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ANTIMICROBIAL PROPERTIES OF *MORINGA OLEIFERA* SEED OIL

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ABSTRACT

Moring oleifera seed oil was extracted by adding hexane to the seed powder in an electro-thermal soxhlet and evaporating the hexane to produce the 100% seed oil. Different concentrations (75%, 50%, and 25%) of the oil were prepared by aseptically dissolving the measured amount of oil into appropriate volume of Dimethyl Sulfur Oxide (DMSO) on volume by volume (v/v) basis. About 0.3mls of the seed oil extract of the varying concentrations were added to four agar wells and to the fifth well, gentamycin was added as a positive control. The agar plates were then incubated at $35^0C \pm 2^0C$ for 24 hours. Effect of the Moring oil extract was assessed by measuring the zones of inhibition to the nearest millimeter, and then compared with the standard gentamycin. The results showed that the 100% seed oil is 78% as effective as the gentamycin against E. coli. It is concluded that the oil can be used as mild antimicrobial agent as skin ointment, hand spray, and sanitizers. It is also recommended that the shelf life of the oil be investigated as well as its action against other common microbes.

Keywords*: M. oleifera, Seed Oil, Antimicrobial Action, E. coli.*

1. INTRODUCTION

1.1 Use of *Moringa Oleifera* Seed Extract in Water Disinfection

Traditionally, water disinfection has been achieved by the use of chlorine in many water supply agencies. However, the production of by-products of chlorination such as halogenated organic compounds has been shown to be associated with various ailments in humans (Hwang *et al*. 2008). Goveas *et al.* (2010) for instance, reported that although chlorine is widely used as an inexpensive and potent disinfectant in the United States for drinking water, it has the potential of forming carcinogenic and mutagenic disinfection by-products (DBPs). These, and other similar problems like the high cost of chlorine in developing countries, necessitates the search for natural disinfectants that are safer and cheaper to use.

Moringa Oleifera (*Zogale*) is cultivated across the whole of tropical belt and used for a variety of purposes (John, 1986). Many researchers (Bina, 1991; Muyibi and Evison, 1995; Falkard et al, 1989; Sutherland eta al, 1990; Bichi, 2013) have reported its great potential for water treatment. Eilert *et al* (1981) also identified an active anti-microbial agent in the seeds. When isolated, it was found to be *4α-4-rhamnosylox.y-benzyl-isothiocynate*, the only known glycosidic mustard oil at present. The compound is readily soluble in water at 1.3µmol/L and is non-volatile.

Thilza, et al (2010) reported that Moringa leaf stalk extract had mild activities against *E. coli* and *Entrobacter aerogenes*. Bukar, et al (2010) also studied the antimicrobial activities of Moringa Seed Chloroform extract and Moringa Seed Ethanol extract. They found both to have inhibitory effects on the growth of *E. coli* and determined the Minimum Inhibitory Concentration (MIC) to be >4mg/ml. Thilza, et al (2010) using extract from Moringa leaf stalk, found that at dilutions of 1000mg/ml, 700mg/ml, 400mg/ml, and 200mg/ml, only mild activity against *E. coli* and *Entrobacter Aerogenes* was noticed. They also found that the highest activity was produced by *E.Coli* at 1000mg/ml which comparatively was less than that of the standard drug tetracycline (250mg/ml).

The application of Moringa seed extract in water treatment entails the removal of the seed oil in order to satisfy the drinking water quality requirement of non-oily. Many seed oils have been found to possess antimicrobial properties (Humeirah et al, 2010 and Oluseyi, et al, 2009). Even though Moringa seed oil needs to be removed

before application in water treatment, it is pertinent to investigate its possible antimicrobial application.

1.2 Use of Seed Oil as an Antimicrobial Agent

Humeirah *et al.* (2010) reported that the essential oils from the twig and root of *Goniothalamusmacrophyllus* (Annonaceae) obtained from Pasoh Forest, Malaysia exhibited the most notable inhibitory activity (0.3 mg/ml) against Vancomycin intermediate-resistance *Staphylococcus aureus* (*VISA 24)*, *Staphylococcusepidermidis* and *Candida albicans*. Oluseyi *et al*. (2009) also reported that *Buchholzia coriacea* (wonderful kola) possess antimicrobial properties. The report showed that the fresh kola, its hexane extract, and its methanolic extracts showed inhibitory zone of 62mm, 21mm, and 30mm respectively with E. Coli.

2. OBJECTIVES OF THE RESEARCH

2.1 Aim of the Research

The aim of this research is to investigate the antimicrobial properties of *Moringa oliefera* seed oil.

2.2 Objectives of the Research

The specific objectives of the research are:

i) To extract *Moring oleifera* seed oil using electro-thermal soxhlet and hexane
ii) To determine the effect of the *Moringa oleifera* seed oil on the death rate of *E. coli*

3. METHODOLOGY OF THE RESEARCH

3.1 Preparation of *Moringa Oleifera* Seeds Powder

Good quality dry *M. oleifera* seeds were obtained locally from a farm house in Maigatari, Jigawa State. Good quality seed was selected and air dried at room temperature under a good condition at the Food science and Technology Laboratory of Kano Universityof Science and Technology, wudil. The seed coat and wings were removed manually, and the kernel was grinded to fine powder using the coffee mill attachment of the Moulinex domestic food blender. The ground powder was then sieved through 210 μm sieve.

3.2 Extraction of *M. oleifera* Seeds Oil

The seed powder was de-fatted using hexane in electro-thermal Soxhlet extractor. Oil extraction was done by adding hexane to the seed powder. To separate the oil from the seeds, the electro thermal Soxhlet was used and hexane was passed in to the apparatus to extract the oil. The hexane was then evaporated leaving the concentrated oil, which was then stored at room temperature. The extracted seed oil was used for the antimicrobial studies.

3.3 Preparation of *Moringa Oleifera* Seeds Oil

Whole Moringa seed oil (100%) was used to serve as stock from which subsequent concentrations of the oil were prepared by aseptically dissolving the measured amount of oil into appropriate volume of Dimethyl Sulfur Oxide (DMSO) on volume by volume (v/v) basis (APHA,1996). The subsequent concentrations were 75%, 50%, and 25%.

3.4 *E. Coli* Test Organisms

Escherichia coli (ER2566) strain was employed as test organisms and was collected from Microbiology Unit of the Food Analysis Laboratory, Department of Food Science, KUST, Wudil. The strain was grown in 10mL broth at 37^0C overnight to obtain an exponential growth phase. This was used for the disinfection studies.

3.5 Preparation of Normal Physiological Saline

This was prepared as described in APHA (1996). 8.5gm of Sodium Chloride was dissolved in 959.0ml of distilled water and toped-up to 1 liter with distilled water. The solution was used for dilution and washing of cells.

3.6 Standardization of Inoculum Concentration

0.5McFarland standard was prepared by adding 0.5ml of 0.048M Barium Chloride ($BaCl_2.2H_2O$; 1.175% W/V) to 99.5ml of 0.18M H_2SO_4 (1% V/V) with constant stirring. The standard was distributed into a screw capped test tube for color comparison of the test inoculums. A loopful of 18-24 hour old colonies saline and the concentration was adjusted to $1*10^6$ colony forming unit per millimeter (Cfu/ml) by comparing with the 0.5McFarland standard as described in APHA (1996).

3.7 Preparation of E. Coli Culture

The E.Coli culture was prepared as described in Obire *et al.* (2005). Nutrient broth (19.0gm) was dissolved in 500mL distilled water by heating slightly. The mixture was then sterilized at 121^0C for 15minutes at 15 SPT in autoclave. The

sterilized broth was cooled to 45^0C and poured (20ml) into sterile petri-dishes and allowed to solidify at room temperature and used to prepare the *E.Coli* culture.

3.8 Test of Antimicrobial Properties of the Extracted Oil

Agar well diffusion method was used to determine the antimicrobial activity of the Moringa seed oil extract. Standardized inoculums of *E. coli* were swabbed with the aid of sterile cotton swabs on to previously prepared and sterilized Mueller Hinton Agar. 10mm diameter wells, about 2.0cm apart, were made in each plate using sterile cork borer. About 0.3mls of the seed oil extract of varying concentrations were added to four of the wells and to the fifth well, gentamycin was added as a positive control. The plates were then incubated at 35^0C ± 2^0C for 24 hours. Effect of the Moring oil extract was assessed by measuring the zones of inhibition to the nearest millimeter, and then compared with the standard gentamycin.

4. DISCUSSION OF RESULTS

Table 1 shows the relative zones of inhibitions of the test conducted on E. coli using the Moring seed oil extract of various concentrations (100%, 75%, 50%, and 25%) and the positive control gentamycin. Whereas gentamycin control gave an average zone of inhibition of 22.27mm, the M. oleifera seed oil gave average zones of inhibitions of 17.7mm, 14.3mm, 11.3mm, and 9.0mm for the 100%, 75%, 50%, and 25% respectively. Thus the 100% concentration gave the highest zone of inhibition and is therefore most effective, even though lower than gentamycin. Table 2 presents the activity indices for the various concentrations of the M. oleifera seed oil compared to the gentamycin control. The 100% concentration exhibited the highest activity index of 0.78 (78%) compared with the gentamycin. The 75%, 50%, and 25% concentrations gave activity indices of 0.64, 0.50, and 0.40 respectively. Thus the 100% M. oleifera seed oil is 78% as effective as the standard gentamycin.

TABLE 1: Relative Zone of Inhibition of Moring oleifera Seed Oil on E. Coli

CODE		Zone of Inhibition (mm)				
	DMSO	Gentamycin Control	100%	75%	50%	25%
E1	00	23	17	14	11	9
E2	00	22	18	15	12	9
E3	00	23	18	14	11	9
Average		22.27	17.7	14.3	11.3	9

TABLE 2: Activity Index of Moring oleifera Seed Oil Compared with Gentamycin

CODE	Activity Index for Various Moringa Oleifera Seed Oil Concentrations			
	100%	75%	50%	25%
E1	0.74	0.61	0.48	0.40
E2	0.82	0.68	0.55	0.41
E3	0.78	0.61	0.48	0.40
Average	0.78	0.64	0.50	0.40

TABLE 3: Comparison of the Antimicrobial Activity of Moring oleifera Seed Oil with Other Seed Oil Extracts

Antimicrobial Agent	Zone of Inhibition (mm)	Activity Index
M. Oleifera Seed Oil (100%)	17.7	0.78
P. macrophylla Oil	17.3	0.76
C. albidum Oil	15.9	0.70
P. gratissama Oil	18.0	0.79
Gentamycin	22.7	1.00

The zones of inhibitions and the activity indices of Moringa and seed oil are compared with the results obtained by Ugbogu and Akukwe (2009) for P. macrophylla Oil, C. albidum Oil, and P. gratissama Oil in Table 3. Taking gentamycin acitivity as 100%, the Moringa seed oil exhibited more activity (78%) than P. macrophylla Oil (76%) and C. albidum Oil (70%). The P. gratissama Oil, however, has higher activity than Moringa with 79%.

5. CONCLUSION

It can be concluded that, even though *Moringa* seed oil is not as effective as gentamycin against *E. coli*, it has achieved up to 78% of its antimicrobial action. Thus it can be used as mild disinfecant such as skin ointment, hand spray, and sanitizers. It is, however, recommended that more work be done to determine its shelf life as the oil used was fresh and the anticrobial action may deteriorate with time. Its action against other common micrbes also needs to be investigated inorder to establish its general effectiveness.

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