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*Full Length Research Paper*

# Phytochemical Screening and Antifungal Activity Of*moringa Oleifera* on Some Selected Fungi in Dutse, Jigawa State

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**Phytochemical screening of the ethanolic extract of *Moringaoleifera* and its antifungal activityon some selected fungi was investigated using the disc diffusion and broth dilution techniques. Resultsof phytochemical screening of the ethanolic extract shows the presence of alkaloids, glycoside, flavonoids, reducing sugar, saponins and tannins. Similarly, it was found that the ethanolic extract has antifungal potential on the tested fungi. The minimum inhibitory concentration for the leaf ethanolic extract was found to be 375mg/ml. therefore *Moringaoleifera* could be used against these fungi pending further research.**

**Keywords:** *Moringaoleifera*, ethanolic extract, phytochemicals, antifungal activity,

## INTRODUCTION

Plants are being used as a source of innovative therapeutic agent for infectious diseases, cancer, lipid disorder and immune modulation. Natural products are being used as source of medicine for thousands of years and still continue today. Various medicinal plants have been used for years in traditional medicine in daily life to treat the different disease and many possess antifungal activities. Natural products and their derivatives have traditionally been the most common source of drugs and still present in more than 30% of the current pharmaceutical market (Kirkpatrick, 2000). According to observations of the World Health Organization (WHO), medicinal plants would be the best course for obtaining a variety of drugs (Santos *et al.,* 1995 and Basso *et al*., 2005). Traditionally used medicinal plants are the major source of medicine and approximately 80% of the world population depends on the traditional medicines derived from medicinal plant (WHO data), of the estimated 400,000 higher plant species in the world only about 10% have been characterized chemically to some extent (Basso *et al.,* 2005).

Infectious diseases are big challenge for tropical countries and account for about half of the death (Abghan *et al.,* 1984). Fungi have genetic ability to develop resistance against antibiotics, which is a big threat to the

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society worldwide. Some plants have shown the ability to overcome resistance in such organisms, which led the researchers to isolate active principles and investigate mechanisms. Isolation and identification of secondary metabolites produced by plants has explored their use as active principle in medicinal preparation (Taylor *et al.,* 2001) and Nube *et al.,* 2008). The pharmaceutical industries are looking for new chemical ways against complex diseases. Fungi are little explored and drug developers from all over the world are looking towards natural world for developing front line drug to face the challenge (Basso *et al.,* 2005). *Moringaoliefera* Lam is the most widely cultivated species of a monogeric family, Moringaceae, which is native to the sub-Himalaya tract of India, Pakistan, Bangladesh and Afghanistan. A number of medicinal properties have been ascribed to various plants of the highly extend tree. Almost all the part of this root, bark, gum, leaf, fruits, flowers, seed and seed oil have been used for the treatment of various ailments in the indigenous medicine. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern.

Bukar *et al.* (2010) reported that *Moringaoleifera* leaf ethanolic extract had the broadest spectrum of activity on the test fungi. Moringa have been reported to be a rich source of B-carotene, protein and vitamin C, calcium and potassium and act as a good source of natural antioxidants and thus enhance the shelf-like of fat containing foods due for the presence of various types a antioxidants compounds such as ascorbic acid, flavonoids, phenolics and caretenoid (Dillard and German, 2000).

Over the years, plants have been used as valuable source of natural products for maintaining animal and human health. Plants have been reported to contain large varieties of chemical substances that possess important preventive and curative therapies (Nascimento *et al.,* 2000). About 80% of individuals from developed countries used traditional medicines which have compounds derived from medicinal plants (Igbinosa *et al.,* 2009). Despite the presence of various approaches to drug discovering, plants still remain the main reservoir of natural medicines (Mahomed and Ojewole, 2006).

Although Moringa Oleifera plant has been reported to possess many antimicrobial activities, there is need to continuously make updates on important pathogenic fungi especially that some dimorphic fungi are difficult to control because of their physiological rate differences.

The aim of the research is to study the phytochemical constituents of leaf Ethanolic extract of *Moringaoleifera* Lam as well as its antifungal properties against some selected fungal isolates.

## MATERIALS AND METHODS

### Collection and Processing of Plant Sample

The leaves of *Moringaoleifera* were collected from Dutse, Gida Dubu Yadi, Jigawa State. The plant specimens were identified by a Lab technician in the Federal University Dutse, Biology lab. The leaves were washed and dried in an airy room for about 3 days. The dried leaves were grounded to powder using a pestile and mortar in the laboratory. The powdered sample was stored in an air-tight bottle for further use.

### Extraction Process

### Ethanolic extract

Fifty grams (50g) of the powdered sample was soaked in 500ml of absolute ethanol and allowed to stand for 24 hours. The mixture was stirred occasionally for 3 days. The sample was double filtered using Whatman No.1 filter paper and collected in an evaporatory dishes. The filtrate was dried in a hot air at temperature of 45$^{o}$C (Handa, 2008).

### Phytochemical Analysis

The extract were analyzed by the following procedures to test for the presence of the saponins, Tannins, reducing sugars, Alkaloids, flavonoids, and glycosides.

### Saponins

This was carried out according to a method of Odebiyi and Ramstard (1978) and Waterman (1993), 2ml of the extract was placed in a take tube and then 2ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15 minutes indicates a positive test for saponins.

### Tannins

To a portion of the extract was diluted with water 3-4 drops of 10% ferric chloride solution was added. A blue colour or green colour indicate the presence of Tannins. Talukdar *et al.,* (2010)

### Reducing sugar

To 0.5ml of plant extract, 1ml of water and 5-8 drops of fehling solution were added and heated over water bath. Brick red precipitate indicate the presence of reducing sugars. Talukdar *et al.,* (2010)

**Alkaloids**

2ml of extract was measured in a test tube to which picric acid was added. An orange colouration indicate the presence of alkaloids (Talukdar *et al.,* 2010)

**Glycosides**

Exactly 25 ml of dilute suphuric acid was added to 5ml of extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5ml of fehling solution was added. Glycosides are indicated by a brick red precipitate. Talukdar *et al.,* (2010)

**Flavonoids**

This was carried out according to a method of Odebiyi and Ramstard (1978) and Waterman (1993). To 3ml of a extract was added 1ml of NaOH. Yellow coloration indicate positive.

**Test Culture**

Two test organism such as *Rhizopousstolonifer* and *Aspergillusniger* were isolated from soil sample using the standard method. While *Candida albican* was collected from Dutse General Hospital Laboratory, Jigawa States. The isolates were maintained in a freshly PDA agar slant and kept for further use.

**Standardization of the Inoculum**

The inocula was prepared from stock cultures which were maintained on PDA slant and subculture on to potatoes destrose agar (PDA) using sterilized wire loop.

**Media for Test Organism**

Nineteen gram of PDA Was added to 500 ml of sterile distilled water and auto clave at 121$^{o}$C for 15 minutes after autoclaving, allowed the media to cool down and mixed, poured in to sterile Petri dishes approximately 4mm and allowed to set at ambient temperature and use.

**Antimicrobial Disk Preparation**

Disk of 6 mm diameter was made from Whatman No. 1 filter paper using paper puncher. Batches of 100 disks were transferred in to Bijou Bottle and sterilized in the autoclave at 121$^{o}$C for 15 minutes. For different concentration were prepared from the leaf ethanolic extract such that each disc absorbed 0.1ml which is equivalent to 5000ug/disk, 4000ugidisk, 3000ug/disk, and 2000ug/disk as described by Bauer *et al*. (1966).

**Antimicrobial Assay**

Disk agar diffusion techniques describe by Bauer *etal*. (1966) was employed. The prepared disks were incorporated in to appropriate medium containing the isolate, cover the petri dish and incubate at 25$^{o}$C for 3days. After incubation, zone of inhibition (diameter) formed in the medium was measured to determine antifungal effectiveness of the different concentrations of the extracts and recorded.

**Determination of Minimum Inhibitory Concentration (MIC)**

The broth (tube) dilution method was used for the determination of the minimum inhibitory concentration (MIC) i.e. the lowest concentration of the extract that completely prevent the growth of the test organism. The following concentrations were prepared; 5000µg/ml, 3000µg/ml, 2000µg/ml respectively using the dilution formula.

**Dilution of the extract**

Eight test tubes were labeled and set up. 1ml of PDA broth was pipetted into tube 1-7. 2ml of PDA broth was pipette into tube 8. Tube 6 is the inoculums' control; tube 8 is the broth control and tube 7 – is the extract control. 1ml of the working extract solution was pipette into tube 1-5 and 7. Doubling dilutions were prepared from tube 1 up to 5 using 1ml amount. O.1ml of the working innoculum was pipette into tubes 1-6. The tubes were incubated at 25$^{o}$c for 3 days. The lowest concentration of the extract that inhibited the growth of the test fungi was noted and recorded as the MIC (Ochei and Kolhatkar, 2008).

**RESULTS AND DISCUSSION**

A total yield of 8g of the ethanolic extract from the original weight of 50g was recovered from the leaf of *Moringaoleifera.* The physical characteristics were indicated in Table (1), table (2) shows the phytochemical composition of plant part screened. The antifungal pattern of the extract was shown on Tables 3-4.

**Phytochemical evaluation of extract of *Moringaoleifera***

Table (2) showed the result of photochemical evaluation of ethanolic extracts of *Moringaoleifera.* The ethanolic extract of *Moringaoleifera* showed the presence of flavonoids, sponnins, tannins, glycoside, reducing sugar and Alkaloids. This result is in line with the report of Bakare *et al*. (2010) and Kasolo *et al.* (2011) on leaves of *Momordicacharantia*

**Table 1:** Physical Characteristic of Leaf Extract of *Moringaoleifera.*

| Plant part | Solvent | Initial Weight(g) | Final Weight(g) | Colour | Texture |
|---|---|---|---|---|---|
| Leaf | Ethanol | 50.00 | 8.00 | Dark Green | Powder |

**Table 2:** Photochemical Evaluation of *Moringaoleifera.*

| S/NO | Constituents | Inference |
|---|---|---|
| 1. | Flavonoids | + |
| 2. | Saponnins | + |
| 3. | Tannins | + |
| 4. | Glycosides | + |
| 5. | Reducing sugar | + |
| 6. | Alkaloids | + |

Key: + = present

**Table 3:** Antifungal activities of leaf ethanol extract of *Moringaoleifera* (measurement zone of inhibition (in mm)/disk potency.

| Test fungi | 5000 | 4000 | 3000 | 2000 |
|---|---|---|---|---|
| *Candida albican* | 22 | 19 | 15 | 10 |
| *Aspergillusniger* | 18 | 16 | 10 | 05 |
| *Rhizophousstolonifer* | 20 | 17 | 11 | 06 |

and *Moringaoleifera* respectively. The presence secondary metabolite such as flavonoid, Alkaloids, Tannins, saponin, and glycoside of leaf of *Moringaoleifera* contribute to its medicinal value Bakare *et al.*(2010).

Alkaloids are natural chemical compound containing basic nitrogen atoms, they often have antifungal activity and pharmacological effects and are used as medication and recreational drugs (Rhoades *et al.,*1979).

Flavonoids enhance the effects of vitamins C and function as antioxidant. Tannin have shown potential Antiviral, Antibacterial and antiparasitic. Saponin causeshaemolysis of red blood cells(Winter *et al.,*1993).

## Antifungal activities of *Moringaoleifera*

Table 3 showed the measurement zone of inhibition of *Moringaoleifera* against tested fungi. The ethanol extract of plant *Moringaoleifera* possessed antifungal activity against *Candida albicans*, *Aspergillusniger, Rhizophousstolonifer*. The ethanol extract produced an inhibition zone on *Candida albican* of 22mm at 5000µg/ml, 19mm at 4000µg/ml, 15mm at concentration of 3000µg/ml and 10ml at 2000µg/ml, *Aspergillusniger,* showed zone of 18mm at concentration of 5000µg/ml, 16mm at 4000µg/ml, 10mm at 3000µg/ml and 05mm at 2000µg/ml. And *Rhizophousstolonifer* showed zone of 20mm at 5000µg/ml, 17ml at 4000µg/ml, 11mm at 3000µg/ml and 02mm at 2000µg/ml.

At 5000µg/ml and 4000µg/ml concentration, the tested organisms are more sensitive and out of this *Candida albicans* was seen to be more sensitive to the extract at concentration of 5000µg/ml with zone of inhibition of 22mm as shown in table 3. These tested fungi are known to cause infections in human except *Rhizophousstolonifer.* The zones of inhibition observed, indicated that they are susceptible to the extract and these extracts could be used on the treatment of unitary tract infection, Aspergillosis associated with *Candida albicans* and *Aspergillusniger* respectively and can be used to treat soft rot in plant associated with *Rhizophousstolonifer.*

This result is also in line with the work of Bauer *et al.* (1966) that reported the ethanol and methanol extract of

**Table 4:** Minimum Inhibitory Concentration (mg/ml) of leaf ethanol Extract of *Moringaoleifera*

| Test fungi | MIC value (mg/ml) |
|---|---|
| *Candida albican* | 625 |
| *Aspergillusniger* | 375 |
| *Rhizophousstolonifer* | 500 |

deride leaf of *Moringaoleifera* has activity against *Candidaalbicans* at a concentration of 4000µg/ml and 5000µg/ml. The antifungal activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Moringaoleifera* showed a good antifungal activity against the tested organisms.

**Minimum Inhibitory Concentration (MIC)**

Table 4 showed the lowest MIC at concentration of 375mg/ml against *Aspergillusniger* and the highest was seen at concentration of 625mg/ml against *Candida albicans*.

## CONCLUSION

The ability of this plant extract to inhibit these organisms indicates the presence entities capable of suppressing the growth of the test fungi. Therefore further photochemical analysis is recommended to assess the presence of other bioactive compounds of chemotherapeutic potentials from the plant part.

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