In vitro Antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases

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Abstract: The *in vitro* antibacterial activity of the water extract of *Moringa oleifera* leaf stalk extract was conducted. Paper disc diffusion method was used to assess the effect of the extract on *Pseudomonas aerogenosa, Staphylococcus albus, Staphylococcus aureus, Escherichia coli, Staphylococcus pyogenus* and *Enterobacter aerogenes*. At dilution of 1000mg/ml, 700mg/ml, 400mg/ml and 200mg/ml only mild activity against *Escherichia coli* and *Enterobacter aerogenes* was noticed. *Pseudomonas aerogenosa, Staphylococcus albus, Staphylococcus aureus* and*, Staphylococcus pyogenus* was resistant at these concentrations. The highest activity was produced by *Escherichia coli* at 1000mg/l which comparably is less than that of the standard drug tetracycline (250mg/ml). in conclusion, this study has shown that the water extract of *Moringa oleifera* posses some degree of antimicrobial activity especially at high dose. [Academia Arena, 2010;2(6):80-82] (ISSN 1553-992X).

Keywords: *In vitro;* Antimicrobial activity; *Moringa oleifera;* diseases

Introduction

Moringa oleifera also known as Drumstick (India), Nebedy (Senegal), Benzolive tree (Haiti), Marum (Thailand) and Malunggay (Philippine) (EL-Awady, 2003). In Nigeria, it is called Zogale, Zogale gandi and Bagaruwar makka (Hausa), Ewe igbale and Idagbo monoye (Yoruba), Ikwa oyibo (Igbo) and Kabi (Kilba).

Moringa oleifera is a well documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria. It is also used in water purification and therefore helps in reducing the incidence of water borne diseases (Marcu, 2004).

Eye infections may be caused by bacteria, fungi, Chlamydia or virus with bacteria being the most common. This seriously affects the activity of the affected subject and may a times lead to vision impairement or blindness.

Kilba people of Adamawa state, Nigeria use the fluid from the stalk of *Moringa oleifera* in treating eye infections.

This study is therefore designed to investigate the invitro activity of *Moringa oleifera* leaf stalk extract on bacterial organisms normally implicated in eye diseases.

Methodology

The leave stalks of *moringa oleifera* were collected from Hong, Adamawa state, Nigeria and identify by the department of biological sciences, University of Maiduguri. The stalk was air dried and pounded into a coarse powder using laboratory pestle and mortar. To 100g of the powder leaf stalk was added 1.5 litres of distilled water and was thoroughly mixed and allowed to stand for one hour before filtering with the aid of whatman filter paper number 1. The filterate was dried in hot air oven (45^{o}C). The extraction yielded 24.891% w/w of water extract.

Laboratory isolates of the pure cultures of *Pseudomonas aerogenosa, Staphylococcus albus, Staphylococcus aureus, Escherichia coli, Staphylococcus pyogenus* and *Enterobacter aerogenes* were obtained from the Department of Veterinary Medicine Research Laboratory, University of Maiduguri, Nigeria. The isolates were propagated on nutrient agar plate (Oxoid, 40, England) according to the manufacturer's specification. The stock cultures were stored at 4^{o}C on nutrient agar. They were then subcultured in

nutrient broth (Oxoid, 40, England) at 37°C for 8 hours prior to antimicrobial testing.

Extract concentrations were prepared by dissolving known weight of the stock solutions of crude aqueous extract in known volume of distilled water to give 200mg/ml, 400mg/ml, 700mg/ml and 1000mg/ml of the crude extract. 250mg/ml of the standard antibacterial agent (tetracycline, cipla ltd, Mumbai, India) was similarly constituted.

Disc diffusion method as described by National Committee of Clinical Laboratory Standard (1993a) was used to determine the antimicrobial activity of *Moringa oleifera*. Disc containing different concentrations of dissolved extract (200mg/ml, 400mg/ml, 700mg/ml and 1000mg/ml) were prepared with sterilized filter paper (Whatman no.1, 6 mm in diameters) soaked in different beakers. The disc was dried at 50°C.

Overnight cultures of each bacterial isolates was diluted with sterile normal saline to give an inoculums size of 1,000,000 cfu/ml. the inocula were spread on the surface of the dried nutrient agar plate with cotton wool swabs which have been dipped in the diluted suspensions of the organisms. The plates were incubated at 35°C for 30 minutes before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 hours. The same procedure was carried out using tetracycline as control. The zone of inhibition above 6mm diameter of each isolate was used as a measure of susceptibility to the extract and was compared to that of the standard drug.

Results

The results of the antimicrobial test using water extract of *Moringa oleifera* leaf stalk and tetracycline are presented in Table 1.

The water extract inhibited the growth of *Escherichia coli* and *Enterobacter aerogenes*. The zone of inhibition of *Escherichia coli* were 7mm, for 200mg/ml, 400mg/ml, 700mg/ml and 10mm for 1000mg/ml of the extract as against 12mm produced by the standard drug, tetracycline (250mg/ml).

The extract did not produce any effect against *Pseudomonas aerogenosa, Staphylococcus albus, Staphylococcus aureus* and *Staphylococcus pyogenus*. However, the standard drug tetracycline (250mg/l), produced zones of inhibition 17mm, 17mm, 25mm and 15mm for *Staphylococcus aureus, Staphylococcus albus, Staphylococcus pyogenus* and *Pseudomonas aerogenosa* respectively.

Table 1. *In vitro* antibacterial effect of *Moringa oleifera* leaf stalk at various concentrations on bacterial organism

Extract/ Antibiotic	*Escherichia coli*	*Enterobacter aerogenes*	*Staphylococcus albus*	*Staphylococcus aureus*	*Staphylococcus pyogenus*	*Pseudomonas aerogenosa*
Extract: 1000mg/ml	10mm	7mm	R	R	R	R
Extract: 700mg/ml	7mm	7mm	R	R	R	R
Extract: 400mg/ml	7mm	7mm	R	R	R	R
Extract: 200mg/ml	7mm	7mm	R	R	R	R
Tetracycline: 250mg/ml	12mm	16mm	17mm	17mm	25mm	15mm

Key: R-Resistant

Conclusion

The result of this study showed that *Moringa oleifera* leaf stalk water extract had no antibacterial activity against *Staphylococcus aureus, Staphylococcus albus, Staphylococcus pyogenus* and *Pseudomonas aerogenosa* and only a mild activity against *Escherichia coli* and *Enterobacter aerogene*. It has been reported that crushed seed extract of *Moringa oleifera* had bactericidal activity against *Staphylococcus pyogenus* and *Pseudomonas aerogenosa* (Suarez *et al.,* 2005). Harvey (2005), also reported that Pterygospermin, a bactericidal and fungicidal compound contained in an aqueous extract made from seed of *Moringa oleifera* was effective against *Staphylococcus aureus* as the antibiotic neomycin. However, this does not in any way indicate that the results of these studies are

scientifically divergent, as plants have different organic compounds stored in them but their concentration in different parts of the plant may not be the same. Harbone (1982), has documented that active principles are stored in different plants and released in varying combination and strength. Miller (1973) has also documented that fats occur in all proportion of plant, but in general, the major accumulations are found in tissues of fruits and seeds. The phytochemistry of *Moringa oleifera* shows that several organic compounds found in the pods and leaves differ despite their closeness (Duke, 1983).

With these, it can be deduced that the active antibiotic principle, Pterygospermin has a very low concentration in the leaf stalk of *Moringa oleifera* and that was why the activity against the test micro-organisms differed from other works.

Suggestion and conclusion

This result showed that *Moringa oleifera* leaf stalk water extract had antimicrobial effect not up to the extent claimed by its traditional users. However, attempts should be made to conduct *in vivo* studies with the extract so as to confirm the present *in vitro* findings as the diameter of the zone of inhibition is not only affected by sensitivity of the micro-organisms alone but concentration of the extract in the discs is used and it's rate of diffusion in the media as well. This will aid in giving a clear evidence for condemning its traditional usage or supporting it to some extent.

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