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Inhibitory activity of Moringa seed extracts on micro-organisms

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Abstract:
M*icro-organisms constitute a major cause of post-harvest food losses. Microbial resistance to antibiotics and antibiotic sensitivity has led to the search for natural alternatives. Inhibitory activity of Moringa (Moringa oleifera) seed extracts were examined to determine their effect on diameter of inhibitory zone of various micro-organisms such as Escherichia coli, Staphylococcus aureus, and Streptococcus. Five (5) treatments of both water and ethanol extracts A= control, B= 25g + 200ml, C= 50g + 200ml, D= 75g + 200ml, and E= 100g + 200ml were tested against the organisms.*
Ethanol extract were effective for Staphylococcus at concentration level 50g/200ml weight to volume ratio with a value of 12.30mm and E.coli having a value of 12.10mm at the same concentration. Diameter of inhibitory zone value for Staphylococcus aureus indicated a much lower value of 11.13mm. The aqueous extract of moringa seed showed lower values with E.coli having a value of 10.30mm, Staphylococcus aureus 8.37mm and Streptococcus 3.40mm.

Keywords: *Antimicrobial agents, Moringa seed extracts, Food pathogens,*

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I. Introduction

Food security is essential for the continued existence and well-being of humanity. Food pathogens pose a constant threat to food security by negatively impacting on crop yield and causing enormous crop losses. Therefore, it is necessary to investigate ways of mitigating this situation using safeantibacterial and antifungal derivatives from plant sources. Microbial pathogens do not only threaten crop yield but also pose a serious health hazard to humans. In order to reduce food poisoning, safe antimicrobial agents must be used. Increasing interest is now being shown in finding safe substances for fighting microbes that damage crop yield. Spoilage of crops in the field constitutes a major problem in African leading to food wastage, seasonal scarcity and food insecurity.

Recently, there has been an increased trend towards natural anti-microbial agents which are cheap, available and effective in control of both plant diseases and spoilage caused by micro- organisms. The development of a natural anti-microbial agent will reduce cost and enhance food security.

The tree is one of the world's most beneficial trees; every part of the tree has been used for food, medication and industrial purposes (Khalafalla*et al.,* 2010). Moringa oleifera is an important tree plant found in most parts of tropical and sub-tropical regions of the world. The plant has a wide range of uses which include medicinal and nutritional functions (Farooq et.al, 2007). Nutritionally they constitute a good source of minerals and β- carotene, phytochemical such as quercetin and kaempferon. The seeds of the plant have been shown to have water purification properties and this occurs by flocculating gram positive and gram- negative bacterial cells (Broin et.al, 2002). The seeds of the plant can also be used as a less expensive bio-absorbent for the removal of heavy metals as reported by Sharma et.al, (2006). The high oleic acid content of moringa seed oil makes it suitable as edible oil, cosmetic oil, bio-diesel and lubrication for machinery (Rashidi et.al, 2008).

The seeds are round with a brownish semi-permeable seed hull. The hull has three white wings which run from top to bottom. The average weight per seed is 0.3 gram. (Meena *et al*., 2010).

The *M.oleifera*is an outstanding source of nutrition; and grows in a wide range of different climate condition such as semi-arid and tropical conditions (Fahey, 2005). All parts of the *M.oleifera* are edible as result it is used as source of nutritional component . *M. oleifera* has been used to combat malnutrition, particularly among infants and nursing mothers in different developing countries in Africa (Elkhalifa*et al.,* 2007).

Streptococcus is a genus of *coccus* (spherical) Gram-positive bacteria belonging to the phylum *firmicutes* (Ryan and Ray, 2004) and the other lactobacillales (Lactic acid bacteria). Cell division in this genus occurs along a single axis in these bacteria, thus they grow in chains or pairs, meaning easily bent or twisted, like a chain (twisted chain). Most are oxidase-negative and catalase-negative, and many are facultative anaerobes.

Currently, over 50 species are recognized in this genus. This genus has been found to be part of the

salivary microbiome (Wang *et al.,* 2016).

Streptococcus species are Gram-positive, cocci, facultative anaerobic bacteria. Some *Streptococcus* groups have also been reported to occasionally result in food borne illnesses (Rajasekhar & Clancy, 2010; Scallan*et al.,* 2011). Symptoms of illnesses caused by these organisms are sore throat; complications include acute rheumatic fever and kidney inflammation. Group C *Streptococcus* has been known to cause meningitis (Rajasekhar & Clancy, 2010).

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans. *S. aureus* is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning(Montville and Matthews 2008; Le Loir *et al.*, 2003).

Escherichia coli are a Gram-negative, none sporulating and facultative anaerobic rod. It is about 2.0 micrometers (μm) in length and its diameter is 0.25-1.0 μm . Those strains which have flagella are motile. Structurally flagella have peritrichous arrangement (Darnton *et al.,* 2007).The goal of this research is to examine the inhibitory activity of both aqueous and ethanol extracts of moringa seed on specific food pathogen such as E.coli, Staphylococcus aureus and Streptococcus.

II. Materials And Method

Experimental laboratory

This study was carried out at the Microbiology and Fermentation Laboratory, Department of Food Science and Technology, Rivers State University of Science and Technology.

Sub-culturing and confirmation of bacteria isolates

Cultures of *E. coli ,Staph.Aureus and Streptococcus*were carefully selected from the properly identified culture plates and were sub-cultured into a fresh prepared nutrient agar plate by streaking to get pure cultures. The cultures were then incubated at room temperature.For the confirmation of the isolates the following biochemical tests; oxidase test, mortality test, catalase test, indole and coagulase test were carried out.Gram staining reaction was also carried out on the isolates, *E.coli* had a negative gram staining while *S. aureus* and streptococcus was gram positive.

Preparation of Moringa seed(Moringa oleifera)sample

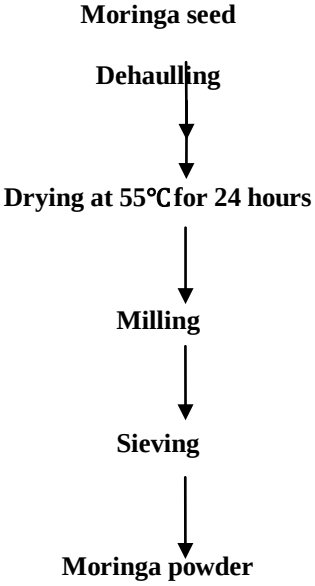

Fig 1: Flow diagram of moringa seed flour production

Source: Ogunsina*et al.*, (2011).

Extraction of active compound.

The method as modified by Patil and Rasika (2013) was used in the experiment. Moringa seed of various weight 25g, 50g, 75g and 100g were weighed in duplicates and each dissolved in either 200ml of ethanol (99.5% laboratory grade) or 200 ml of distilled water for ethanol and water extraction, respectively. The

solution was allowed to stand for 5 hours and filtered using Whatman filter paper number 4. The filtrate was concentrated using rotary evaporator thereafter 1ml of theextracts was then used to inoculate the plate containing the nutrient agar and the organisms

Experimental Laboratory

This study was carried out in the microbiology and fermentation laboratory, Department of Food Science and Technology, Rivers State University located at NkpoluOroworukwo, Port-Harcourt, Rivers State.

Experimental design

Completely randomized design (CRD) consisting of ten (10) treatments(5 each of water and 5 of ethanol extract) replicated two (2) times was used for the study

Treatments

Table 1 Treatments with moringa seed ethanol and water extract on *E.coli*

Treatments	*Escherichia coli* (Ethanol)	*Escherichia coli*(Water)
A	Control	Control
B	25g + 200ml	25g + 200ml
C	50g + 200ml	50g +200ml
D	75g + 200ml	75g + 200ml
E	100g + 200ml	100g + 200ml

Table 2 Treatments with moringa seed ethanol and water extract on *Staphylococcus aureus*

Treatments	*Staphylococcusaureus* (Ethanol)	*Staphylococcus aureus* (Water)
A	Control	Control
B	25g + 200ml	25g + 200ml
C	50g + 200ml	50g +200ml
D	75g + 200ml	75g + 200ml
E	100g + 200ml	100g + 200ml

Table 3 Treatments with moringa seed ethanol and water extract on *Streptococcus spp*

Treatments	*Streptococcus spp*(Ethanol)	*Streptococcusspp*(Water)
A	Control	Control
B	25g + 200ml	25g + 200ml
C	50g + 200ml	50g +200ml
D	75g + 200ml	75g + 200ml
E	100g + 200ml	100g + 200ml

III. Results And Discussion

The study revealed antimicrobial effect of the ethanol and aqueous extract of moringa seed on gram positive bacteria (*Staphylococcus aureus* and *Streptococcus spp*) and gram negative (*Escherichia coli*). The sensitivity pattern of *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus spp* to the ethanol extract of the moringa seed (fig.1) at different concentration showed that *Escherichia coli* exhibited a diameter of inhibitory zone ranging from 5.05mm to 12.10mm, with concentration of 50g/200ml(weight : volume) being higher than all treatments and the treatment containing 100g/200ml (weight: volume) extracts being the least.

Similarly, the inhibitory zone of ethanol extracts for Staphylococcus aureus showed values of 12.30mm for 50g/200ml concentration and 11.10mm for 75g/200ml concentration respectively.

Gram positive Streptococcus showed an inhibitory diameter zone of 10.30mm. The results obtained showed indicated that the concentration level of 50g/200ml, all the tested organisms(E. coli, Staphylococcusaureus, Streptococcus) displayed varying degrees of sensitivity when exposed to ethanol extract of moringa seed. The results obtained are in agreement with those reported by Kalpana *et.al (*2013) who investigated the anti-microbial activity of moringa seed.

Table 4 Biological Test

	E. coli	***Staph aureus***	***Streptspp***
Gram staining	-	+	+
Oxidase	-	-	-
Motility	+	-	-
Catalase	-	+	+
Coagulase	-	+	+

Key + =positive, Key - =negative

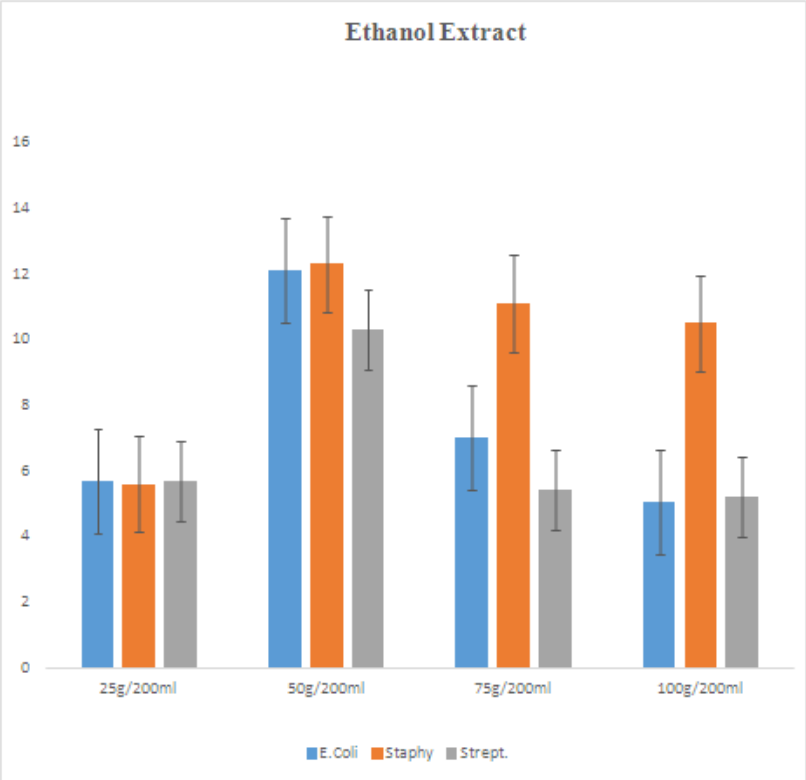

Fig 2: Showing diameter of inhibitory zone of ethanol extract.

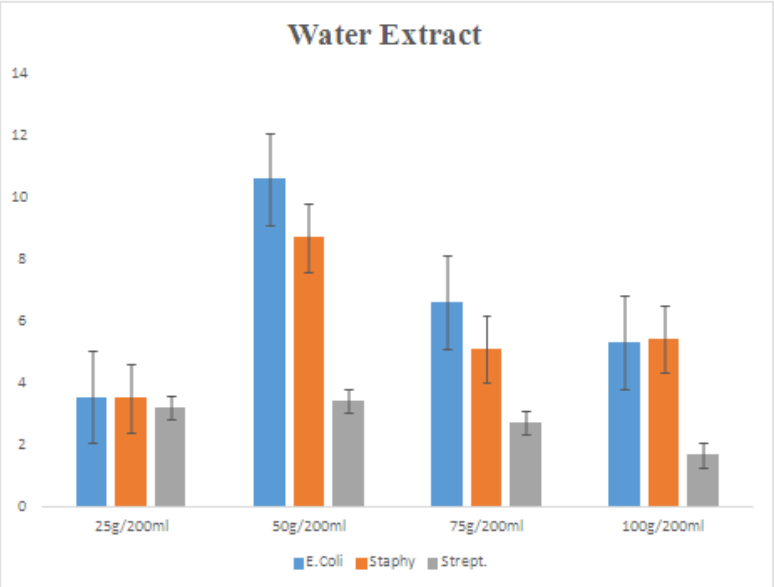

Fig 3: Diameter of inhibitory zone of aqueous extract of moringa seed

The results indicate that the diameter of inhibitory zonedepended on the concentration of crude extracts as the different concentrations of the extracts both aqueous and ethanol exhibited different levels of inhibitory zone. Similar anti- bacterial properties of moringa seed have been reported by Chelliah*et.al*, (2017) who attributed the mechanism of action to the presence of benzlisothiocynate which have the capacity to disrupt microbial cell membrane. Further investigations also revealed that the ant-i microbial activity of moringa seed could be due to the presence of quercetin which have been shown to hinder bacterial growth by blocking nucleic acid synthesis and biofilm formation in fungi (Yang*et.a*l, 2020). However, there were decreasing values of diameter of inhibitory zone from 75g/200ml to100g/200ml for all the pathogens tested both aqueous and ethanol extracts. It would appear that higher concentrations above 50g/200ml contributed to higher nutrient content of

the medium leading to decreasing inhibitory effect.

Results of biochemical test shown in Table 4 indicated that Staphylococcus aureus and Streptococcus were coagulase, catalase, and gram staining positive organisms with both organism being oxidase and motility negative. Biochemical test on E.coli revealed that the organism had gram staining, oxidase, catalase, and coagulase negative properties.

Table 5 shows the result of antibiotic sensitivity testing against the micro-organisms used in the experiment. Amoxacillin had highest diameter of inhibitory zone on gram negative organisms(Staphylococcus aureus and Streptococcus) with a value of 22mm while Pefloxacillin and Tarivi showed least sensitivity with 10mm diameter of inhibitory zone.

Table 5: Showing antibiotic sensitivity

Antibiotic	Gram +ve (mm)	Gram –ve (mm)
E	4	
PEF	8	10
CN	18	R
APX	14	
Z	7	
AM	8	22
R	R	
CPX	12	12
S	10	R
SXT	R	12
SP		15
AU		14
OFX		10

Key
E- Erythromycin
PEF- Pefloxacillin
CN- Centamycin
APX- Ampliclox
Z- Zinox
AM- Amoxacillin
R- Rifaximin
CPX- Ciproflaxacin
S- Streptomycin
SXT- Septrin
AU- Augmentin
OFX- Tarivi

IV. Conclusion:

The result obtained show that ethanol extract of moringa seed exhibited a higher diameter of inhibitory zone than the aqueous extract. Moringa seed extracts from the results had varying degrees of inhibition to all the microbial pathogens tested. The concentration of the extracts affected the affected microbial sensitivity as reflected in the diameter of inhibitory zone values.

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