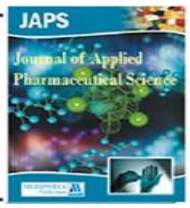
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 17-04-2012
Revised on: 28-04-2012
Accepted on: 02-05-2012
DOI: 10.7324/JAPS.2012.2510

Preliminary Phytochemical Screening, *In-Vitro* Antioxidant and Cytotoxic Activity of Five Different Extracts of *Moringa Oleifera* Leaf

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ABSTRACT

The purpose of this study was to judge the antioxidant activity and brine shrimp lethality bioassay followed by phytochemical screening of five different extracts of *Moringa oleifera* leaves. Preliminary Phytochemical screening of the crude extracts of *Moringa oleifera* leaf revealed the presence of different kind of chemical groups such as Flavonoids, tannin, Saponin, alkaloids, glycosides, carbohydrate and Triterpenoids. The leaf exhibited significant DPPH free radical scavenging activity with highest IC_{50} value showed by chloroform extract with a value of 47.481 µg/ml followed by ethanol and methanol having value of 62.09 and 68.321 respectively as opposed to that of the scavenging effects of ascorbic acid and BHT of 5.698 and 8.816 respectively. Dried leaf of *Moringa oleifera* were subjected to brine shrimp lethality bioassay and the LC_{50} values of methanol, ethanol, petroleum ether, n-hexane and chloroform were found to be 0.747µg/ml, 0.712 µg/ml, 1.632 µg/ml, 2.163 µg/ml and 0.633 µg/ml respectively. The data obtained in the present study suggests that the extracts of Moringa oleifera leaves have potent antioxidant activity against free radicals and significant cytotoxic activity that can be used in disease prevention.

Keywords: Phytochemical screening, antioxidative activities, DPPH, Brine shrimp lethality bioassay.

INTRODUCTION

Moringa oleifera (Moringaceae) is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands (Iqbal *et al*, 2006). Literature revealed that the Moringa tree was introduced to Africa from India at the turn of the twentieth century where it was to be used as a health supplement (Muluvi *et al*., 1999). The Moringa plant has been consumed by humans throughout the century in diverse culinary ways (Iqbal *et al.*, 2006). Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste and flavor as a vegetable and seed. The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value (Arabshahi *et al.*, 2007*;* Fahey, 2005).

With the leaves being rich in nutrients, pregnant women and lactating mothers use the powdered leaves to enhance their child's or children's nourishment, especially in under developed countries suffering from malnutrition (McBurney *et al.,* 2004; Lockett *et al.,* 2000). Moringa leaf has been purported to be a good source of nutrition and a naturally organic health supplement that can be used in many therapeutic ways (McBurney *et al.,* 2004; Fahey, 2005; DanMalam *et al.,* 2001). The leaves are a very rich source of nutrients and contain the essential vitamins A, C and E. Though not proven, it is has been considered by many to contain as much vitamin A as a carrot, vitamin C as an orange and vitamin E as a pomegranate. Leaves rich in biologically active carotenoids, tocopherols and vitamin C have health promoting potential in maintaining a balanced diet and preventing free-radical damage that can initiate many illnesses (Smolin *et al.,* 2007). While the provitamins cannot be identified in the leaves, they can be monitored after conversion to their respective vitamins within the body. The edible Moringa leaves contain essential provitamins, including ascorbic acid, carotenoids (Lako *et al.,* 2007) and tocopherols (Gomez- Coronado *et al.,* 2004; Sánchez-Machado *et al*., 2006). Epidemiological studies have indicated that *M. oleifera* leaves are a good source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities (Chumark *et al.,* 2008; DanMalam *et al*., 2001; Dahiru *et al*., 2006). Considering the traditional uses of this Ayurvedic drug, the aim of this study was to investigate the antioxidant and cytotoxic activity of *Moringa oleifera* leaves.

MATERIALS AND METHODS

Chemicals

1, 1 diphenyl-2-picrylhydrazyl (DPPH), Vincristine sulfate was obtained from Sigma Aldrich. Other chemicals, Methanol, Butylated hydroxytoluene (BHT), Concentrated H_2SO_4 (96%) were obtained from (Merck KGaA, Darmstadt, Germany). Ascorbic acid, Ferric Chloride, Picric acid, Lead acetate from Loba Chemie Pvt.Ltd, India. Sodium chloride crystal, Sodium hydroxide pellets, Gelatin, Chloroform, Dimethyl sulfoxide (DMSO), 1-Naphthol from Merck Specialities Private Limited, India. All other reagents and chemicals used were of analytical grade.

Collection of plant sample

Plant sample of *Moringa oleifera* was collected from suburb of Dhaka, Bangladesh. After complete cleaning and ringing with water, leafs were sun dried for seven days. The dried leafs were then grinded in coarse powder using high capacity grinding machine which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction procedure

The powdered plant material (20 gm) was successively extracted in a Soxhlet extractor at elevated temperature using 200 ml of distilled petroleum ether (40-60°C) which was followed by n-hexane, ethanol, chloroform and methanol. All extracts were filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvents from the extract to get dry extracts. After drying in desicator, crude extracts were weighed and stored in stock vials and kept in refrigerator (0- 4°C) for farther use.

Phytochemical screening

Preliminary phytochemical screening was performed to identify the various classes of active chemical constituents such as flavonoids, tannins, saponins, alkaloids, glycosides, carbohydrates and triterpenoids.

DPPH free radical scavenging assay

The free radical scavenging capacity of the extracts was determined using DPPH (Braca *et al.,* 2001). Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (15.625 µg/ml to 250 µg/ml) to every test tube so that the final volume was 5 ml and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid and Butylated hydroxy toluene (BHT) was used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 517 nm using a spectrophotometer. Methanol was served as blank.

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer *et al.,* 1982). The brine shrimp, *Artemia salina*, was used as a convenient monitor for the screening. The eggs of the brine shrimp, *A. salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution, pH 8.5) for 48 hr to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. The test samples (extract) were prepared by dissolving in DMSO (not more than 50 µl in 5 ml solution) with sea water (3.8% NaCl in water) to attain concentrations 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/ml. In the present study Standard vincristine sulfate was used as positive control. As vincristine is a very cytotoxic alkaloid and it was evaluated at very low concentration (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 µg/ml). Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial were counted. The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC50) from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis. The LC50 was derived from the best-fit line obtained.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary Phytochemical screening of the crude extracts of leaf of *Moringa oleifera* revealed the presence of different kind of chemical groups that are summarized in table 1. Methanol and

ethanol extracts of leaf of *Moringa oleifera* contain phenols, Flavonoids, tannin, Saponin, alkaloids, glycosides, carbohydrate and Triterpenoids. Pet-ether extract of leaf of *Moringa oleifera* contains phenols, Flavonoids, tannin, alkaloids, glycosides, carbohydrate, fats and oils and n-hexane extract contains, tannin, carbohydrate, Flavonoids and Triterpenoids. Chloroform extract contains Flavonoids, tannin, Triterpenoids and alkaloids. Water extract of leaf of *Moringa oleifera* contains phenols, Flavonoids, tannin, Saponin, alkaloids, glycosides and carbohydrate. Flavonoids present in all six extracts of leaf of *Moringa oleifera* and steroid is present only in methanol extract.

Table. 1: Result of chemical group test of various extracts of leaf of *Moringa oleifera.*

TESTS	EXTRACT					
	Metha-nol	Etha-nol	Pet-ether	n-hexane	Chloro-form	Water
Phenols	+	+	+	-	+	+
Flavonoids	+	+	+	+	+	+
Tannin	-	+	+	+	+	+
Saponin	+	-	-	-	-	+
Alkaloids	+	+	+	-	+	+
Glycosides	+	+	+	-	-	+
Carbohydrate	+	+	+	+	-	+
Steroids	+	-	-	-	-	-
Amino acids	-	+	-	-	-	-
Fats and Oils	-	+	+	-	-	-
Triterpenoids	+	+	-	+	+	-
Reducing sugars	-	-	-	-	-	-

+++: Present in high concentration, ++: Present in moderate concentration, +: Present in low concentration and -: Absent

DPPH free radical scavenging assay

The free radical scavenging activity of different extracts of *Moringa Oliefera* leaf was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. The chloroform, ethanol and methanol extracts showed maximum activity of 73.90%, 69.35% and 67.59% respectively at 250 µg /ml, where as ascorbic acid and BHT at the same concentration exhibited 96.66% and 92.59 % inhibition respectively. Five extracts exhibited considerable DPPH free radical scavenging activity as indicated by their IC_{50} values and this has been showed in (Table 2 and Figure 1).

Table. 2: IC_{50} values of different extracts of *Moringa oleifera* in DPPH scavenging assay.

Extracts/standard	IC_{50} µg/ml
Methanol	68.321
Ethanol	62.09
Pet-ether	10028.15
n-hexane	12365.42
Chloroform	47.481
Ascorbic acid	5.698
Butylated hydroxy toluene (BHT)	8.816

IC_{50} Indicate the potency of scavenging activity. Standard ascorbic acid and BHT were found to have an IC_{50} of 5.698 µg/ml and 8.816 µg/ml. In comparison to standard ascorbic acid and BHT, chloroform, methanol and ethanol extract of *Moringa Oliefera* leaf showed IC_{50} of 47.481, 68.321 and 62.09 respectively. Petroleum ether and n-hexane fraction is seen to have the least free radical scavenging activity.

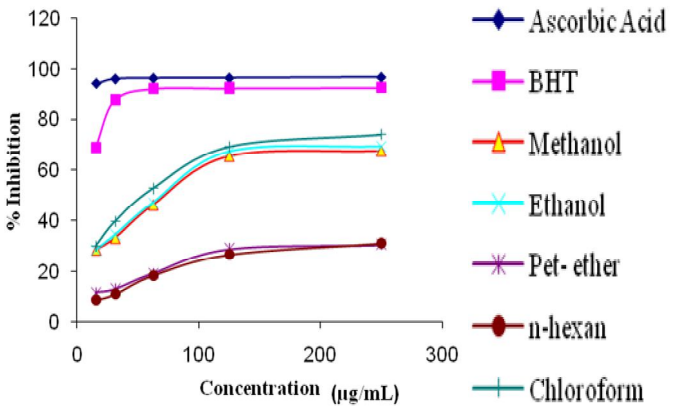

Fig. 1: Comparative DPPH radical scavenging activity of *Moringa Oliefera* leaf extract, Ascorbic acid and Butylated hydroxy toluene (BHT)

Brine shrimp lethality bioassay

In the present bioactivity study the five crude extracts and pure compounds showed positive results indicating that the test samples are biologically active. The methanol, ethanol, petroleum ether, n-hexane and chloroform extract of the dried leaf of *Moringa oleifera* were subjected to brine shrimp lethality bioassay following the procedure which has been utilized by Meyer *et al.*, 1982. The lethality of the extractives to brine shrimps was determined and the results are given in Table 3.

Table. 3: LC_{50} values of the five extracts of *Moringa oleifera* and standard.

Test Samples	Regression line	R^2	LC_{50} values
Vincristine	y = 29.79x + 64.62	R^2 = 0.927	0.323
Methanol	y = 18.92x + 52.39	R^2 = 0.942	0.747
Ethanol	y = 17.91x + 52.64	R^2 = 0.905	0.712
Pet-ether	y = 19.32x + 45.89	R^2 = 0.872	1.632
n-hexane	y = 19.73x + 43.38	R^2 = 0.921	2.163

The lethal concentration (LC_{50}) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. Vincristine Sulphate (VS) was used as positive control and the LC_{50} was found as 0.323 µg/ml. Compared with the negative control, VS (positive control) gave significant mortality and the LC_{50} values of the different extractives were compared with negative control.

The LC_{50} values of methanol, ethanol, petroleum ether, n-hexane and chloroform were found to be 0.747µg/ml, 0.712 µg/ml, 1.632, 2.163 µg/ml and 0.633 respectively (Table 3). However, varying degree of lethality of *Artemia salina* was observed with exposure to different dose levels top the test samples ranging from 0.781-400 µg/ml. The degree of lethality shown by the extractives was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (0.781 µg/ml) to the highest concentration (400 µg/ml). This concentration dependent increment in percent mortality of Brine Shrimp nauplii

produced by the *Moringa oleifera* extracts indicates the presence of cytotoxic principles in these extractives. There was no mortality in the negative control groups indicating the test as a valid one and the results obtained are only due to the activity of the test agents.

CONCLUSION

Further work is needed to isolate the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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