INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

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

**Jackie K. Obey [2], Anthoney Swamy T[1*], Lasiti Timothy[1] and Makani Rachel[1]**

[1]Department of Chemistry, School of Science and Technology, University of Eastern Africa, Baraton, P.O. Box 2500, 30100 Eldoret, Kenya.

[2]Department of Medical Laboratory Sciences, School of Health Sciences; University of Eastern Africa, Baraton; P.O. Box 2500, 30100 Eldoret, Kenya.

**Received for publication:** March 10, 2016; **Accepted**: March 15, 2016

**Abstract:** The determination of the antibacterial activity (zone of inhibition) and minimum inhibitory concentration of medicinal plants 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 (Zelipha, 2015).

*Myrsineafricana* also called Cape Myrtle or African boxwood is a *Myrinaceae* 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. pillansii*are indigenous to South Africa. The name *Myrsine*is derived from the Greek name for myrtle, *myrtus*. *Myrsinea fricana*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 (*Gathuma, 2004)*. The fruits of myrsine Africana 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 feaces. 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 aqua 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 powdery form using a laboratory mortar and pestle, a modification of Victor et al (Miura, 2002) technique. Eighty-six grams (86 g) of the powdery form was extracted using ethanol and water in the

***Corresponding Author:**
**Dr. T Anthoney Swamy, Ph.D**
Head, Department of Chemistry,
University of Eastern Africa, Baraton,
P.B No. 2500 – 30100, Eldoret, Kenya.

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$^0$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$^0$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 logarithm 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 strains of pathogenic organisms.

| Bacterial Organism | Extract (500mg/ml) | Penicillin | DMSO |
|---|---|---|---|
| *E. coli* | 22.00±0.00 | 28.00±0.00 | 0.00±0.00 |
| *B. cereus* | 20.33±0.33 | 27.33±0.33 | 0.00±0.00 |
| *S. epidermidis* | 25.00±0.17 | 30.50±0.28 | 0.00±0.00 |
| *S. pneumonia* | 18.17±0.17 | 25.04±0.87 | 0.00±0.00 |

**Table 2:** Tukey's honesly significant difference of the zones of inhibition among *M. africana* extract and penicillin controls.

| Comparison | P-value | Significance |
|---|---|---|
| *E. coli vs B. cereus* | 0.001 | S |
| *E. coli vs S. epidermidis* | 0.000 | S |
| *E. coli vs S. pneumoniae* | 0.000 | S |
| *E. coli vs. E. coli control* | 0.000 | S |
| *B. cereus vs. S. epidermidis* | 0.000 | S |
| *B. cereus vs. S. pneumoniae* | 0.000 | S |
| *B. cereus vs. B. cereus control* | 0.000 | S |
| *S. epidermidis vs. S. pneumoniae* | 0.000 | S |
| *S. epidermidiis vs. S. epidermidis control* | 0.000 | S |
| *S. pneumiae vs. S. pneumoniae control* | 0.000 | S |

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

| Bacterial Organism | MIC (mg/ml) |
|---|---|
| *E. coli* | 7.81 |
| *B. cereus* | 7.81 |
| *S. epidermidis* | 15.62 |
| *S. pneumonia* | 15.62 |

The ethanol extract of *M. africana* inhibited the growth of *E. coli, B. cereus, S. epidermidis* and *S. pneumoniae* 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. pneumoniae,* 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. pneumonia*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


1. Gathuma JM, Mbaria JM, Wanyama J, Kaburia HF, Mpolce LJN. Samburu and Turkana healers. Efficacy of Myrsineafricana, Albizia anthelmintica and Hilderbrantia sepalosa herbal remedies against mixed natural sheep helminthosis in Samburu district, Kenya. J Ethnopharmacol. 2004; 91:7–12.

2. Mbaabu M, Matu EN. Medicinal plants utilization in the treatment of human and livestock diseases in Meru. Pharm J. 2013;1(2):18–24.

3. Zhong HB. State administration of traditional Chinese medicine of the people's republic of China, third edition. China: Shanghai Science and Technology Press; 1985. p. 245–95.

4. Kokwaro JO. Medicinal plants of East Africa. 2nd ed. Nairobi: Kenya literature Bureau; 1993. p. 401–50.

5. Zabta KS, Ashiq AK, Toshiyuki N. Medicinal plants and other useful plants of District Swat. Peshawar, Pakistan: Pakistan. Al Aziz Press; 2003. p. 79–210.

6. Beentje H. Kenya Trees, Shrubs, and Lianas; National Museums of Kenya. 1994.

7. Schorderet M. Pharmacologie des concepts fondamentaux aux applications theurapeutiques. Editions Frison-Roche Paris: Editions Slatkine Genève; 1992. p. 33–4.

8. Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, et al. Antisickling property of Carica papaya leaf extract. Afric J Biochem Res. 2009;3(4):102–6.

9. World Health Organization (WHO). Herbal medicine research and global health; an ethical analysis. Bulletin of the. 2008;86(8):577–656.

10. Stanley B. "Recognition and respect for African Traditional Medicine". National Coordinating Agency for pupation and Development (NCAPD), "Draft Policy on Traditional Medicine and Medicinal Plants", Nairobi 2004.

11. Killick, D.J.B. 1969. Myrsineafricana. The Flowering Plants of Africa 40: t.1564.

12. McClintock, E. 1994. African Box: Myrsineafricana, Pacific Horticulture 56,2: 46.

13. Pooley, E. 1998. A field guide to wild flowers of KwaZulu-Natal and the eastern region. Natal Flora Publications Trust, Durban.

14. Pooley, E. 2003. Mountain flowers. Field guide to the flora of the Drakensberg and Lesotho. Flora Publications Trust, Durban.

15. Watt, J.M. & Breyer-Brandwijk, M.G. 1962. Medicinal and poisonous plants of southern and eastern Africa. Livingstone, Edinburgh.

16. Miura T, Nosaka K, Ishii H, and Ishiada T, Antidiabetic effect of Nitobegiku (Tithonia diversifolia) in KK-Ay diabetic mice. Biol. Pharm. Bull, 2005, 28(11): 2152-2154.


**Cite this article as:**
Jackie K. Obey, Anthoney Swamy T, Lasiti Timothy and Makani Rachel. *In Vitro* Antibacterial activity of ethanolic extract of *Myrsine africana* against laboratory Strains of Pathogenic Organisms. *International Journal of Bioassays* 5.4 (2016): 4512-4514.

**Source of support:** Nil
**Conflict of interest:** None Declared