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Antifungal and antiradical potential of *Moringa stenopetala* (Baker f.) Cufod (Moringaceae)

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ABSTRACT

In the present investigation, we determined antifungal and antiradical activity of leaf extract of Moringa stenopetala (Baker f.) Cufod (Moringaceae). Extraction was carried out by maceration technique using methanol as solvent. Antifungal efficacy was determined by poisoned food technique against 8 seed borne fungi. Antiradical activity of extract was screened by DPPH and ABTS free radical scavenging assays. The extract was effective in inhibiting the growth of all test fungi and the effect was concentration dependent. Species of Aspergillus were found to be more susceptible to leaf extract. The extract exhibited marked dose dependent scavenging of DPPH and ABTS radicals. Scavenging of ABTS radicals was marked when compared to DPPH radicals. The plant appears to be promising resource of bioactive principles having antifungal and radical scavenging potential. The plant can be used against phytopathogenic fungi and oxidative stress.

Key Words: *Moringa stenopetala, Antifungal, Poisoned food, Free radical, DPPH and ABTS*

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I. Introduction

Fungi are well known to be the aetiological agents of a number of plant diseases and the fungal infections of plants results in considerable economic loss to the farmer. Besides, fungi are also implicated in the spoilage of grains or seeds during storage, rendering them unfit for human consumption by reducing their nutritive value. Some fungi are known to produce toxic secondary metabolites called mycotoxins (for example, Aflatoxins by *A. flavus*) which on consumption lead to mycotoxicoses. Immense interest in plants with antifungal property has been triggered due to several drawbacks such as high cost, adverse health effects and environmental pollution that are associated with the use of synthetic fungicides. Studies have shown the inhibitory effect of several botanicals

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against a number of seed borne phytopathogenic fungi (Satish et al., 2007; Avasthi et al., 2010; Amrouche et al., 2011; Chandra and Mahesh, 2013).

Free radicals are produced during normal metabolism and are associated with various degenerative disorders such as neurodegenerative diseases, carcinogenesis, cardiovascular disturbances and ageing. Antioxidants combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process, viz., initiation, propagation and termination. These antioxidants are also produced by biological system. The balance between oxidants and antioxidants decides the health and disease. In pathophysiological conditions, there is an extract need for antioxidants from external sources, mainly through diet. Plants are considered as an important source of antioxidants (mainly polyphenolic compounds, ascorbic acid and tocopherols) and their consumption results in health benefits (Gulcin et al., 2011; Kedare and Singh, 2011; Raghavendra et al., 2015).

Moringa stenopetala (Baker f.) Cufod belonging to the family Moringaceae is an underutilized, fast growing, domesticated *Moringa* species in East African countries. It is indigenous to southern Ethiopia and is used as indigenous vegetable food crop in south western Ethiopia. Local tribes of Ethiopia consume the leaves as vegetable especially during the dry season. The plant is adapted to semi-arid areas. The leaves and fruits are consumed as vegetables as they are nutritionally rich (in particular proteins). Often, the leaves and pods are used as animal fodder. The seeds of *M. stenopetala* have flocculating property and are promising as purifying agent in turbid water. Various parts of the plant have been used traditionally to treat malaria, hypertension, asthma, diabetes, common cold, wounds, retained placenta and stomach problem (Mekonnen and Gessesse, 1998; Abuye et al., 2003; Melesse et al., 2011; Seifu, 2012; Seifu, 2014).

Extract of leaf and root of *M. stenopetala* caused concentration dependent antileishmanial activity (Mekonnen and Gessesse, 1998). The ethanol extract of fresh root wood and the acetone extract of dried leaves were found to possess antitrypanosomal activity (Mekonnen et al., 1999). Extracts and fractions from various parts of the plant have shown antimicrobial activity (Walter et al., 2011; Chekesa and Mekonnen, 2015). Leaf extracted in water caused significant fall in blood pressure of experimental animals (Mengistu et al., 2012). Leaf powder and extracts exhibited anticoccidial activity against Eimeria tenella infection in broiler chickens (Meskerem and Boonkaewwan, 2013). The hydroalcoholic extract of leaves was shown to inhibit intestinal α-glucosidase, pancreatic cholesterol esterase and pancreatic lipase activities (Toma et al., 2014). The seed powder was found to be effective in the removal of several heavy and toxic metals (Degefu and Dawit, 2013; Sajidu et al., 2013). In the present study, we screened antifungal (against seed borne fungi) and antiradical activity of leaf extract of *M. stenopetala*.

II. Materials and Methods

Collection and extraction of plant material: The fresh leaves of *M. stenopetala* leaves were collected form Ambo, Ethiopia. Ambo city which is located 112 km south of Addis Ababa, the capital city of Ethiopia. Identification of plant was made by referring regional flora (Verdcourt, 1986). The leaves were washed well, dried under shade and powdered in a blender. Extraction of powdered leaf (10g) was carried out by maceration process using methanol (100ml). After filtration through Whatman No. 1 filter paper, the filtrate was evaporated to dryness at 50°C in an oven. The condensed extract was stored in refrigerator until use (Raghavendra et al., 2015).

Antifungal activity of leaf extract: Inhibitory potential of leaf extract of *M. stenopetala* was assessed by Poisoned food technique against seed borne fungi namely *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Penicillium* sp., *Fusarium oxysporum*, *Rhizopus* sp., *Alternaria alternata* and *Curvularia lunata*. These fungi were isolated from moldy grains of sorghum and ground nut by standard blotter technique. Identification of these fungi was made on the basis of cultural and microscopic characteristics. The test fungi were aseptically inoculated on control (without extract) and poisoned (0.5, 1.0 and 1.5mg extract/ml of medium) potato dextrose agar plates by point inoculation method. The plates were incubated in upright position for 72 hours at room temperature. The diameter of fungal colonies (in cm) on control and poisoned plates was measured in mutual perpendicular directions using a ruler.

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The antifungal effect of leaf extract (in terms of inhibition of mycelial growth) was determined using the formula:

Inhibition of mycelial growth (%) = (C – T / C) x 100, where C and T denotes the diameter of colonies on control and poisoned plates respectively (Raghavendra et al., 2015).

Antiradical activity of leaf extract: Two *in vitro* antiradical assays namely 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) assays were performed to investigate the efficacy of leaf extract of *M. stenopetala* to scavenge free radicals. The protocol employed in our earlier study was followed to determine antiradical activity (Raghavendra et al., 2015).

DPPH radical scavenging activity: DPPH radical solution (0.1mM) and various concentrations of leaf extract of *M. stenopetala* and ascorbic acid (3.125-100µg/ml) were prepared in methanol. In labeled tubes, 1ml of different concentrations of leaf extract and ascorbic acid was mixed with 3ml of DPPH radical solution. The tubes were kept in dark for 30 minutes and the absorbance of each tube was read at 517nm spectrophotometrically. Radical scavenging efficacy of various concentrations of extracts/ascorbic acid was determined using the formula:

Radical scavenging activity (%) = (C – T / C) x 100, where C and T refers to absorbance of DPPH control and DPPH and extract/ascorbic acid combination. IC_{50} value was calculated (using Origin 6 software) which denotes the concentration of extract needed to scavenge 50% of free radicals.

ABTS radical scavenging activity: Different concentrations of leaf extract of *M. stenopetala* and ascorbic acid (3.125-100µg/ml) were prepared in methanol. In brief, 1ml of different concentrations of leaf extract and ascorbic acid was added to 3ml of ABTS radical solution in labeled tubes. The tubes were incubated for 30 minutes at room temperature and the absorbance was measured spectrophotometrically at 730nm. The radical scavenging activity of extract and ascorbic acid was calculated using the formula:

Radical scavenging activity (%) = (C – T / C) x 100, where C and T refers to absorbance of DPPH control and DPPH and extract/ascorbic acid combination. IC_{50} value was calculated (using Origin 6 software) which denotes the concentration of extract needed to scavenge 50% of free radicals.

III. Results and Discussion

Antifungal activity of leaf extract

In the present study, we evaluated the antifungal activity of three concentrations of extract (0.5, 1.0 and 1.5mg extract/ml of medium) by Poisoned food technique. This method is one of the widely used methods to screen antifungal activity of a variety of samples including plants and their components. Reduction in the size of colonies of test fungi on poisoned plates when compared to control plates indicates antifungal activity (Satish et al., 2007; Avasthi et al., 2010). All concentrations were effective in inhibiting the growth of test fungi. The leaf extract of *M. stenopetala* was found to exhibit concentration dependent inhibition of mycelial growth of test fungi. A considerable reduction in the size of the fungal colonies was observed with an increase in the concentration of extract. Among fungi, marked susceptibility was recorded in case of *Aspergillus* species. Among the species of *Aspergillus*, *A. flavus* was inhibited to higher extent. An inhibition of 50% and higher of all test fungi was observed at 1.5 mg/ml extract concentration. Least inhibitory effect of extract was observed in case of *F. oxysporum* and *C. lunata* (Table 01).

Table 01. Antifungal activity of leaf extract of *M. stenopetala*

Test fungi	Colony diameter at different concentrations of extract (% inhibition of test fungi)			
	0 mg/ml	0.5 mg/ml	1.0 mg/ml	1.5 mg/ml
A. niger	3.6	3.0 (16.66)	2.4 (33.33)	1.4 (61.11)
A. flavus	2.8	2.1 (25.00)	1.4 (50.00)	0.5 (82.14)

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A. fumigatus	3.1	2.4 (22.58)	1.6 (48.38)	0.6 (80.64)
Penicillium sp.	2.6	2.2 (15.38)	1.8 (30.76)	1.2 (53.84)
Rhizopus sp.	5.6	4.8 (14.28)	3.8 (32.14)	2.6 (53.57)
F. oxysporum	4.4	4.1 (06.81)	3.6 (18.18)	2.2 (50.00)
A. alternata	3.3	2.8 (15.15)	2.4 (27.27)	1.6 (51.51)
C. lunata	3.6	3.3 (08.33)	2.8 (22.22)	1.8 (50.00)

Antiradical activity of leaf extract

DPPH assay is one of the most widely used in vitro radical scavenging assays and is considered as a valid, accurate, easy and economic method to evaluate radical scavenging activity of various types of samples including plant extracts. It is a stable radical (by virtue of the delocalisation of the spare electron over the molecule as a whole) and need not to be generated and the results are reproducible. The assay was developed by Blois (1958) and the radical has a strong absorption at 515-520nm in alcoholic solution. The assay is done in dark (incubation) because the absorbance of DPPH decreases in the presence of light. Substances capable of donating hydrogen atom (often termed antioxidants) will reduce the purple colored DPPH to DPPHH which has yellow colored (Blois, 1958; Kedare and Singh, 2011; Raghavendra et al., 2015). In the present study, we evaluated radical scavenging efficacy of leaf extract of *M. stenopetala* by DPPH assay and the decrease in absorbance of DPPH radical solution was monitored at 517nm. Extract was found to exhibit marked dose dependent scavenging of radicals (Figure 01). A scavenging effect of >50% was observed at concentration 25µg/ml and higher. At concentration 100µg/ml, a scavenging effect of 96.55% was observed. The IC_{50} value for extract and ascorbic acid was found to be 12.59µg/ml and 6.14µg/ml. Although the scavenging potential of leaf extract of *M. stenopetala* was lesser than that of reference standard (ascorbic acid), it is evident that the leaf extract possess antioxidant principles having hydrogen donating property which possibly could act as free radical scavengers. In a previous study, Tebeka and Libsu (2014) observed dose dependent scavenging of DPPH radicals by 80% aqueous methanolic extract of leaves. Assefa et al. (2015) showed DPPH radical scavenging activity of cold water extract and decoction of leaves of *M. stenopetala*. In terms of IC_{50} value, the scavenging activity observed was lesser when compared to our study.

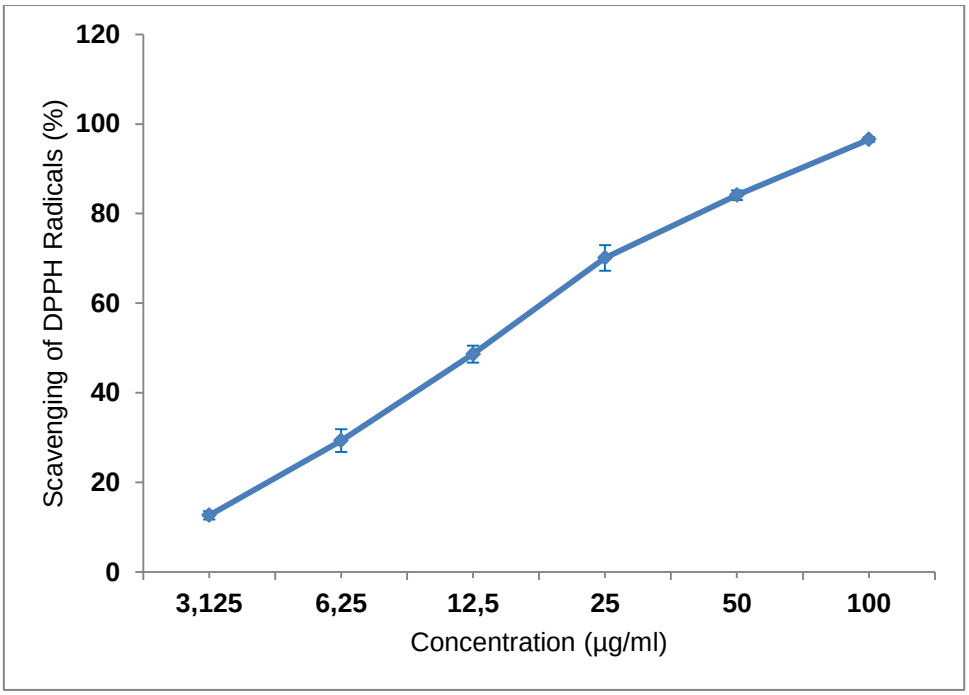

Figure 01. Scavenging of DPPH radicals by different concentrations of leaf extract.

Like DPPH assay, an assay that involves scavenging of ABTS radical cation is another routinely used in vitro radical scavenging assay which is used to evaluate radical scavenging potential of wide range of samples including plant extracts. However, this assay differs from DPPH method as ABTS assay requires generation of ABTS radical cation. This is accomplished by allowing ABTS stock solution

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(7mM) and postassium persulphate (2.45mM) to react in dark condition at room temperature for 12-16 hours. The resulting blue-green radical solution is diluted with water to an absorbance of 0.70 at 734 nm. The ABTS assay can be used to determine the antioxidant capacity of both lipophilic and hydrophilic antioxidants. Substances capable of donating electrons will reduce the blue-green ABTS radical solution to colorless neutral form which is highlighted by suppression of characteristic long wavelength absorption spectrum of radical (Re et al., 1999; Wangcharoen and Morasuk, 2007; Kekuda et al., 2015; Raghavendra et al., 2015). In the present study, we determined the efficacy of leaf extract of *M. stenopetala* to scavenge ABTS radical cations and the result is shown in Figure 02. The extract and ascorbic acid scavenged ABTS radicals in a dose dependent manner. A scavenging potential of >50% was observed at extract concentration 12.50µg/ml and higher. Leaf extract displayed marked scavenging activity with an IC_{50} value of 9.10µg/ml. However, ascorbic acid exhibited stronger scavenging activity (IC_{50} value 3.90µg/ml) when compared to leaf extracts. Although leaf extract of *M. stenopetala* exhibited lesser scavenging effect when compared to ascorbic acid, it is evident that the leaf extract contain compounds having electron donating property and hence the extract can act as potent free radical scavengers.

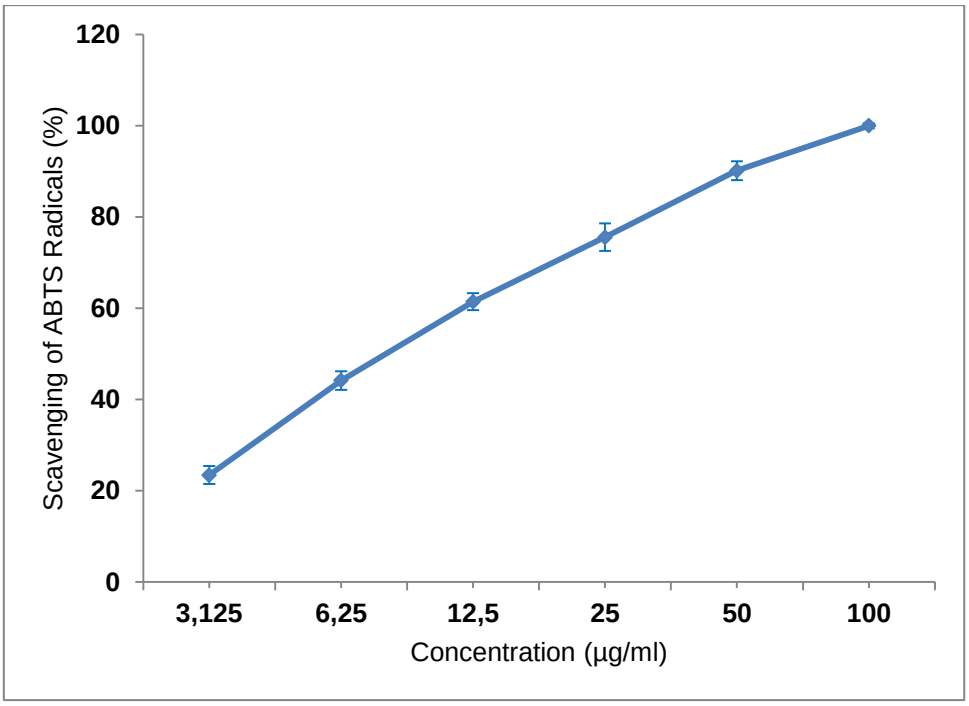

Figure 02. Scavenging of ABTS radicals by different concentrations of leaf extract.

IV. Conclusion

Leaf extract exhibited marked antifungal activity which is dose dependent. Use of the leaves in suitable formulation can possibly reduce the incidence of seed borne fungi which are implicated in deterioration of seed or grain quality. Extract was shown to exhibit an appreciable radical scavenging efficacy. Leaf of *M. stenopetala* can be used to prevent and control oxidative damage induced by free radicals. Presence of active principles in the leaf extract accounts for the observed bioactivities which are to be isolated and characterized.

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