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Antifungal Activity of Petroluem Ether Extracts of Moringa oleifera Leaves and Stem Bark against Some Plant Pathogenic Fungi

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Abstract
The petroleum ether extracts of the leaves and stem bark of *Moringa oleifera* Lam. were evaluated for their antifungal activity against *Sclerotium rolfsii* Sacc., *Botryodiplodia theobromae* Pat., Van Tiegh, *Penicillium oxalicum* Currie Thom. and *Aspergillus niger* Van Tiegh. The phytochemical analysis of the crude extract of the plant parts received the presence of saponins, alkaloids, tannins, flavonoids, steroids, anthraquinones, reducing sugars, phlobatannins, cardiac glycosides, terpenes and phenols. The petroleum ether extracts of the plant parts were effective against all the fungi tested. *Sclerotium rolfsii* was the most susceptible, while *A. niger* was the least susceptible. The inhibition of *S. rolfsii* to both extract was significantly ($P<0.05$) different compared to the other fungi tested. The leaf extract exhibited more significant activity than the bark extract. The results obtained suggest that *M. oleifera* can be used in controlling the test fungal pathogens in plants.
Keywords: *Moringa oleifera*, antifungal activity, leaves, stem bark, pathogenic fungi.

1. Introduction

The obvious pollution problems in the environment and the toxic effects of synthetic chemicals on non target organisms have led to global effort at screening various plants for bioactivity against plant pathogenic organisms (Onifade, 2000; Nwachukwu and Umechuruba, 2001; Okungbowa and Edema, 2007; Gupta and Tripathi, 2011). Studies have shown the importance of natural chemicals as a possible source of non toxic, safe and easily biodegradable alternative pesticides (Singh, 1994; Ghorban and Salary, 2004; Stompor-Chrzan, 2004). Pesticides of plant origin are available and cost effective in countries where synthetic pesticides are expensive and difficult to obtain (Mason and Mathew, 1996).

Many natural products of plant origin are pesticidal and have the potentials to control fungi diseases of crops (Okwu *et al.,* 2007). However Earnsworth (1990), reported that just very few plants; about 10% have been investigated for their anti-microbial activity. Therefore, a large reservoir of potential sources of botanical fungicides such as *Moringa oleifera* still exists especially in tropical forests awaiting exploitation.

Moringa oleifera belongs to the family Moringaceae (Nadkarni, 1976). It is a low growing tree that s native to Indian subcontinent but now widely spread in tropical and subtropical areas of the world. Its trunk is soft, white corky and branched bearing a gummy bark. The leaves are tripinnate bearing several small leaflets. The flowers are white and the three wing seeds are scattered by the winds (Farooq *et al.,* 2012). The plant grows well in the humid tropic and can survive in harsh climate condition (Morton, 1991). *Moringa oleifera* has enormous medicinal potential, which has long been recognized in many parts of the world. Nearly every part of this plant, including root, bark, gum, leaf, fruit (pods) flowers, seeds and seed oil have been used for various ailments in the indigenous medicine (Odebiyi and Sofowora, 1991). In Nigeria, poultice of the leaves is applied to wounds, boils and swellings. A concoction of the stem bark is given as a cardiac stimulant in asthma and cough and the seed oil is used for the treatment of goiter and acute rheumatism (Gill, 1992). The current investigation was undertaken to evaluate the antifungal activities by petroleum ether extracts of *Moringa oleifera* leaves and stem bark against *Botryodiplodia Theobromae, Sclerotium rolfsii, Penicillium oxalicum and Aspergillus niger* causing diseases in plants.

MATERIALS AND METHODS

Collection of plant materials

The leaves and stem of *Moringa oleifera* were collected in March, 2012 from Asaba in Delta State, Nigeria. They were identified by a taxonomist in the Department of Forestry and Wildlife, Delta State University, Asaba Campus, Asaba, Nigeria.

Extraction

The stem bark was carefully peeled from the stem.. The leaves and stem bark were air-dried on the laboratory bench for 7 days and then ground into a uniform powder using a Tower blender (Model BL-NC-6802D, Italy). Each of the powdered plant materials (650g) was extracted exhaustively and successively with Petroleum ether (60 – 80°C) to yield petroleum ether extract. The extracts were concentrated to dryness *in vacuo* at 40°C to

obtain dry petroleum ether extract of leaves (17.8g) and stem bark , (14.5g).

Phytochemical screening
Phytochemical screening was carried out on part of the dry extracts to reveal the presence of secondary metabolites in them according to the methods of Trease and Evans (1989) and Poongathai *et al.,* (2011).

ungi
The fungi used were *Sclerotium rolfsii* Sacc., *Botryodiplodia theobromae* Pat., *Aspergillus niger* Van Tiegh, and *Penicillium oxalicum* Currie Thom. They were obtained from the National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria. Organisms were maintained on Potato Dextrose Agar (PDA)(Oxoid, England) at 4°C and revived twice on fresh PDA before used.

Effect of extract on mycelial growth and spore germination
Food poisoning technique described by Okigbo *et al*. (2009) was used in this study. Solution of each extract previously obtained by Petroleum ether extraction was prepared by dissolving 5g of crude plant extract in 20 ml sterile distilled water in a bath at 80°C to give 25% extract concentration. One milliliter of each extract was pipetted aseptically into 9 ml of cooled PDA medium in Plastic Petri dishes. Each plate was gently swirled on the table to ensure even dispersion of the extract and allowed to solidify. Three replicate plates for each extract were then inoculated at the centre with 2mm diameter mycelial discs obtained from the colony edge of 5-day old culture of each test fungus. Plates without extract but similarly inoculated with mycelial discs were included as controls. Plates were incubated at 28°C and radial growth was measured daily for 5 days. Colony diameter was taken as the mean growth along two pre-drawn perpendicular lines on the reverse side of each plate.

For the effect of extracts on spore germination, plates with each extract were inoculated with one drop (0.1 ml) of conidial suspension (5.0 x 10^4 conidial ml^{-1}) of each test fungus. Spore suspensions were obtained from a 10-day old culture of the fungi. Spore concentration was measured using haemocytometer. Plates were inoculated at the centre of each of four sectors in each Petri dish Plates without extract but similarly inoculated with spore suspension of each fungus were included as controls. Counts of germinating spores were made under the low power (x 100) of microscope after 24 hours incubation at 28°C. Germination was based on the mean of 100 conidia counted per sector in each plate. There were three replicates of each extract per fungus. For *S. rolfsii*, 10 sclerotia were placed per plate and germination was assessed after 72 hours incubation at 28°C (Wokocha and Okereke, 2005). Percentage inhibition of mycelia growth or spore germination was calculated using the formula of Wokocha and Okereke (2005) as follows:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where:
dc = average diameter of fungal colony/average number of spore germination in control plates.
dt = average diameter of fungal colony/average number of spore germination in treated plates.

Statistical analysis
The data collected were subjected to analysis of variance (ANOVA) using SAS (2000). Significant means were separated using the Duncan's Multiple Range Tests (DMRT) ($P<0.05$).

RESULTS AND DISCUSSION
The leaves and stem bark of *Moringa oleifera* showed variations in the distribution and concentration of classes of secondary metabolites. As the leaves contained anthraquinones in high concentration, the stem bark showed it in moderate quantity (Table 1). Also, the leaves possessed tannins flavonoids, steroids, phlobatannins, cardiac glycosides and terpenes in moderate or low concentration as opposed to their absence in the stem bark. Other constituents such as saponins, alkaloids reducing sugar and phenols were present either in moderate or low quantity in both plant parts. The presence of these secondary metabolites could be responsible for their antifungal activity. Burapedaja and Bunchoo (1995) reported the presence of tannins in *Terminalia citrine* extracts and explained that they inhibited cell wall formation in fungi, leading to the death of the organism. The antifungal activities of *Spilanthes calva* on *Fusarium oxysprum* and

Table 1: Phytochemical analysis of the petroleum ether extracts of different parts of *Moringa oleifera*

Test	Inference	
	Leaves	Stem bark
Saponins	++	++
Alkaloids	++	++
Tannins	+	-
Flavonoids	++	-
Steroids	++	-
Anthraquinones	+++	++
Cardiac glycosides	+	-
Carotinoids	-	-
Reducing sugar	++	+
Phlobatannins	+	-
Terpenes	++	-
Phenols	+	+

Trichophyta on *mentagrophytes* was attributed to the presence of an alkaloids known as spilanthol (Rai *et al.* 2004). Ejechi *et al.* (1999) reported the antifungal activities of phenolic extracts of pepper fruit on tomato rot fungi. They reported that phenolics interfere with the integrity of the cell membranes or inhibit the germination of spores. Giordani *et al.* (2008) reported that there was a positive correlation between the antifungal activity and phenolic content of the essential oil of *Thymus mumidicus*. Oyewale and Audu (2007) reported that the presence of saponins, alkaloids, flavonoids and tannins in the extracts of six tropical African flora accounted for the board spectrum of activities on the microorganisms tested.

The petroleum ether extracts of the leaves and stem bark of *M. oleifera* elicited antifungal activity against the mycelia growth and spore germination of all the fungal pathogens tested (Tables 2 and 3). This is in agreement with reports of some earlier workers on different plants and pathogens (Al-Abed *et al.,* 1993; Qasem and Abu-Blan, 1996; Amadioha, 1998; 2000; Onifade, 2000; Nwachukwu and Umechuruba, 2001; Udo *et al.* 2001; Gupta and Tripathi, 2011; Okigbo *et al.,* 2013). *Sclerotium rolfsii* was observed as the most susceptible fungi, while *A. niger* was the least susceptible showing resistance to the petroleum ether extracts of the plant parts. The observed variation in susceptibility of the pathogens may be due to the difference in the chemical, physiological and structural integrity of the organisms (Alade and Irobi, 1993) The leaf extract exhibited more significant activity than the bark extract. This could be attributed to the fact that more phytochemicals were deposited on the leaves. Plant stores these antifungal chemicals on the leaves to protect the leaves from microbial attack. This agreed with the findings of Okwu and Emenike (2006) who reported that phytochemicals are reserved in plants to protect the plant against the attack and inversion of microorganisms.

Table 2: Antifungal susceptibility test of the petroleum ether extracts of different parts of *Moringa oleifera* on mycelia growth of four plant pathogenic fungi

Test fungi	Growth inhibition (%)	
	Leaves	Stem bark
Sclerotium rolfsii	67.42^{a}	55.34^{a}
Botryodiplodia theobromae	38.32^{c}	24.85^{c}
Penicillium oxalicum	44.49^{b}	41.57^{b}
Aspergillus niger	28.12^{d}	16.24^{d}

Means in the same column with different superscripts are significantly different (P<0.05)

Table 3: Antifungal susceptibility test of the petroleum ether extracts of different parts of *Moringa oleifera* on spore germination of four plant pathogenic fungi

Test fungi	Growth inhibition (%)	
	Leaves	Stem bark
Sclerotium rolfsii	54.06^{a}	46.38^{a}
Botryodiplodia theobromae	22.61^{c}	15.56^{c}
Penicillium oxalicum	35.32^{b}	29.12^{b}
Aspergillus niger	6.25^{d}	5.52^{d}

Means in the same column with different superscripts are significantly different (P<0.05)

5. Conclusion

This study has shown that the petroleum ether extracts of *Moringa oleifera* leaves and stem bark contain antifungal compounds and were effective against *B. theobromae, S. rolfsii, P. oxalicum* and *A. niger* on PDA. It has also revealed that *S. rolfsii* was the most susceptible while *A. niger* was the least susceptible. Thus, the two

plant parts have inhibitory potential and could be used as a biological fungicide to control these pathogens in plants.

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