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ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS ON EXTRACTS OF *Moringa oleifera* Seeds COLLECTED AT THE UNIVERSITY OF ILORIN BOTANICAL CENTER, KWARA STATE, NIGERIA

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ABSTRACT

There had been a rise in the research interest on alternative potential therapeutic agents with specific focus on natural occurring bioactive agents. One of such widely source are seeds of *Moringa oleifera*, which has buffered phytomedical relevance of the plant. The study evaluates an antimicrobial and phytochemical assessment on extracts of *M. oleifera* seeds at the University of Ilorin Botanical center, Kwara State, Nigeria. It employed phytochemical analysis of the extracts using both cold ethanolic and N-hexane extracts. A brief standard microbiological/antimicrobial susceptibility testing (using agar well diffusion) was applied as infected wound microbial potential pathogens used were both bacterial and fungus. The presumptively identified bacterial strains used include: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* while the fungi strain is *Aspergillus niger*. Our report showed presence of saponins, flavonoids, tannins, and alkaloids in both extraction-solvents used. The four strains used were highly senceptible to *M. oleifera* seed crude extracts at 150 mg/ml and 200 mg/ml concentration, among which *P. aeruginosa* showed least sensitivity even at highest concentration of extract in comparison with the antibiotics used as control (ciprofloxacin). It is indicative that extracts of *M. oleifera* seeds possess relevant therapeutic components and shown the antioxidant property due to the presence of specific antioxidising properties. The results of this study have shown the potentials of *M. oleifera* seed extracts as a good antimicrobial lead agent since it inhibit orthopaedic wound associated potential pathogens which suggest their therapeutic relevance in disease control cases.

Keywords: Antibacterial property, Isolates, *Moringa oleifera* seeds, Phytochemical, Phytomedicine

INTRODUCTION

Several plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes (Mehnaz-kamal, 2008). Some plants recognized to be medicinal in populations of wild animals like gorillas and chimpanzees have also been reported to be medicinal in human; effective against various parasitic infections, inflammation, pain and related illnesses (Cousins and Huffman, 2002).

Multi-resistant bacterial strains are a growing public health concern worldwide (Albuquerque *et al.*, 2007; Igere et al., 2020,2022) justifying investments in the search for alternative forms of treatment of infections. As a result, a number of medicinal plants used in indigenous medicine have been tested and found to possess bactericidal properties (Chea *et al*., 2007; Onohuean *et al*., 2022).

Moringa originated from the southern hills of the Himalayas and was introduced in many tropical and subtropical areas, largely by migrant Asian populations (Radovich, 2009; Biswas et al., 2020).

Every part of *M. oleifera*is a storehouse of important nutrients and antinutrients. The leaves of *M. oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron, copper (Kasolo *et al*., 2010). Vitamins like beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E also present in *M. oleifera* (M. Mbikay, 2012). Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present along with anti-cancerous agents like glucosinates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate (Berkovich *et al*., 2013).Different parts of the *M. oleifera* plant is known to possess medicinal properties such as treatment of dysentery, colitis and other health cases (Tankur *et al*., 2016; Zhang *et al*., 2020) which is exhibited by other parts (leaf, pod, bark, gum, flower, seed, seed oil and root) of the plant (Stohs *et al*., 2015). These include pods usage to treat hepatitis and relieve joint pain (Gopalakrishnan *et al*., 2016); roots usage to treat kidney stones, liver diseases, inflammation, ulcers and pain associated with the ear and tooth (Mahajan *et al.*, 2007); laxative activity of seeds and usage in the treatment of tumors, prostate and bladder problems (Popoola *et al*., 2020) etc. Recent pharmacological studies have revealed its antimicrobial potential (Mishra *et al*., 2011) against Gram-positive and Gram-negative bacteria (Adnan *et al*., 2018), antifungal, anti-inflammatory, antioxidant (Banik *et al*., 2018) anticancer (Parvathy *et al*., 2007) fertility and wound healing.

Traditional medicine has been practiced by 80% of the world population, especially in developing countries (Moshi, 2005), and its practice is responsible for 90% of the pharmacological discoveries in the world (Moshi 2005; Fokunang 2011). Moreover *M. oleifera* is a relevant food source for the natural nutrition of the tropics that provides health benefits, as sources of proteins, essential minerals and antioxidants (Fahey, 2005; Anwar *et al*., 2007). Seedextracts of *M. oleifera* tree have been found to be one of the most effective clarifiers and its effectiveness in treating water since early 1970's (Henderson *et al.*,2010). Such early studies established its effectiveness as a coagulant for treatment of water with high

levels of turbidity. Furthermore, toxicological assessments indicate that the use of *M. oleifera* as a primary coagulant does not pose a human health threat (Rondeau *et al.,* 2001). Although much has been reported, the seed extract of *M. oleifera* grown in western region of Nigeria has poorly been reported in litertures since most of the growth is within the region. It is to this end the study evaluates the antimicrobial and phytochemical analysis on extracts of *M. oleifera* seeds collected at the University of Ilorin Botanical center, Kwara State, Nigeria.

MATERIALS AND METHODS

The materials used for this research include: *M. oleifera* seeds, petri dishes, sterile swab-sticks, sensitivity disks, inoculating loop, glass wares, autoclave, incubator, rotary evaporatorand other chemicalsagents (Draggendorff's reagent, dimethyl sulfoxide, ammonium hydroxide, ethanol and n-hexane).Media used were Mueller-Hinton agar (Lab M Expiry date 10-2015), Nutrient Agar (Lab M Expiry date 12-2015).The seeds of *M. oleifera* were collected at University of Ilorin Botanical center, Kwara State, Nigeria and allowed to air dry for 3 days. The pods of the seeds were manually peeled and 100g of the seeds was weighed and left to air dry for 3 days, the weight reduced after air drying.

Ethanol and n-hexane Extraction of *Moringa oleifera* oil

Extraction and isolation: The dried seed was ground into a fine powder using a scientific electric blender. The 30g of the seed powder were soaked separately in 300ml of ethanol and N-hexane respectively for 72 h at room temperature. Obtained filtered extracts were concentrated using a rotary evaporator.
Microorganisms: Bacterial isolates used for the antibacterial assay were isolated from the wounds of some orthopaedic in-patients of the Igbobi General Hospital, Lagos, Nigeria. Wound swab samples of the patients were cultured on nutrient agar.

Phytochemical Constituent

An initial qualitative phytochemical screening of the extract of the *Moringa oleifera* plant reported the presence of alkaloids, tannin, saponin and flavonoids and it was performed on the extracts as follows:

Determination of Saponins

The ability of saponins to produce frothing in aqueous solution was used as screening test for saponins. About 0.5 g of each plant extract was shaken with distilled water in a test tube, frothing which persisted on warming was taken as evidence for the presence of saponins. The determination of the foam index was made adaptingthe methology described in Brazilian Pharmacopoeia (Brasil, 2010a).

Determination of Tannins

Five grams of each portion of plant extract was stirred with 100 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black green precipitate indicated the presence of tannins. (Jonathan, 2009)

Determination of alkaloids

A 0.5 g of extract was diluted with 10 ml of acid alcohol, boiled and filtered. Two milliliter of diluted ammonia was added to 5 ml of the filtrate. Five milliliter of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Meryer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Meryer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was taken as positive for the presence of alkaloid (Aiyelaagbe and Osamudiamen, 2009).

Determination of flavonoids

A 2 g of powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5ml of 20 % sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids (Trease and Evans,1989).

RESULTS AND DISCUSSION

Results

Our results are presented in tables and reported in simple percentage as shown in Table 1 (showing antimicrobial activity of ethanolic extract of *Moringa oleifera* seed oil); Table 2 (showing antimicrobial activity of N-hexane extract of *Moringa oleifera* seed oil); Table 3 (showing *Moringa oleifera* seed oil ethanolic and N-hexane extracts concentrations at 50,100, 150, 200 mg/ml); Table 4 (showing phytochemical analysis of *Moringa oleifera* seed oil using two solvent extractions).

Table 1: Antimicrobial activity of ethanolic extract of *Moringa Oleifera* seed oil

Organisms	Ethanolic acid extract Diameter of zone of inhibition (mm)				Control (Ciprofloxacin) Diameter of zone of inhibition (mm)
	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	
Staphylococcus aureus	5.2	9.0	11.0	16.0	16.0
Pseudomonas aeruginosa	5.1	5.6	5.9	6.2	12.0
Bacillus Subtilis	5.5	6.2	7.4	11.0	R
Escherichia coli	5.7	6.4	10.0	15.0	14.0
Aspergillus niger	6.0	10.0	11.0	14.0	None used

Key: numbers indicate measured one of inhibition in mm, R represents resistance

Table 2: Antimicrobial activity of N-hexane extract of *Moringa Oleifera* seed oil

Organisms	N-Hexane extract Diameter of zone of inhibition (mm)				Control (Ciprofloxacin) Diameter of zone of inhibition (mm)
	50mg/ml	100mg/ml	150mg/ml	200mg/ml	
Staphylococcus aureus	6.2	7.0	11.0	17.0	16.0
Pseudomonas aeruginosa	6.4	6.2	8.5	13.0	12.0
Bacillus Subtilis	5.1	7.5	10.0	12.0	R
Escherichia coli	6.1	8.0	11.0	14.0	14.0
Aspergillus niger	6.5	8.5	10.0	15.0	None used

KEY: numbers indicate measured one of inhibition in mm, R represents resistance

Table 3: *Moringa oleifera* seed oil ethanolic and N-hexane extracts concentrations at 50, 100, 150, 200 mg/ml

Ethanolic oil extract Stock solution (282.5mg/ml)				N-hexane oil extract Stock solution (415mg/ml)			
50	**100**	**150**	**200mg/ml**	**50**	**100**	**150**	**200mg/ml**
0.71	1.42	2.12	2.83	0.24	0.48	0.72	0.96

Table 4: Phytochemical analysis of *Moringa oleifera* seed oil using two solvent extractions

Solvent used	Component	% composition
Ethanolic acid extract	Saponins	2.0
N-hexane extract		5.0
Ethanolic acid extract	Flavonoids	1.0
N-hexane extract		0.6
Ethanolic acid extract	Tannins	-
N-hexane extract		-
Ethanolic acid extract	Alkaloids	
N-hexane extract		0.4

Discussion

Moringa oleifera seed ethanol extract (MSE) showed little inhibitory effect on the entero-pathogens at extract concentration of 50 mg/ml with zone of inhibition <6mm which was in comparison to (Napolean *et al*., 2009) that shows *Moringa oleifera* seed ethanol (MSE) extract was active against three bacterial isolates with *S. aureus* (10mm) and *E. coli* (07mm) being sensitive to the lowest concentration of 50mg/ml. The *Moringa oleifera* seed ethanol extract had its highest inhibitory effect against *Staphylococcus aureus* and *Aspergillus niger* at extract concentration of 100, 150, 200mg/ml with zone of inhibition ranging between 9.0-16.0mm as indicated in Table 4.1. This shows the seed extract has high antibacterial and antifungal potential. The MSE also had a good inhibitory effect against *Escherichia coli* and *Bacillus subtilis* at extract concentration of 100mg/ml and 150mg/ml with zone of inhibition ranging between 10mm to 15mm which indicated that the extract was highly effective against enteropathogens and could be applied in the treatment of enteric infections and food-borne infections. A fact noted was that *Pseudomonas aeruginosa* was resistant to MSE at all concentrations showing that there was little or no inhibitory effect indicating that *M. oleifera* extracts tested might have limited effect on the proliferation and activities of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is well known as a hardy and difficult organism that constitutes problems even to researchers (Odjadjare *et al*., 2012). It was also observed that the organisms were sensitive except *Bacillus subtilis* which was resistant to the control (ciprofloxacin) which belongs to a broader bacterial spectra (quinolones, they work by interfering with bacterial DNA gyrase, preventing the super coiling of DNA, a required step for packaging DNA in the bacterial cell) exhibited a great effectiveness in the treatment of enteric infections, food-borne related infections and gastro-intestinal infections. Therefore *Moringa oleifera* seed ethanol extract in comparison with *Moringa oleifera* seed n-hexane extract had highest inhibitory effect against all organisms at extract concentration of 150mg/ml and 200mg/ml as indicated in Table 2. N-hexane also showed little inhibitory effect at extract concentration of 50mg/ml with zone of inhibition <6.5mm. At extract concentration of 100mg/ml, the inhibitory effect was considerably good with zone of inhibition of ≤8.5mm. The N-hexane extract also had the highest inhibitory effect against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* (at extract concentration of 150mg/ml and 200mg/ml with zone of inhibition between 8.5-17mm which are among the most common pathogenic organisms that cause infection and therefore this result yield indicates *Moringa oleifera* seed N-hexane extract as a good antimicrobial agent with less adverse effects and can be used in the treatment of wound infections, skin infections and this was inconcordance with (Elijah *et al*., 2025) who evaluated wound healing property from the aqueous extract of leaves of *M. oleifera* on male Swiss albino mice and reported significant increase in wound closure rate, skinbreaking strength, fungal infections and enteric infections. Similarly, (Dollah *et al*., 2020) investigated antipyretic and wound healing activity from the ethanolic and ethyl acetate extracts of *M.oleifera* seeds and reported that the ethanolic and ethyl acetate extracts of seeds showed significant antipyretic activity in rats. The oil extract also exhibited a high inhibitory effect against *Pseudomonas aeruginosa* and *Bacillus subtilis* with zone of inhibition ranging between 8.5-13mm which is indicative that it could be used in the treatment of food-borne infections. The antimicrobial activity of the extracts tested, which reveal bioactivity on organisms such as *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *S. typhimurium* and *E. aerogenes* is encouraging as these organisms range from pathogenic and toxigenic organisms liable to cause food - borne illnesses to spoilage-causing organisms liable to spoil food products, The control of these organisms the extracts in foods would reveal the potentials of these extracts as preservatives (Fokunang *et al*., 2011; Adnan *et al*., 2018). In comparison with the control (ciprofloxacin) used it is indicative that the antibiotics as well as the extracts are both good mediums that can be used in the

treatment of infections, Phytochemical analysis for quantitative detection of saponins, flavonoids, tannins and alkaloids were perfomed on the extracts, The phytochemical activity of the ethanolíc and n-hexane extracts of *M. oleifera* seed is presented in Table 4.4, The table indicates presence of saponins due to the presence of frothing and flavonoids which was as a result of a yellow solution formed in both extracts with a percentage of <5%, Saponins were detected in MSE in agreement with report by (Napolean *et al*., 2009).Alkaloids were reported in the present study which was not determined by (Napolean *et al*., 2009),Tannin was absent in both extracts, 0.4% of alkaloid was found in the n-hexane extract only after the formation of a reddish-brown precipitate (with Draggendorff's reagent). (Farooq *et al*., 2007) reported that plants occur in varying habitats, a great magnitude of variation in the concentration and composition of phytochemical ingredients in the different parts of such plant is expected. Moreover, (Wang et al 2020) reported that phytochemicals are produced in response to perceived threats by the plants, therefore variation exist in the production of these phytochemicals depending on the type and amount of threat encountered by the plant, The presence of phyto-chemicals in the seed (saponin, flavonoids, alkaloids) % yield indicated its antioxidant effect and its nutraceutical use.

CONCLUSION

The results of the present study have shown the potentials *Moringa oleifera* seed ethanol and n-hexane extracts possess as good antimicrobial agent and phytochemical source. This study has been able to justify the investments in the search for alternative forms of treatment of infections. Therefore *Moringa oleifera* being one of researched medicinal plants is considered a rich source of nutrition and natural energy and is also known to possess anti-oxidant, antibacterial andantifungal activities and is highly recommended for all.

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