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In vitro Antifungal Activity of Extracts of *Moringa oleifera* on Phytopathogenic Fungi Affecting *Carica papaya*

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Abstract

BACKGROUND: Plants remain the natural sources of efficacious phytonutrients with beneficial assets to mankind against microbial disorders. Diverse folklores have reported the roles of medicinal plants in the remedies of various disorders in man and animals. Metabolites and pesticides from the plant origin are considered better alternatives due to favorable environmental impact as compared to the synthetic counterparts. Significant economic losses and hindrance of global papaya production are due to fungal diseases. Phytochemicals have made medicinal plants become sources of environmentally friendly alternative antimicrobials.

AIM: This study aimed at assessing the antifungal activity of leaf extracts of *Moringa oleifera* against phytopathogenic fungi isolated from *Carica papaya*.

METHODS: n-Hexane, ethyl acetate, ethanol, methanol, and aqueous extracts of *M. oleifera* leaves were evaluated for their antifungal properties. Agar well-diffusion method was implemented for *in vitro* screening, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract types against fungal species of *Aspergillus*, *Penicillium*, *Rhizopus,* and *Trichoderma*.

RESULTS: All the extracts evaluated inhibited fungal growth to some degree, with the aqueous extract exhibiting more inhibitory activities than the organic extracts. There was significant inhibition of fungal development by the tested plant extracts at different concentrations. MIC of the extracts was 15.625 mg/ml while the MFC values ranged between 15.625 and 31.25. In this work, the antifungal activity of *M. oleifera* was found to be equal or higher than commercially available fungicide, ketoconazole.

CONCLUSION: The results of this study indicate that foliole extracts of *M. oleifera* have potential for use as biofungicides for plant protection against fungal diseases.

Introduction

The plant kingdom boasts of a broad diversity of integrant that is utilized in the treatment of diverse infections [1]. Flora serve as the wealthiest resource of drugs of conventional methods of healing, modern remedies, nutraceuticals, food augments, folk therapies, pharmaceutical intermediates, and synthetic units for simulated drugs [1]. Plants constitute the inexpensive and secure alternative etymologies of antimicrobials. Production of secondary metabolites by plants, which have been isolated and characterized, is utilized as effectual principles in medicinal preparation [2], [3]. Flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulfur combinations, saponins, cyanogenic glycosides, benzophonones, xanthenes, anthraquinones, and glucosinolates are some of the common phytochemicals of plants [4]. Diverse studies have extensively shown that medicinal plants are regarded as wealthy etymologies of antimicrobial agents [5] with consequent screening for their prospective roles as alternative remedy in the remedy of infections by microorganisms [6] and reports boasts of diverse efficacious effects against both plant and human pathogenic microbes [7]. Reports have revealed the antifungal properties of many essential oils and extracts from plants, which possess no consequent effects on man and animals, hence the belief that diverse plant resources boast of antifungal activity [8]. In plants, the prominent disease-causing agents are fungi that cause alterations both on field and at post-harvest [9]. Research has revealed the quality problems in fruits and vegetables caused by a broad range of diverse fungal species that include features, nutritional benefits, organoleptic traits, and limited shelf life with some cases of allergic or toxic disorders among consumers caused indirectly by fungi from the production of allergens or mycotoxins [9], [10], [11]. Basically, synthetic fungicides are employed to control pest and fungal diseases but with increased restriction in application due to negative impacts on human health and environs [11]. *Moringa oleifera* Lam, a broadly grown strains of Moringaceae family, is popularly referred to as a “Miracle tree,” with virtually all of its parts possessing useful products for humankind. The plant *Moringa* is indigenous to the sub-Himalaya tract of India, Pakistan, Bangladesh, and Afghanistan [12]. Aisha *et al*. [12] stated that in

indigenous medicine, almost all the segments of this plant including the root, bark, gum, foliage, fruits, florets, ovules, and ovule oil have been utilized for the remedy of differential sicknesses. In furtherance, it has been opted for as an excellent aboriginal etymology of highly palatable protein, calcium (Ca), iron (Fe), Vitamin C, and carotenoids acceptable for implementation in numerous economically growing regions where undernourishment is of vital concern [12]. Reports from Aisha *et al*. [12] and Dillard and German [13] and revealed that the plant presents abundant etymology of B-carotene, protein and Vitamin C, calcium, and potassium and serves as a good genesis of natural antioxidants, thereby increasing the shelf-life of fat containing foods as a result of the presence of different types of antioxidants compounds that include ascorbic acid, flavonoids, phenolics, and carotenoid. Ethnobotanically, the plant has been reported in the remedy of ascites, rheumatism, and venomous bites and as cardiac and circulatory restorative [7]. Reports of its hepatoprotective [14] and anti-malignancy [15] roles have been documented. Ovules of *M. oleifera* have been utilized for the effective remedy of athlete's foot and tinea [16].

Studies abound on the antimicrobial activities of diverse plant part extracts of *M. oleifera* but there is a need to continuously conduct updates on its efficacy on evolving phytopathogenic fungi, especially the emerging resistant strains. This study was aimed at identifying the phytochemical components present in the differential leaf extracts of *M. oleifera* and to determine the antifungal activity of *M. oleifera* leaf extracts employing *in vitro* antifungal screening techniques.

Materials and Methods

Plant collection and identification

Leaves of *M. oleifera* were obtained from the Covenant University Farm in Ota, Ogun State, Nigeria. Authentication of the already identified plant species (voucher specimen number: Mo/Bio/H816) was conducted at the Herbarium Section of the Forestry Research Institute of Nigeria, Ibadan, Nigeria, and allocated forestry herbarium identification number of *M. oleifera*-FHI No: 110313.

Preparation of aqueous and organic extracts

Mature disease-free leaves were rinsed to eliminate dust and other foreign particles, air-dried in a shaded area at room temperature for a period of 3 weeks [17]. Dried materials were grounded into powder employing a blender and stored in airtight bottles at room temperature (25–30°C) until use. Weighed amount of 300 g of the dried powder leaf was extracted by soaking and macerating in 1.5 L of n-hexane, ethyl acetate, ethanol, methanol, and water at ambient temperature for 72 h for each solvent used. Mixtures were vigorously agitated and subsequently filtered through cheese cloth and Whatman No.1 filter paper. Resultant filtrates were subsequently concentrated using a rotary evaporator and the solvents used were recovered under pressure until slurried/dry extracts were achieved. The five different crude extracts obtained were then kept at 4°C for subsequent use. Concentrated extracts were later dissolved in appropriate volumes of dimethyl sulfoxide (DMSO) to make the various concentrations for antifungal screening. All the stock solutions were stored in sterile capped bottles, labeled accordingly, and stored at 4°C for analysis. Each antifungal test was carried out in three replicates against each fungal isolate.

Phytochemical screening of plants extracts

Assessments were carried out on the crude extracts of the five crude extracts to detect the presence of phytochemicals according to protocols previously described by Sofowora [18] and Trease and Evans[19].

Fungal isolates and inoculum quantification

Fungi were obtained from pawpaw samples from the Pawpaw research demonstration farm, Covenant University, Ota. *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Rhizopus* spp., *Penicillium* spp., and *Trichoderma* spp. were identified, maintained, and later stored in cryovials at −20°C at the Microbiology Laboratory, Covenant University, Ota. Fresh fungal isolates of 72 h were prepared on PDA by subculturing and fungal spore suspensions were obtained after filtration. Spore suspension for the different fungal isolates was evaluated using hemocytometer cell counting chamber and final inoculum was adjusted to obtain 0.5 McFarland's standard (10^6 spores/ml).

Antifungal activity of crude extracts of M. oleifera

Potato dextrose agar medium (Oxoid) was made ready following the manufacturer's instructions. Antifungal activity of the crude extract was carried out using the agar well-diffusion technique as described by Vollekova *et al*. [20]. Standard dose was constituted by dissolving 1000 mg of crude extract in 1 ml of DMSO (1:1) for all the extracts. The antifungal agent ketoconazole (100 mg/ml) was utilized as the positive control while DMSO was used as the negative control. Surface of medium was streaked with the standardized fungal spore suspension for uniform distribution of the inoculum on the agar. The seeded

plates were let to dry. Wells were then bored into agar media utilizing a sterile cork borer of 10 mm and these wells were thus filled with 0.2 ml of the various extract concentrations. Sequel to the diffusion of the extracts into the agar at ambient temperature, the bored agar plates were incubated at room temperature for 3–5 days and observations made at 24–72 h. Antifungal activity of the leaf extracts was determined by measuring zone of inhibition surrounding the agar wells. The sensitivity tests were conducted in triplicates and the average diameter of the zones of inhibition was recorded accordingly.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of plant extracts

The MIC and MFC of the plant extracts were evaluated using agar well diffusion as described by Samie and Mashau [21] and agar dilution method by Sule *et al*. [4]. Different concentrations of the extracts were constituted (125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml) from which the MIC and MFC of the plant extracts were determined. Potato dextrose agar was made ready according to the manufacturer's instructions and apportioned into Petri plates. Agar surfaces were seeded with 0.1 ml of standardized fungal spores and let to dry. Sterile 10 mm diameter cork borer was used to punch two equidistant holes in the middle of the inoculated agar plates. These holes were then supplied with 0.2 ml of the various extract concentrations. After the diffusion of the extracts into the agar at room temperature, the bored agar plates were incubated at ambient temperature for 3–5 days and observations made at 24–72 h. The antifungal activity of the leaf extracts was determined by measuring zone of inhibition surrounding the agar well. Negative and positive control plates were without any plant extracts but ketoconazole (positive control) and DMSO (negative control) were added, respectively. The sensitivity tests were conducted in triplicates and the mean diameter of the zones of inhibition was recorded accordingly.

Data analysis

The diameters of zones of inhibition for the *in vitro* antifungal activity were expressed as means of three replicates. Significant differences between and within the averages of treatments and controls were analyzed using ANOVA at $p \leq 0.05$ and *post hoc* tests. Statistical analyses were computed using SPSS version 20 software package.

Results and Discussion

The alternative control methods for plant diseases are required due to biohazards, pollution, resistance to fungicides by the fungal pathogens, and high cost of producing novel chemicals. Studies have been orchestrated on the employment of plant procured products as disease control agents because of their minimal toxicity to mammals, environmentally friendly roles, and broad public acceptance [22]. Abundant aromatic and medicinal plants are bestowed with phytochemical components that include polyphenols, flavonoids, saponins, alkaloids, and others in their different parts (leaves, bark, flowers, seeds, wood, and branches) which possess extensive applications as antioxidants and antimicrobials and are acknowledged for their pharmaceutical and biopesticide potentials [23]. This medicinal plant was selected based on it ethnobotanical and pharmacological usage as well as antimicrobial properties [12], [22], [24]. The phytochemical screening of the different plant extracts showed saponins, tannins, anthocyanin and betacyanin, cardiac glycosides, phenols, carbohydrates, coumarins, and terpenoids and triterpenoids in all the leaf extracts as presented in Table 1. This finding agreed with the reports of earlier researchers [12], [24] who found alkaloids, saponins, tannins, anthocyanin and betacyanin, cardiac glycosides, phenols, carbohydrates, coumarins, and terpenoids. The results obtained portrayed that the five crude leaf extract types (n-hexane, ethyl acetate, ethanol, methanol, and aqueous) at 1000 mg/ml concentration of *M. oleifera* evaluated had antifungal activity against all the fungal genera tested, as shown in Figure 1. The highest antifungal activity was seen in *M. oleifera* against *Penicillium* spp. with a 33 mm zone of inhibition while the least zone of inhibition was seen in the n-hexane extract against *A*. *flavus* (20 mm). For the antifungal activity of control antifungal agents, the zones of inhibition measured between 11–13 mm and 0 mm for the positive and negative controls, respectively.

Paray *et al*. (2018) carried out a study on the petroleum ether extracts of folioles and stem bark of *M*. *oleifera* against the growth of *A. niger*, *Sclerotium rolfsii*, *Botryodiplodia theobromae,* and *Penicillium oxalicum* with extracts of both plant parts showing inhibitory activity. In furtherance, the study observed that the leaf extract showed more significant inhibitory action compared to the bark extract. Research by Aisha

Table 1: Results for phytochemical screening of n-Hexane, ethyl acetate, ethanol, methanol, and aqueous extracts of *Moringa oleifera*

Tests	n-Hexane	Ethyl acetate	Ethanol	Methanol	Water
Alkaloids	−	−	+	−	−
Quinones	−	−	−	−	+
Glycosides	−	−	−	−	−
Flavonoids	−	−	−	−	−
Saponins	+	−	−	−	+
Tannins	−	−	+	−	+
Carbohydrates	−	−	−	−	+
Anthocyanin and betacyanin	−	−	−	+	++
Cardiac glycosides	−	+	+	+	+
Terpenoids	−	+	−	+	−
Triterpenoids	−	−	+	+	−
Phenols	−	−	−	+	−
Coumarins	−	+	−	+	+
Steroids	−	−	−	−	−
Acid	−	−	−	−	−

+ = Present, −=Absent, ± = Weakly positive.

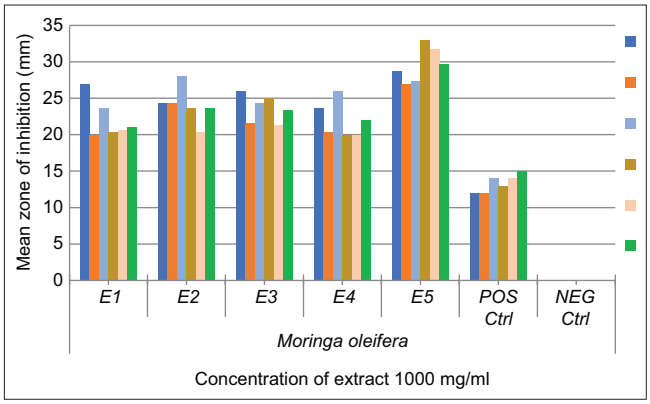

Figure 1: Mean zones of inhibition for antifungal activity of Moringa oleifera against fungal isolates from pawpaw (Carica papaya) samples. E1-n-hexane, E2-ethyl acetate, E3-ethanol, E4-methanol, E5-aqueous, POS Ctrl-Ketoconazole and NEG Ctrl-DMSO

et al. [12] revealed that the ethanolic extract had high inhibitory activity against *A. niger*, *Rhizopus stolonifer,* and *Candida albicans* at varying concentrations with *C. albicans* being the most sensitive and closely followed by the afore-mentioned fungi. A significant difference at $p \leq 0.05$ for all the extracts tested against *A. niger*, *A. flavus*, *Rhizopus oryzae*, *Penicillium* sp., *Trichoderma* sp., and *A. fumigatus* using a two-way ANOVA with treatment (test extracts and control) as the predictor and fungal growth as the response variable followed by *post hoc* tests for comparison. Results also show that the aqueous extract of *M. oleifera* was most significant ($p < 0.05$). In addition, significant difference was observed across all the extract types ($p = 0.000$) as compared to the controls, but no significant difference was observed for the isolates ($p > 0.05$). The results for MIC and MFC were established for the leaf extracts of the plant. MIC values for the extract types were 15.625 mg/ml while the MFC values ranged from 31.25 to 62.5 mg/ml.

Conclusion

Medicinal plants still subsist as the best resource for provision of diverse drugs and pesticides. *M. oleifera* leaf extracts have significant antifungal activity against common fungal isolates cultured from samples of *Carica papaya*. This shows that its extracts can serve as an alternative and ecofriendly means of controlling and treating fungal diseases in plants.

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