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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EVALUATION OF *MORINGA OLEIFERA* SEED EXTRACTS

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Abstract

Infectious diseases caused by pathogens such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, etc. are a major public health concern worldwide. Moringa oleifera having been used as medicinal plant was subjected to extraction with polar (methanol) and nonpolar (chloroform) solvents. The extracts were used for phytochemical screening and antimicrobial analysis following standard procedure. Phytochemical screening results of methanolic extract showed that Moringa oleifera contains the presence of alkaloids, saponins, flavonoid, terpenoids and steroid while tannins, phenol and glycosides were absent. However, alkaloids and saponins were the only substances present in the chloroform extract. The antibacterial result showed that methanol extract inhibits the action of Escherichia coli (16 mm), Staphylococcus (12 mm), Klebsiella (4 mm), Salmonella typhi (10 mm), Proteus (18 mm) and Streptococcus (15 mm). The chloroform extract has no zone of inhibition (activity) with Salmonella typhi and Proteus, but showed zone of inhibition with Escherichia coli (15 mm), Staphylococcus (9 mm), Klebsiella (6 mm) and Streptococcus (10 mm). However, both extracts show no zone of inhibition on Pseudomonas. Antifungal activities showed that both the methanol and chloroform extracts demonstrated inhibitory effects against Rhizopus oryzae, with inhibition zones of 10 mm and 15 mm, Fusarium oxysporum 20 mm and 16 mm, Aspergillus parasiticus 8 mm and 11 mm respectively. This indicates that the activity of the extract is influenced by the solvent used for the extraction. It is recommended that Moringa oleifera be employed in the production of antimicrobial medications due to its antibacterial action and phytochemical composition.

Keywords: *Moringa oleifera, antifungal, antibacterial, Chloroform*

Introduction

Over 80% of the global population depend on medicinal plants for curing and maintenance of their healthcare needs (Shanmugavel *et al.*, 2018). The plant-source medicines are known for their reliability, accessibility, and affordability. These therapeutic benefits of these plants are associated to the presence of bioactive phytochemicals, which have specific physiological effects on the human body. Major bioactive secondary metabolites found in plants include alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds (Helal *et al.*, 2019;). Infectious diseases have been found to be caused by pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Streptococcus pyogenes* and has been are a major health concern worldwide (Mestrovic *et al.*, 2019). *Moringa oleifera* commonly known as the drumstick tree, a member of the

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Moringaceae family, native to Northern India's sub-Himalayan tracts. It is one of 13 species in the genus and has spread to tropical and subtropical regions up to 2000 m in elevation (Leone *et al.*, 2015). It is widely recognized for its nutritional and medicinal benefits, the seeds of *Moringa oleifera* are particularly noteworthy due to their high content of essential nutrients, bioactive compounds, and potential health benefits (Rockwood *et al.*, 2013). It is a powerful antidote to malnutrition because almost all its parts (leaves, pods, and seeds) contain a range of vital phytochemicals. The resistance of antibiotic by pathogens has resulted to the high rate of mortality and morbidity rates (Singh *et al.*, 2018). The WHO estimates that infections caused by multidrug-resistant (MDR) bacteria result in approximately 700,000 deaths globally each year, affecting people of all ages, including 200,000 newborns (Romandini *et al.*, 2021). Thus, the quest for natural products from botanical source used by traditional healers represent a potential treatment for diseases caused by these MDR bacteria. Many foods, including fruits and vegetables, are rich in antioxidants. Plants and animals maintain sophisticated systems of various antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E, as well as enzymes like catalase, superoxide dismutase, and various peroxidases. Traditional herbal treatments and dietary foods were the primary sources of antioxidants and antimicrobials for ancient peoples, protecting them from free radical damage and antibiotic resistance (Yadav *et al.*, 2016). In response to these challenges, the present work is investigating the polar and nonpolar constituents of *Moringa olifera* as a potential antimicrobial agents

MATERIALS AND METHOD

Sample Collection

Moringa oleifera seeds was gotten from Surulere street, Odo Emure, Emure Ekiti Local Government area, Nigeria and was brought to Chemistry laboratory, Federal Polytechnic Ado Ekiti., Ekiti State Nigeria.

Sample Preparation

Moringa oleifera seeds were air-dried at room temperature and pulverized into powder for extraction. The powder (500g) was macerated in 80% methanol and allowed to stand for 72h at room temperature. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator to get a golden semi-solid extract. Solvent partitioning of the crude methanol extract was performed as demonstrated by Kupchan and Tsou [12]. Fractionation was carried out using chloroform, and 20% aqueous methanol (v/v). Crude extract (25 g) was weighed and dissolved in 250 ml of 20% aqueous methanol (v/v) to form a stock solution. Then, 250ml of chloroform was added to the solution and poured into a separating funnel. The mixture was allowed to stand for 20 min for proper separation, and the upper part was collected in a beaker. The lower part being the aqueous methanol part was collected.

Phytochemical Screening of Extracts

Phytochemical profiling of methanol and chloroform extract of *Moringa oleifera* seeds lwere carried out using the procedures as performed by Harborne.

Microbial strains used for Anti-microbial activity

The bacterial strains of *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Proteus*

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vulgaris and *Aspergillus niger* were obtained from Ekiti State University Teaching Hospital (EKSUTH) and bring to Microbiology Laboratory at Federal Polytechnic, Ado-Ekiti. Every single bacterial sample was kept and maintained in nutrient agar slants. On PDA medium plates, the fungus sample was kept and maintained.

Anti-bacterial activity

For anti-bacterial activity, the cup plate agar diffusion method is used. A Petri plate was filled with the prepared nutrient agar medium after being inoculated with 18-hour-old cultures of the test organism. In a laminar airflow, at room temperature, the plates' medium was given time to harden. Four 5mm-diameter cups were created on each plate at an identical spacing when the media solidified. The stock solutions with different concentrations were prepared (800 µL, 600 µL and 400 µL). Using sterile micropipettes, 50 µL of each concentration was added to the cups. Each plate containing one cup was utilized for the standard drug. The prepared Petri plates were placed in an incubator and kept at 37°C for 24 hours before being measured for inhibitory zones and analyzed.

Anti-fungal activity

For anti-fungal activity, potato dextrose agar medium is utilized and 50 µL of the fungal test organism, *Aspergillus niger*, *Aspergillus Flavus*, *Aspergillus parasiticus*, *Aspergillus sclerbtiicarbonarius*, *Rhizopus oryzae* and *Fusarium oxysporum* which was made from 48-hour-old cultures, is inoculated with it and transferred into sterile petri plates. The same procedure is repeated as anti-bacterial activity, from solidification of the medium to preparing different concentrations of stock solutions. Stock concentrations were put into the cups using sterile pipettes. In each plate, one cup is left for control. As a result, the plates were incubated at 35 °C for 24 hours. The studies were carried out in duplicate, and tabular records of the average diameters of the zones of inhibition were made.

Preparation of the Media

Nutrient agar was prepared according to manufacturer's instruction which says dissolve 28g in 1000ml of distilled water by using 25 plates and 20 ml of water that will be 20 multiply by total number of plates which will be 20 × 25 = 500 ml

Therefore $\frac{28\ g}{1000\ ml} \times 500\ ml$ = 14 g

Potato dextrose agar was prepared according to manufacturer's instruction which says dissolve 39 g in 1000 ml of distilled water by using 25 plates and 20 ml of water per plate that will be 20 multiply by total number of plates which will be 20 × 25 = 500 ml

Therefore $\frac{39\ g}{1000\ ml} \times 500\ ml$ = 19.5 g

14g of nutrient agar was dissolved in 500ml of distilled water inside conical flask and it was allowed to dissolved uniformly. It was then homogenized and sterilized using autoclave for 15minutes. Similarly, 19.5 g of potato dextrose agar was dissolved in 500ml of distilled water inside conical flask and it was allowed to dissolved uniformly. It was then homogenized and sterilized using autoclave for 15minutes.

Sterilization and preparation of plate for microbial analysis

Petri dish was washed with soap and rinse with running water, it was then sterilized using hot air at 160 ^{0}C for 1 hour. After, it was then removed and it was allowed to cool. The sterilized media was poured into the sterilized dish in a sterilized manner like nose mask, gas on and swab the table using ethanol. The media (nutrient agar and potato dextrose gar) was allowed to

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solidified. Stricking plate technique used to introduced the organisms on the solidified media. The introduction of the species (*Moringa oleifera* seed extracts) was done using well diffusion technique. The well was bored using 6mm cork borer and 200 ml micropipette was used to pipette each species to the well and was well labeled according to the species it was then incubated at 37 ^{o}C for 24 hours for bacteria and for 48 hours for fungi and zone of inhibition were measured in mm.

STATISTICAL ANALYSIS

The data obtained from the various experiments were computed and subjected to statistical analysis using SPSS 20.0 (2014).

RESULTS AND DISCUSSION

Phytochemical screening results of methanolic extract showed that *Moringa oleifera* contains the presence of alkaloids, saponins, flavonoid, terpenoids and steroid while tannins, phenol and glycosides were absent. However, alkaloids and saponins were the only substances present by using chloroform as a solvent while the rest investigated were absent. Therefore, methanol is the best solvent for the extraction of these phytochemical from *Moringa oleifera*. Similar studies have been reported (Uttu *et al.,* 2022). The presence of these diverse secondary metabolites in the plant justifies its use in folklore medicine.

Table 2 and 3 showed the antimicrobial sensitivity test of *Moringa oleifera* leaf extracts against species of bacteria and fungi. In table 2, methanol extract showed (16 mm) with *Escherichia coli*, *Staphylococcus* (12 mm), *Klebsiella* (4 mm), *Salmonella typhi* (10 mm), *Proteus* (18 mm) and *Streptococcus* (15 mm). The chloroform extract has no zone of inhibition (activity) with *Salmonella typhi* and *Proteus*, but showed zone of inhibition with *Escherichia coli* (15 mm), *Staphylococcus* (9 mm), *Klebsiella* (6 mm) and *Streptococcus* (10 mm). However, both extracts show no zone of inhibition on *Pseudomonas*. This indicates that the activity of the extract is influenced by the solvent used for the extraction (Malliga *et al.,* 2014).

The table 3 presents the antifungal activities of *Moringa oleifera* leaves extracts using methanol and chloroform against various fungal species. Both methanol and chloroform extracts showed no inhibition against *Aspergillus Flavus*. This indicates that *Moringa oleifera* extracts may not be effective against this particular fungal strain. The methanol extract displayed a zone of inhibition of 8 mm against *Aspergillus parasiticus*, while the chloroform extract exhibited a slightly higher inhibition zone of 11 mm. This suggests that the chloroform extract may be more potent in inhibiting the growth of *Aspergillus parasiticus* compared to the methanol extract. The chloroform extract showed a substantial inhibition zone of 15 mm against *Aspergillus sclerbtiicarbonarius*, while the methanol extract did not exhibit any inhibition. The chloroform extract's efficacy could be due to the presence of non-polar compounds that are more effective against *Aspergillus scleroticarbonarius.* Both the methanol and chloroform extracts demonstrated inhibitory effects against *Rhizopus oryzae*, with inhibition zones of 10 mm and 15 mm, respectively. This indicates that both extracts have antifungal properties against this species, with the chloroform extract showing slightly higher efficacy. The methanol extract displayed a significant inhibition zone of 20 mm, while the chloroform extract exhibited an inhibition zone of 16 mm against *Fusarium oxysporum*. This suggests that the methanol extract of *Moringa oleifera* seed may be

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more effective in inhibiting the growth of this fungal strain compared to the chloroform extract. Ojiako (2014) in his study of phytochemical and antimicrobial screening of *Moringa oleifera* leaves extracts found similar results. In his study, ethanol extract showed (9 mm) with *Staphylococcus* (9mm), *E. Coli* 4 mm, *Salmonella typhi* 6mm, *Mucor* 3 mm and *Candida* 3 mm. The n-hexane extract has no zone of inhibition (activity) with *Staphylococcus aureus* and *Escherichia coli*, but showed zone of inhibition with *Salmonella typhi*, *Mucor*, and *Candida* 2 mm. Malliga *et al.*, (2014) reported that the activity of the extracts is influenced by the solvent used for the extraction. All the antimicrobial activities was none the less connected with the presence of the phytochemicals (alkaloids, flavonoids, saponins, among others) identified in the methanol extract.

CONCLUSION

The study on the antimicrobial activity of polar and nonpolar phytoconstituents extracted from *Moringa oleifera* leaves provides evidence that the plant exhibits significant antimicrobial properties, influenced by the solvent used for extraction. The methanol extract showed higher antibacterial activity against *Escherichia coli*, *Proteus*, and *Streptococcus* while the chloroform extract was generally less effective compared to the methanol extract. The methanol extract exhibited strong antifungal activity against *Fusarium oxysporum* and moderate activity against *Rhizopus oryzae* and *Aspergillus parasiticus*. The chloroform extract, on the other hand, showed higher efficacy against fungi such as *Aspergillus scleroticarbonarius*, *Aspergillus parasiticus*, and *Rhizopus oryzae*. The study showed that methanol, a polar solvent, and chloroform, a nonpolar solvent, each extracted different sets of bioactive compounds, resulting in varied antimicrobial activities against different microorganisms.

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Table 1: Results of phytochemical screening of *Moringa oleifera* leaves extracts

Secondary metabolites	**Test**	**Methanol extract**	**Chloroform extract**
Alkaloid	Wagner's test	+	+
Steroid	Liebermann-burchard test	+	–
Saponin	Foam test	+	+
Phenol	Ferric chloride test	–	–
Flavonoid	Alkaline test	+	–
Glycosides	Keller test	–	–
Terpenoid	Salkowki's test	+	–

Key: + -= present, - =Absent

Table 2: Result of antibacterial activities of *Moringa oleifera* leaves extracts

	Zone of inhibition (mm)	
Organisms	**Methanol extract**	**Chloroform extract**
Escherichia coli	16± 1.11	15± 0.13
Staphylococcus	12± 1.00	9± 0.19
Klebsiella	14± 1.15	6± 0.11
Pseudomonas	–	–
Salmonella typhi	10± 1.15	–
Proteus	18± 1.00	–
Streptococcus	15± 1.10	10± 0.10

Table 3: Result of antifungal activities of *Moringa oleifera* leaves extracts

	Zone of inhibition (mm)	
Organisms	**Methanol extract**	**Chloroform extract**
Aspergillus Flavus	–	–
Aspergillus parasiticus	8± 0.10	11± 0.13
Aspergillus sclerbtiicarbonarius	–	15± 0.11
Rhizopus oryzae	10± 0.12	15± 0.16
Fusarium oxysporum	20± 0.17	16± 0.15

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