7.81mg/ml, 7.81mg/ml, 15.63mg/ml and 15.63mg/ml respectively. Crude extracts are considered active when they inhibit microorganisms with zones of inhibition of 8mm and above. Therefore, this study has shown that the ethanol extract of *M. africana* can control the growth of the four organisms tested.

**Key words:** Zone of Inhibition; MIC; *Myrsine africana*; Pathogenic-organisms; Ethanol; Aqua.

**Introduction**

Since antiquity, man has used plants to treat common diseases even long before mankind discovered the existence of microbes. An 80% of the population in the non-developed countries depends on traditional medicine for their primary health care this is according to WHO in 2008. In an increasing search of new antimicrobial agent to cope with the microbial resistance to antibiotics, scientists are searching from different sources including plants. Plants from different genera and species were found to have antimicrobial potentials which lead to the discovery and development of new antimicrobials or drugs (Hammer et al., 1999; Sharififar et al., 2009; Ilesanmi and Olawoye, 2011). Natural medicinal products have gained recognition worldwide in the treatment and control of diseases. One of the major concerns as they are used is the lack of adequate pharmacological and toxicological data to support their use (Zelilha, 2015).

*Myrsine africana* also called Cape Myrtle or African boxwood is a *Myrtaceae* and is an evergreen shrub growing to 2 m at a slow rate. The plant native to Africa and Asia and usually grows well in dry parts. *Myrsine africana* typically has dense, dark-green to red foliage and produces tiny bright purple berries which are edible. The small genus *Myrsine* has about ten species that occur from Africa to China. Two species, *Myrsine africana* and *M. pilcomii* are indigenous to South Africa. The name *Myrsine* derived from the Greek name for myrtle, *myrtos*. *Myrsine africana* was introduced from the Cape into England in the late seventeenth century where it was cultivated at Hampton Court in 1691 (McClintock, 1994).

The seeds and roots of *M. africana* are widely used for livestock and human as an anthelmintic, especially in the treatment of tapeworms (Gallhanna, 2004). The fruits of myrsine African are used traditionally in many cases such as treatment of roundworm and tapeworm and as the remedy for chest pains and stiff joints. In the case of worms 2-3 handfuls of ripe fruits are eaten and later the worms are released in the faeces. For chest and stiff joints treatment, ripe fruits are dried and ground into a fine powder. A tablespoonful of this powder is taken either in milk or cold water (Kokwaro, 2008). This plant is also used for the treatment of diarrhea, rheumatism, toothache pulmonary tuberculosis and relieving hemorrhage (Zhong, 1985). *M. africana* is traditionally used as a fragrance in tea, carminative, spice, appetizer and flavoring agent. Birds love the fleshy fruits of *Myrsine africana*, helping to disperse the seed (Killick, 1969). In this study, the antibacterial activity and minimum inhibitory concentration of the ethanolic extract of *Myrsine africana* fruits against selected pathogenic organisms.

**Materials and Methods**

**Extraction procedures**

The fresh *Myrsine Africana* fruits were air-dried at room temperature and reduced to powder form using a laboratory mortar and pestle, a modification of Victor et al (Miura, 2002) technique. Eighty-six grams (86 g) of the powdered form was extracted using ethanol and water in the...
ratio 9:1. The macerated mixture was carefully filtered through What man filter paper size 0.1 micrometer. The filtrate was evaporated in well regulated water bath maintained at 45°C, and the dilutions of the extract yielded a yellowish solid extract weighing 4.35 g. The ethanol extract was stored in a refrigerator at 4°C, and the dilutions of the extract were made with distilled water.

Source of test organisms:
The test organisms include, Escherichia coli, Bacillus cereus, Staphylococcus epidermidis, Streptococcus pneumonia. These organisms were obtained from the stock culture of the Medical Laboratory Department, University of Eastern Africa, Baraton.

Standardization of microorganisms:
Culture was standardized according to the methods described by Baker and Thomsberg (1983) and the National Committee for Clinical Laboratory Standards (NCCLS, 2002). About 0.2 mL of an 18 hrs old culture of each bacterium was suspended into sterile universal bottles containing 20 ml nutrient broth and incubated for 5 hrs at 37°C to obtain a logarimath growth phase. Normal saline was gradually added so as to compare its turbidity to McFarland Standard of 0.5 which corresponds to approximately 1.0 × 108 CFU/mL.

Preparation of the extract concentration and antibiotic:
Stock solutions of the extracts were prepared by dissolving 500mg in 1ml of dimethylsulfoxide (DMSO). A serial double dilution was prepared for each extract to obtain 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml, 7.81mg/ml, 3.91mg/ml, and 1.95mg/ml respectively. An antibiotic control was made by dissolving 1µg of positive control in 1ml of sterile distilled water. DMSO served as a negative control.

Susceptibility testing of bacteria species:
Susceptibility was determined using the Agar cup diffusion technique. (Adeniyi et al., 2004) A 0.1 mL aliquot of logarithmic phase broth culture of each bacterium (optical density equivalent to 107-108 CFU/mL) was used to seed sterile molten Mueller-Hinton agar (Oxoid) medium. The seeded plates were allowed to dry in the dryer for 20 min. A standard cork borer (6 mm diameter) was used to cut uniform wells on the surface of the agar, into which was added increasing concentrations of reconstituted test extract. A pre-incubation diffusion of the extracts into the seeded medium was allowed for 1 hr. Bacteria plates were incubated at 37°C in an incubator for 18-24 hours after which diameters of zones of inhibition (mm) were measured. Since each of the extracts was reconstituted in solvents, those diluents were included in each plate as controls

Results and Discussion

Table 1: Zone of Inhibition(mm±S.E.) of M. africana ethanol extract against selected pathogenic organisms.

<table>
<thead>
<tr>
<th>Bacterial Organism</th>
<th>Extract (500mg/ml)</th>
<th>Penicillin</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>22.00±0.00</td>
<td>28.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>B. cereus</td>
<td>20.33±0.33</td>
<td>27.33±0.33</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>25.00±0.17</td>
<td>30.50±0.28</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>18.17±0.17</td>
<td>25.04±0.87</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Table 2: Tukey’s honestly significant difference of the zones of inhibition among M. africana extract and penicillin controls.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli vs B. cereus</td>
<td>0.001</td>
<td>S</td>
</tr>
<tr>
<td>E. coli vs S. epidermidis</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>E. coli vs S. pneumonia</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>E. coli vs E. coli control</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>B. cereus vs S. epidermidis</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>B. cereus vs S. pneumonia</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>S. epidermidis vs S. pneumonia</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>S. epidermidis vs S. epidermidis control</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>S. pneumonia vs S. pneumonia control</td>
<td>0.000</td>
<td>S</td>
</tr>
</tbody>
</table>

Table 3: Minimum Inhibitory Concentration (MIC) (mg/ml) of the ethanol extract of M. africana extract Against Selected Pathogenic organisms.

<table>
<thead>
<tr>
<th>Bacterial Organism</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>7.81</td>
</tr>
<tr>
<td>B. cereus</td>
<td>7.81</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>15.62</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>15.62</td>
</tr>
</tbody>
</table>

The ethanol extract of M. africana inhibited the growth of E. coli, B. cereus, S. epidermidis and S. pneumonia with average zones of inhibition of 22.00±0.00, 20.33±0.33, 25.00±0.00 and 18.17±0.00 respectively. The antibiotic control showed large zones of inhibition while the DMSO control did not show any zone of inhibition for the organisms (Table 1).

Analysis of variance (ANOVA) results showed that the zones of inhibition differed significantly from each other for the extract against each organism and for the antibiotic control (p<0.0001, F=473.67). A pairwise comparison using Tukey’s honestly significant difference test showed that the zones of inhibition were significantly different for all the pairs of organisms and against the organism and the antibiotic control (Table 2).

The minimum inhibitory concentration (MIC) was determined using the agar well diffusion method. The ethanol extracts of M. Africana showed the best activity with lowest MIC against E. coli and B. cereus, both with MIC of 7.81mg/ml. The extract also showed good activity against S. epidermidis and S. pneumonia, both with MICs of 15.62mg/ml (Table 3).

Caution should be exercised when using this natural product extract as was indicated by the altered biochemical parameters; AST, urea and
creatinine. It was recommended that lower doses than the studied ones should be used for treatment. Further studies to evaluate sub-acute and chronic effect of this extract are recommended (Mbaabu, 2013).

The zone of inhibitions of S. epidermidis was significantly bigger than S. pneumoniae but was significantly smaller than those of B. cereus, S. pneumoniae and its control. The zone of inhibitions of S. pneumoniae was significantly smaller than those of all the other organisms tested and its control. However, there was no zone of inhibitions produced by negative control DMSO against any of the organisms.

The result obtained from the bioassay study has shown that it is possible to control the spread of pathogenic microorganisms such as E. coli, B. cereus, S. epidermidis and S. pneumoniae using ethanol-water extract of Myrsine Africana fruits.

Conclusion
These results have shown that the ethanol extract of M. africana has active ingredients against both gram positive and gram negative bacteria. The extract also showed MIC values against the organisms that were very low. These results are pertinent in addressing problems like drug resistance in treating diseases caused by the selected microorganisms using conventional antibiotics. The extract can be considered in drug development after toxicity studies have been conducted in animal models.

Reference

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