# In *vitro* evaluation of antifungal activity of *Moringa oleifera* Lam extracts against coffee wilt pathogen, *Gibberella xylarioides* Heim and Saccas

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## Abstract

The *Moringa oleifera* extracts were investigated for antifungal activity against coffee wilt pathogen G*ibberella xylarioides* as a first step in the screening of the extracts for preliminary fungicides properties on coffee using micro dilution method. *In vitro* evaluation revealed that, *M. oleifera* leaf, seed, flower and stem bark extracts exhibited broad spectrum activity against the test organism with minimum inhibition concentration (MIC) range from 0.37 mg/mL to 3.125mg/mL. *M. oleifera* roots back extracts appeared to be less effective against *G. xylarioides* with MIC at 6.25 mg/mL. Results of this study have shown the potentials of *M. oleifera* extracts as fungicides by inhibiting the growth of the test organism.

**Key Words;** Coffee wilt disease; fungicides; *Moringa oleifera*; minimum inhibition concentration (MIC)

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## Introduction

Coffee is vital to the economy of Eastern and Central Africa, providing a major source of foreign exchange earnings as a cash crop (Rutherford, 2006). It supports the livelihood of millions of people who are involved in cultivation, processing, marketing, and exportation (Bacon, 2005). However coffee production and development is now threatened by various disease-causing organisms such as fungi (De la Rosa *et al.,* 2000). One of the most common fungi diseases to coffee plant is wilt disease (Tracheomycosis) caused by *Fusarium xylarioides* Steyaert imperfect stage (*Gibberella xylarioides* Heim and Saccas perfect stage) (Alemu, 2013). Coffee wilt disease (CWD) was first noticed around 1927 in Oubangui-Chari (Central African Republic) on *Coffea excels (Afrasa, 2012), but b*etween 1937 and 1939 the disease spread to *C. canephora* and *C. liberica* in Cameroon, Guinea, Côte d'Ivoire, Democratic Republic of Congo (DRC) and Uganda as a major threat to Robusta coffee (Lepoint *et al.,* 2005). Tanzania particularly, CWD is one of the major threat to coffee industry and livelihoods of more than 90,000 that depend on the crop in Kagera Region (Kilambo *et al.,* 2010).

Although, lacking an immune system comparable to animals, *plants have* developed *mechanisms* that help defending themselves *against* infections (Kiraly *et al.,* 2007). In the case of fungal infection, these mechanisms include synthesis of bioactive organic compounds (Morrissey and Osbourn, 1999) such as antifungal proteins, saponin and peptides (Osbourn *et al.,* 2003). However, quantity and quality of these active compounds depend on the plant species, plant tissue and environmental factors, some plants produce inefficient amount and hence become vulnerable to be attacked by various infection including fungus (Ncube *et al.,* 2008). Current recommended techniques for fungi management is by uprooting the diseased tree, burning, and the use of copper based fungicides for stem painting to prevent landing of *G. xylarioides* spores (Kilambo *et al.,* 2012). These approaches all have drawbacks in terms of efficiency and cost to coffee farmers (Rutherford and Phiri, 2006). Likewise, the use of copper based fungicides has led to soil copper toxicity and resistance to the pathogen hence posing another challenge to coffee industry as well as environment concern (Zala and Penn, 2004). Since the use of synthetic fungicides is becoming increasingly restricted due to their high cost, the unavailability on the

local market, emergence of fungi resistant and their damaging effects on the environment and health, justifies the search for plant based materials as a new control strategies.

Several works demonstrated in laboratory trials reveal that natural products of higher plants may possess a new source of antimicrobial agents (Shu, 1998). There are several reports on the presence of several groups of compounds in various plant parts like leaves, bark, fruit, root and flowers of *Moringa oleifera* with medicinal value (Anwar *et al.,* 2007; Caceres *et al.,* 1991; Chuang *et al.,* 2007; Oliver-Bever, 1986). *M. oleifera* is recognized for its antispasmodic, anti-inflammatory, diuretic, obortificient, emmenagogue and ecbolic properties and useful in treatment of many diseases including fungi (Talreja, 2010). In this study, *M. oleifera* was chosen based on ethnobotanical information of antimicrobial activity and previous studies that have demonstrated antifungal properties using different kinds of extracts (Dahot, 1998; Nikkon *et al.,* 2003), as well, the *M. oleifera* is easily available in many areas of Tanzania where coffee farming is the major economic activity (Kakengi *et al.,* 2007; Foidl *et al.,* 2001). Hence, the present study was undertaken specifically to speculate the antifungal activity of sequential extracts of *M. oleifera* (Lam) against an important phytopathogenic fungi, the coffee wilt pathogen *Gibberella xylarioides* Heim and Saccas.

## Methodology

### Collection and Preparation of Plant Materials

Leaves, seeds, flower, roots and stem bark of *M. oleifera*, were collected from in Lushoto, Tanga Region, Tanzania. The plant was identified by Mr. Haji Selemani, a botanist from the Department of Botany, University of Dar es Salaam and the voucher specimen (MOLT 2140) was kept at the Nelson Mandela African Instituttion of Science and Technology, Arusha. The leaves and flowers collected were kept under a room temperature at ($29^{O}$ C-$31^{O}$C till dry completely. The root bark were washed with clean running tap water to remove soil while stem bark and seeds separately were exposed under the sun until they dried and plant materials were pulverized to get powders for extraction.

**Reagents, media and test organism.**

Ethanol (absolute), petroleum ether and ethyl acetate were bought from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands). Dimethylsulphoxide (DMSO) was obtained from RFCL Limited (Haryana-India), In addition, Iodonitrotetrazolium chloride was purchased from SIGMA (Sigma Aldrich, St Louis, USA). But potato dextrose agar and broth were bought from Hi Media Laboratories Pvt Ltd (Mumbai-India). *Gibberella xylarioides* (clinical isolate) were obtained from Tanzania Coffee Research Institute (TaCRI) Moshi, Tanzania.

**Extraction**

Sequential extraction was done using Petroleum ether, ethyl acetate and methanol for all plant parts. Briefly, 1000g of plant parts were dissolved in 2.5 litres of solvent for 24h. After 24h the extracts were sieved using funnel and then concentrated in *vicuo* using rotary evaporator. The crude extracts were collected, poured into air tight bottles and stored in refrigerator at -20°C until the time for testing.

**Testing for antifungal activity**

Minimum inhibitory concentrations (MICs) were determined by microdilution method (Eloff, 1998) using 96-well microtitre plates with minor modification. The plates were first preloaded with 50 µL of the potato dextrose broth media in each well followed by an addition of 50 µL of the extract (100 mg/mL) into the first wells of each row tested to make a total volume of 100 µl in the first wells. After thorough mixing 50 µL was drawn from each of the first row wells and put into the next row wells. The process was repeated down the columns to the last wells at the bottom where 50 µL was discarded. Thereafter, 50 µL of the fungi suspension (0.5 Mac Farhland standard turbidity) was then added in each well to make the final volume of 100 µL in each well. On the other side, DMSO was used as negative control while the rows with broth and fungi were only used to monitor fungi growth. All assays for antifungal activity were carried out at least in triplicate for significance statistical results. The plates were then incubated at 37°C for 48 h. For each extract, MICs were determined by adding 40 µL of 0.02% *p*-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1 h at 37°C. In this regard, fungi growth was indicated by a change of color to pink. The lowest concentration which showed no fungi growth was considered as MIC (Aligiannis *et al.*, 2001).

**Table 1: Minimum Inhibition Concentration (MIC) of *M. oleifera* extracts against *Gibberella xylarioides***

| Plant part | Extracts | MIC (mg/mL) |
|---|---|---|
| Leaf | Petroleum ether | 3.125 |
| | Ethyl acetate | 1.5125 |
| | Methanol | 0.37381 |
| Stem bark | Petroleum ether | 1.5125 |
| | Ethyl acetate | 0.37381 |
| | Methanol | 0.75625 |
| Root bark | Petroleum ether | 6.25 |
| | Ethyl acetate | 6.25 |
| | Methanol | 6.25 |
| Seed | Petroleum ether | 1.5125 |
| | Ethyl acetate | 3.125 |
| | Methanol | 1.5125 |
| Flower | Petroleum ether | 1.5125 |
| | Ethyl acetate | 0.75625 |
| | Methanol | 1.5125 |
| DMSO (-ve control) | | 12.50 |
| BROTH | | 25.00 |
| LSD (0.05) | | 3.06 |
| Sx | | 1.53 |
| Mean | | 4.34 |

**Key**; DMSO= Dimethylsulphoxide

**Results and Discussion**

Antifungal test of petroleum ether, methanol and ethyl acetate extracts of *Moringa oleifera* showed that the plant exhibits antifungal activity against *Gibberella xylarioides.* From the total of fifteen (15) extracts evaluated (Table 1), thirteen (13) extracts indicated variable sensitivity to different extracts of *M.oleifera* against *Gibberella xylarioides* with MIC values ranged from 0.38 mg/mL to 3.125mg/mL. Likewise, leaves and stem bark ethyl acetate extracts, were found to have maximum antifungal activity of MIC 0.38 mg/mL in comparison to flower and seed extracts. Similar results have been reported by Senguttuvan *et al*. (2013) and stated that ethyl acetate extracts of *Hypochaeris radicata* inhibited the fungal growth effectively rather than the ethyl acetate and methanolic solvent extract against *Aspergillus niger* and *Mucor sp*.

Leaf ethyl acetate and stem bark petroleum ether extract exhibited moderated antifungal activity against *Gibberella xylarioides* with MIC at 1.31mg/mL concentration. This difference can be explained by the nature of the compounds found in the different extracts since the three extracts used in this study vary in terms of their polarity (Akowuah *et al.,* 2005). Seed and flower methanolic and petroleum ether extracts showed almost similar antifungal activity against *Gibberella xylarioides* with MIC 1.5mg/mL concentration. Equally, several reports show that, plant extracts have remarkable biological activity when tested against fungus (Ali-Shtayeh and Abu Ghdeib, 1999; Hammer *et al.*, 1999; Shivpuri *et al.*, 1997).

*M. oleifera* stem bark methanolic and ethyl acetate extracts revealed broadest spectrum of activity on the tested organism since they both exhibited relatively high activity of MIC at 0.78 mg/mL and 0.37 mg/mL respectively against *G. xylarioides* which is comparable to the results obtained by Sheik and Chandrashekar, (2013) on *in vitro* antimicrobial, antioxidant, antiarthritic and phytochemical evaluation of *Pscychotria flavidatalbot*. However, root bark ethyl acetate and methanolic extracts proved the least antifungal activities with MIC 6.25mg/mL concentration. The poor performance of root extract used in this study also was verified by Rahul Shaw and Singh, (2014) were it was found that the root extacts of *Leptadenia pyrotechnica* had no activity against the fungi *Aspergillus niger*.

The fact that the results of this study showed that *Moringa oleifera* exhibits antifungal properties justify their traditional use as medicinal plants. This may be due to synergistic effect of several compounds that are in various proportions in a *M. oleifera* which constitute an important source of microbicides, pesticides and many pharmaceutical drugs such as saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts (Zaffer *et al.,* 2012; Igbinosa *et al.,* 2009).

## Conclusion

Exploitation of naturally available chemicals from plants, which retards the reproduction and growth of undesirable microorganisms remains the most realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies. Findings of the present *in vivo* investigation are an important step towards isolation and characterization of the antifungal agent. This makes the *Moringa oleifera* a good candidate for bioprospecting fungicide against *G. xylarioides*.

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