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Phytochemical and Antifungal Efficacy of Different Parts of *Moringa oleifera* Plant Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author TOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NIO and TMA are the supervisors of the work, author OOO managed the literature searches and carried out the laboratory work under the supervision of the supervisors. All authors read and approved the final manuscript.

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ABSTRACT

Antifungal potential of *Moringa oleifera* and the determined Phyto-consituents of its parts in different solvents has shown its prowess economically. *Moringa oleifera Lam (Moringaceae)* seed, leaf and stem bark were evaluated for antifungal efficacy and phytochemical constituents. Phytochemical screening of *Moringa oleifera* and its antifungal efficacy against *Aspergillus flavus* was investigated using poison food technique method. Result of the phytochemical screening exposed the presence of tannins, alkaloids, flavonoids, reducing sugar, saponins and cardiac glycosides. Reducing sugar and steroids were present in all the extract; cardiac glycoside was present in all the extract except stem bark extracts. The antifungal assay result shows that *M.*

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oleifera leaf ethanol leaf extract exhibited broad spectrum activity against the test organism. The physical characteristics of the Moringa is an express indication of the presence of acidic compounds which could importantly serve as a natural preservative of fruits. Studies can be carried out on the isolation, purification, and characterization for further expository of the active ingredients.

Keywords: Moringa oleifera; seed; leaf; stem bark; phytochemistry; antifungal.

1. INTRODUCTION

Suffice to note that, Plants and its components have tremendous chemical substances that have medicinal and health benefits, [1]. In recent times, emphasis is now based on using a safer, natural option for extending the shelf-life of fruits, owing to safety on health and lesser side effects [2]. Over the years, medicinal plants with antimicrobial properties have been utilised. However fungal infections on fruits are the major cause of postharvest loss of both qualities as well as the quantity of fruits. *Aspergillus flavus* is highly legion in the climatic region of Nigeria; they are found to be toxin-producing fungi and are associated with the visible mould discolourations which prompt the chemical and nutritional changes in food [3].

The chemical substances that protect fruits from pathogenic attack have some physiological action on the fruits, which has led to the isolation of the chemical components and establishment of their biological potency. The vital secondary metabolites in plants are the alkaloids, tannins, flavonoids and phenolic compounds [4]. The phenolic compounds according to literature are the most numerous and structurally diverse plant phytoconstituents [5]. It presupposes therefore that, the phytoconstituents will help determine their biological activities and medicinal properties.

Moringa oleifera is a multipurpose tree and has enormous antimicrobial properties. It is a fast-growing, deciduous tree growing up to 5-12 m with an open umbrella-shaped crown native to India, Africa, Arabia, South East Asia, South American and the Pacific and Caribbean Islands [6]. It is known as miracle tree, all the plant parts are edible (leaves, seeds, flowers, pods) and derive useful products for human consumption [7]. The plant has deep wells of chemical substances that possess both preventive and curative substances, pending on the organic solvents used to extract them. The leaves are known to be highly nutritional and have antioxidant properties and are a natural antihelmintic antibiotic, detoxifier representing an outstanding immune builder [8]. The objective of this study is aimed to evaluate the efficacy of different parts of *Moringa oleifera* plant extracts against *Aspergillus flavus* and to qualify the phytochemical constituents of the plant extract.

2. MATERIALS AND METHODS

2.1 Collection, Authentication and Processing of Plant Materials

Fresh leaves, seeds and bark of *Moringa oleifera* Lam were harvested from the Experimental Farm of Federal University of Agriculture, Makurdi. The plant materials were identified and authenticated by a botanical taxonomist of the Biological Sciences Department, Benue State University Makurdi. His analysis and description were in consonance with those found in various literature [9] and [10]. The plant materials were washed under running water and dried under shade for about one week and then pounded into powdered form, using a mortar and pestle, then ground using an electric grinder and stored for further use. The powdered plant material (100 g) was extracted in 1 L ethanolic solvents, ethyl acetate solvents, and distilled water by maceration and shaken for 24 hours by a vibrator. The supernatant obtained was filtered with Whatman's No 1 filter paper into evaporating dishes and allowed to dry in hot air at a temperature of 45°C [11].

2.2 Test Culture

The pure fungal cultures of *Aspergillus flavus* was isolated from rotten tomato fruit using the standard method [12]. The isolates were maintained in a freshly Potato Dextrose Agar slant and kept for further use.

2.3 Standardization of the Inoculum

The inoculum was prepared from a stock culture which was maintained on PDA slant and subculture on to PDA using sterilized wire loop.

2.4 Media for Test Organism

A mass of 19 g of PDA was added to 500 ml of sterile distilled water and autoclaved at 121°C for 15 minutes. After autoclaving, the media was allowed to cool down and mixed, poured into sterile Petri dishes approximately 4mm and allowed to set at an ambient temperature and used.

2.5 Phytochemical Analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was carried out on the extracts as described by [13,14,15].

2.5.1 Test for tannins

About 0.01g of the crude extract was boiled in 20ml of water in a boiling tube. Few drops of 0.1% of $FeCl_3$ were added. Formation of brownish green or blue-black colouration indicated the presence of tannins.

2.5.2 Test for saponins

About 0.01g of the crude extract was boiled in 20ml of distilled water in a water bath, and then it was mixed with 5ml of distilled water in a water bath. Then it was mixed with 5 ml of distilled water and it was shaken well stable persistent forth indicated the presence of saponins.

2.5.3 Test for flavonoids

About 0.01 g of the crude extract was dissolved in 2 ml of ethanol solvent. A concentrated HCl and magnesium turning was added. A yellow colouration in the extract indicated the presence of flavonoids.

2.5.4 Test for phytosteroids

About 0.01 g of crude extract was dissolved in 2 ml of ethanol solvent. A volume of 2 ml of acetic anhydride and 2 ml of concentrated H_2SO_4 were added. The colour changes from violet to blue or green indicated the presence of steroids.

2.5.5 Test for cardiac glycosides

Exactly0.01 g of crude extract was dissolved in 2 ml of ethanol and 2 ml of glacial acetic acid which contained a drop of $FeCl_3$ solution was added. This was underplayed with 1ml of concentration H_2SO_4. A brownish ring of the interface indicated the presence of cardiac glycosides.

2.5.6 Test for alkaloids

About 0.01 g of crude extract was dissolved in 2 ml of ethanol and it was divided into parts; few drops of Wagner's reagent along 1 ml of extract.

2.5.7 Test for Phenolics

Ferric chloride test; treat the extract with 3-4 drops of ferric chloride solution, a formation of bluish black coloured indicate the presence of phenols.

2.5.8 Detection of Reducing Sugar

Benedict's test filtrate was treated with Benedict's reagent and boil in a thermostatic water bath for 5minutes. Formation of an orange-red precipitate indicated the presence of reducing sugars.

2.6 Evaluation of Antifungal Activity of Plant Extracts

For evaluating antifungal activity of plant extracts, poisoned food technique adopted from [16] was used. The principle involved in this technique is to poison the nutrient medium with plant extract and to allow a test fungus to grow on such a medium. Potato-Dextrose-Agar (PDA) medium was prepared and sterilized. A volume of 15 ml of PDA was added to 3 ml of the extract in the Petri plates and thoroughly mixed by stirring and allowed to cool down. A small disc of the pure fungus culture (7 mm) was cut with a sterile cork borer and transferred aseptically in the centre of the petri dish containing 18 ml of the medium mixed with leaf extract. Suitable checks were kept where the culture discs are grown under same conditions on PDA medium without extract. This serves as a control. These Petri dishes were then kept in an incubator for 72 hours at 28ºC ± 1ºC. After that, the diameter of the fungal colony was measured and compared with that of control, which was devoid of plant leaf extract. Then the percent mycelial inhibition was calculated using the following formula introduced by [17].

$$\text{Percent inhibition of mycelial growth} = \frac{Gc - Gt}{Gc} \times 100$$

Where,

Gc = Mycelial growth in terms of colony diameter in control set.
Gt = Mycelial growth in terms of colony diameter in treatment set

2.7 Statistical Analysis

Each experiment has three replicates and three determinations were conducted. Means of variable and standard deviations were recorded (R programming language version 3.0.3).

3. RESULTS AND DISCUSSION

Analysis of the physical characteristics of the plant extracts at different solvent showed low yield of the extract because of the method of extraction used (maceration) which agrees with [18]. The water extract of the leaf had the highest yield of 18.57% while seed ethylacetate extract had the least percentage yield of 3.51%. From Table 1 the percentage yield of leaf extracts, extracted with different solvents (water, ethylacetate, ethanol and methanol) were higher than that of the seed and bark extracts. The extracts colour ranged from dark green, light green and creamy for the leaf, bark and seed respectively. The pH values of the *M. oleifera* extracts (4.45 -6.07) were below the neutral value (<7), indicating the plant extracts were found to be acidic. Most plant extracts are weak acids or bases which form ions by dissociating slightly when dissolved in water [19]. The presence of acidic compounds in the samples as observed by Soncini, et al. [20] in *Averrhoa carambola*. This shows that *M. oleifera* could serve as a preservative.

3.1 Antifungal Activity

Table 2, showed the antifungal efficacy of different *M. oleifera* (Bark Seed and Leaf) plant parts extract on mycelia growth of *Aspergillus flavus.*

Methanolic, ethyl acetate, ethanolic and water extract of *Moringa oleifera* leaves, seeds, bark were found to be effective against the test fungi. The water extract of the seed (6.01±0.92) showed the least inhibition against the test fungi. The leaf and bark ethanolic and methanolic extract show the highest inhibition activity (100%) against the test organism respectively. There

Table 1. Physical characteristics of *Moringa oleifera* extract

Plant part	Solvent (l litre)	Initial weight (g)	Final weight (g /% yield)	Colour	pH
Leaf	Water	100.00	18.57	Dark Green	4.45
	Ethylacetate	100.00	3.65		----
	Ethanol	100.00	4.76		5.30
	Methanol	100.00	8.07		6.01
Seed	Water	100.00	9.33	Creamy	5.90
	Ethylacetate	100.00	3.51		5.40
	Ethanol	100.00	4.68		6.07
	Methanol	100.00	6.19		5.95
Stem Bark	Water	100.00	5.25	Light Green	4.98
	Ethylacetate	100.00	6.50		5.06
	Ethanol	100.00	4.50		5.77
	Methanol	100.00	6.00		5.74

Table 2. The antifungal efficacy of different *M. oleifera* (Bark Seed and Leaf) plant parts extract on mycelia growth of *Aspergillus flavus*

Parts/Solvents	Zone of inhibition (%)				
	Control	Water	Ethylacetate	Ethanol	Methanol
Leaf	0.00^{a}±0.00	40.40^{b}±0.99	56.33^{c}±1.28	100.00^{b}±0.00	100.00^{b}±0.00
Seed	0.00^{a}±0.00	6.01^{a}±0.95	33.34^{b}±1.08	38.80^{a}±1.15	9.28^{a}± 0.83
Bark	0.00^{a}±0.00	67.72^{c}±0.92	28.04^{a}±0.92	100.00^{b}±0.00	100.00^{b}±0.00

*Values are mean ± standard deviation of triplicate samples. Means with similar superscripts are not significantly different (p>0.05). LS= level of significance; *=significant at alpha=0.05*

was no significance difference (P≤0.05) in the percentage zone of inhibition among the control, a plate without extract. The highest inhibitory percentage for water solvent extraction was observed in the bark extract (67.72%), while the highest for ethylacetate solvent extraction is in the leaf extract (56.33%). The findings of this present study revealed that the leaf and bark ethanolic and methanolic extract were very active against the test fungus. The result showed a fungicidal effect (no growth) indicating 100 % inhibition rate for both. However, because of the non-edibility of methanol, it is not advisable to use it as a solvent for extraction of extract meant to be used for coating of edible food products.

The present findings showed that the activity is solvent and pathogen dependent. A similar trend was also observed in some previous reports on the antimicrobial activity of different parts of *M. oleifera*, by [21] and [22]. Extracts of various plant extracts were found to have potent bio-regulatory activities against micro-organisms [23] as can be seen in plates 1 to 12. The plates showed outright inhibition i.e. in plates 1, 4, 10 and 11. Showing some degree of inhibition were plates 2, 6, 7, 8 and 12; while, 3, 5 and 9 which are control showed normal growth. It is discovered that toxicity of extracts is higher if purified, and reduced if considering the pool of compounds. The antifungal activity of the crude extracts might be due to the presence of lipophilic compounds that might bind internally to the cytoplasmic membranes [24] and affect the growth of the filamentous fungi causing membrane permeabilization. The antifungal activity of *Moringa oleifera* leaf is due to the presence of a significant phytochemical of a

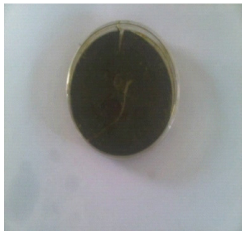

Plate 1. Methanol Leaf extract; No growth

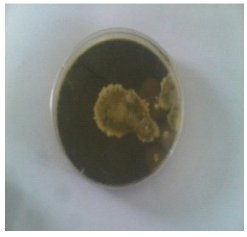

Plate 2. Ethylacetate Leaf Extract: Growth Observed with inhibition

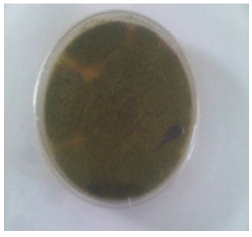

Plate 3. Control with Normal growth and Black

Plate 4. Ethanol leaf extract; No growth

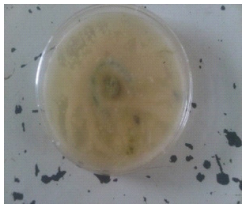

Plate 5. Control with Normal growth

Plate 6. Ethanol seed extract; growth with contaminant

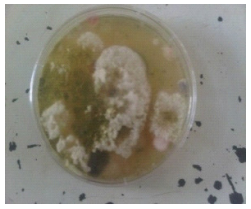

Plate 7. Ethylacetate seed extract; growth with contaminant

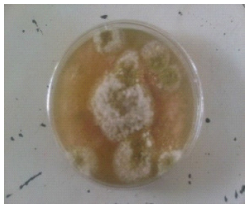

Plate 8. Methanol seed extract; growth with contaminant

Plate 9. Control at 48 hours

Plate 10. Ethanol Bark extract at 48 hours

Plate 11. Methanol Bark extract at 48 hours

Plate 12. Water Bark extract at 48 hours with contaminant

Table 3. Phytochemical Screening of *M. oleifera* (Leaves, Seed, and Stem Bark) in different solvents

Phytochemical constituents	Leaves				Seed				Stem bark			
	W	ET	E	M	W	ET	E	M	W	ET	E	M
Tannins	+				+				+			
Saponins	+	+	+	-	+	++	-	+	+	-	+	-
Phenols	++	-	+	+	-	-	-	-	-	-	+	+
Phytosterols	++	++	+	++	++	+	+++	+	+++	+	++	++
Flavonoids	++	++	+	+	+	-	++	-	-	+	+	+
Reducing sugar	++		+	++	++	+	+	+	++	++	++	++
Cardiac glycosides	++	++	++	++	++	++	++	++	-	++	++	++
Alkaloids	-	++	++	+	++	++	++	++	++	++	++	++

W: Water, E: Ethanol, ET: Ethylacetate, M: Methanol

+ : Slightly present, ++ : Moderately Present, +++: Highly Present, - : Absent

short polypeptide, quercetin-3-O-β-d-glucoside also known as isoquercitrin or isotrifolin, this peptide act directly on fungi and result in growth inhibition by disrupting cell membrane synthesis and/or synthesis of essential enzymes [25].

After that, the diameter of the fungal colony was measured and compared with that of control, which was devoid of plant leaf extract (Plates 1-12).

3.2 Phytochemical Analysis

The result of the phytochemicals screening of *M. oleifera* leaf extracts is presented in Table 3. The screening showed that the plant contained alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, reducing sugar and phenolic compounds.

Reducing sugar and steroids were present in all the extracts, cardiac glycosides were present in all the extracts except the stem bark of water extracts, alkaloids were also present in all the extracts except the water extracts of the leaf. Flavonoids are present in all the leaves extracts, but absent in the seed methanolic, ethanolic extracts and water extracts of stem bark. Phenols are present in all the leaf extracts but absent in the seed extracts, methanolic and ethanolic stem bark extracts. Tannins are present in the crude extracts of the leaves, stem and bark, this is in agreement with Abdulkadir et al. [26].

Phytochemical components are responsible for the biological activities and medicinal properties of plants. These medicinal bioactive constituents exert microbiological action through various pathways [27]. Flavonoids and tannins antimicrobial activity are due to their ability to complex with extracellular and soluble protein and to complex with fungal cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell wall envelope proteins [27]. The phenolic compounds are known to possess antimicrobial activities such as anti-inflammatory, antioxidants, anticancer etc.

4. CONCLUSION

The result of the antifungal assay of different extracts and their phytochemical analysis showed that combination of different compounds may be responsible for the bioactivity of the plant extracts. The study has shown that the presence of phytochemicals in different parts of *M. oleifera* and the acidic nature of the different compounds conferred natural preservative properties on the extracts from different parts of *M. oleifera* plants. The ethanolic extract of the leaf had the best antifungal inhibitory potentials of all the plant extracts tested. Further studies can be carried out on the isolation, purification and characterisation of the active ingredients present in it.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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