ANTIOXIDANT POTENTIAL AND SKIN IRRITANCY OF *MORINGA OLEIFERA* LAMK.

Muhammad Usman, Safdar Ali and Zaheer-ud-din Khan

Department of Botany, GC University, Lahore, Pakistan.
Corresponding: safdaralimirza@gmail.com

ABSTRACT

The present study was undertaken to evaluate the antioxidant activity and irritancy or dermatological effects of extracts of *Moringa oleifera* Lamk. belonging to family Moringaceae. The crude extracts of powdered plant material were obtained in non-polar and polar solvents such as petroleum ether, chloroform, methanol, and distilled water, The %age yield of distilled water extract of flower was highest (85%) among all the four extracts. All the extracts of leaves were found highly antioxidant showing greater activity than the scavenging activity where chloroform extract showed higher value (1.6±00) than standard BHT (0.905±00). But in case of flower extracts only distilled water extract was recorded to hold highest antioxidant activity (1.246±00) as compared to standard (0.676±00). The tests of dermatological activity indicated that only the chloroform extract of flower caused slight redness on rabbit ear's skin after 24 to 48 hours.

Keywords: *M. oleifera*, antioxidant activity, dermatological effects

INTRODUCTION

Generation of ATP and consumption of oxygen within the body of animals is accompanied with the formation of Reactive Oxygen Species (as free radicals) as a result of redox mechanisms occurring in cell. The Reactive Oxygen Species (ROS) when produced in larger quantities interfere through oxidation of fundamental biomolecules mandatory for the metabolic operations inside the living systems resulting in the cell annihilation, tissue affliction or variant ailments such as arteriosclerosis, cancer, cardiovascular diseases, inflammations, neural disorders and skin irritations. The amount of free radicals can be demilitarized and prowled by the assistance of antioxidant composites. These conglomerates either contribute their hydrogen atoms or chelate metals to avert the consequences of the oxidants (Koksal *et al*., 2011). The paramount commencement of antioxidants in the human body is the nourishing subsistence consumed by the individuals, encircling appropriate expense of plant commodities. These antioxidants then get incorporated in the quintessence and execute the key responsibility of contradicting the oxidative deterioration to human body. The most important source of antioxidants is the derivatives of the phenolic amalgamations and flavonoids which occur naturally in the crude entities of the plants (Koksal *et al*., 2011). Due to the substantial articulation of the antioxidants in the metabolism and the immunity of animals including human beings results in conspicuous conservation from the afflictions, the concern has been evolved to assess the endeavor for the discernment of the more and more antioxidant phytochemicals (Ebrahimzadeh *et al*., 2010). In the same perspective as described above, the present study was undertaken to explore the antioxidant potential and skin irritatancy potency of *M. oleifera* plant material.

MATERIALS AND METHODS

Preparation of crude extracts of *M. oleifera* plant material

Moringa oleifera plant material was obtained from District Bahawal Pur. The plant identification was authenticated from Department of Botany, GCU, Lahore. Its leaves were shade dried and ground (max. particle size 1mm). Fifty gram ground material was soaked in 250 ml petroleum ether for one week. The filtered plant residue was re-soaked in 500 ml methanol for one week and filtered extract was taken.

DPPH free radical scavenging activity

In order to evaluate the free radical scavenging activity of the leaves and flowers extracts of *M. oleifera*, each extract was allowed to react with a stable free radical, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) according to Blois (1958), Brand-Williams *et al.* (1995) and Scanchez *et al.* (1998). The extract solutions were prepared by dissolving 0.5 mg/ml of each dry extract in the respective extraction solvent.

Each extract (0.2 ml) was added to 3.8 ml of 0.025% (w/v) solution of DPPH in 95% ethanol. The reaction mixture was incubated at 28^{o}C for 40 minutes. The scavenging activity on DPPH free radical was determined by measuring the absorbance at 517 nm. The antioxidant activity was expressed as %age of scavenging activity on

DPPH free radical: SC%= [1-(absorbance of sample)/(absorbance of control)] ×100. The control contained all reagents except the relevant extract. The DPPH free radical scavenging activity of BHT (0.5 µg/ml) was also assayed for comparison. All tests were performed in triplicate and the means were calculated.

Determination of total antioxidant capacity

The total antioxidant capacity of each extract was assayed according to the method of Prieto *et al*. (1999). 0.1 ml of each extract of *M.oleifera* (0.5mg/ml) was combined with 1.9 ml of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The reaction mixture was incubated at 95^{o}C for 60 minutes. After the mixture had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed as the absorbance of the sample. The antioxidant activity of a standard antioxidant, BHT (0.5 mg/ml) was also assayed for comparison. (Table 2 and 4).

Skin irritant activity

To test the skin irritancy of the crude extracts of leaves and flower, albino rabbits were used. They were first acclimatized in the animal house for four days prior to their use. Standard green food and water was available *ad libitum*. The following procedure was adopted for skin irritancy studies:

Each of the solvent extracted material was accurately weighed (20 mg) and made 20 mg/10 ml (w/v) with acetone. One of the extract solutions (10 µL) was applied to the inner surface of an ear of a rabbit using a Drummond's microcap, while the other ear was used as a control. Similar procedure was adopted for the other solvent extracts of each plant part. For comparison, simple acetone was applied on one of the ears of a rabbit as a negative control (vehicle control). While the latex of *Euphorbia splendens* (20 mg/10 ml in acetone w/v) was applied to one of the ears of another rabbit as a positive control [(caustic latex of *Euphorbia* species often gives 100% positive reaction) (Evans and Schmidt, 1974; Evans and Soper, 1978)]. The ears were examined for redness after 30 minutes intervals until two of the examinations indicated that further redness would not occur. The ears were also examined after 24 and 48 hours to ascertain any chronic inflammatory reaction. The responses observed were graded in a similar way as described by Evans and Schmidt (1980) on mice. The time that showed +++ response was regarded as the positive peak irritancy time after Hecker (1971) and Evans and Soper (1978). - No reaction, + Doubtful reaction, + Slight reddening of main vessel, ++ Marked reddening of the main vessels, with reddening of the area in between +++ Intense reddening of entire ear, ++++ Macroscopically visible hyper-plasia. (Table 5).

RESULTS

Antioxidant activity of *M. oleifera* plant materials

A flat concentration Assay was carried out with four extracts of the leaves and flowers of *M. oleifera*. The results thus obtained provided a direct comparison of antioxidant activity shown by the extracts and by the standard antioxidants, such as BHT. All the extracts of leaves showed scavenging activity, e.g. 95% of methanolic extract, 53.19% of petroleum ether, chloroform 60.99% and distilled water 78% (Table 1). Moreover the /flower extracts showed scavenging activity i.e. methanolic extract 73.55%, Petroleum ether 1.442%, chloroform extract 21.15% and distilled water 85% as compared with BHT standard with 80.2% scavenging activity as shown in (Table 3).

Table 1. Scavenging activity of leaf extracts on DPPH free radical.

Leaf Extracts	Value	Percentage
Methanol	0.007±00	95%
Petroleum ether	0.066±00	53.1%
Chloroform	0.055±00	60.99%
Distilled water	0.031±00	78%
BHT	0.004±00	97.1%
Blank(solvent)	0.141±00	--

Table 2. Total antioxidant activity of extracts of leaves.

Leaf Extracts	Value
Methanol	0.922±00
Petroleum ether	0.789±00
Chloroform	1.614±00
Distilled water	0.671±00
BHT	0.905±00
Blank	0.195±00

Table 3. Scavenging activity of flower extracts on DPPH free radical.

Flower Extracts	Value	Percentage
Methanol	0.055±00	73.55%
Petroleum ether	0.205±00	1.422%
Chloroform	0.252±00	21.15%
Distilled water	0.031±00	85%
BHT	0.041±00	80.2%
Blank(solvent)	0.208±00	

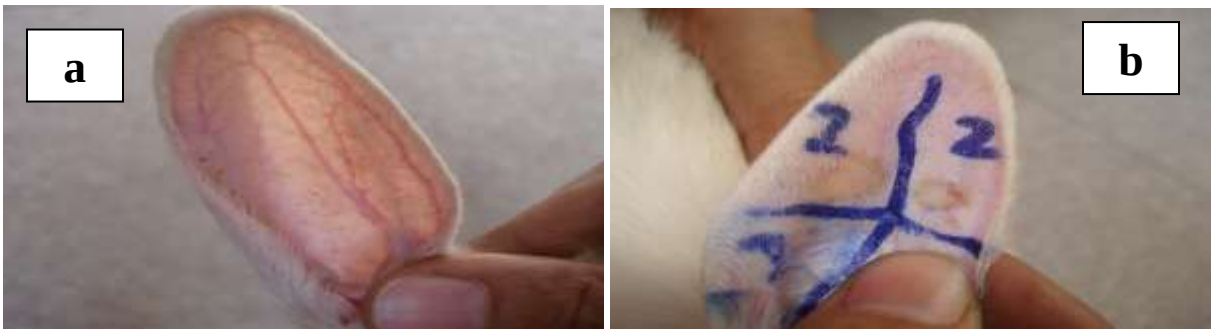

Fig. 1. Skin irritancy test for *M. oleifera* flower extracts**.**
(a) Rabbit's ear before applying the extracts of *M. oleifera* for studying skin irritancy
(b) Irritation caused by chloroform extract of flower of *M. oleifera* on rabbit's ear within one hour.

Fig. 2. Effect of leaf extract of *M. oleifera* on the rabbit's ear skin (No irritation response within 24 hours).

Table 4. Total antioxidant activity of extracts of flowers.

Flower Extracts	Value
Methanol	0.365±00
Petroleum ether	0.142±00
Chloroform	0.201±00
Distilled water	1.241±00
BHT	0.676±00
Blank	0.109±00

Table 5. Skin irritation response of the extracts of leaves and flowers of *M. Oleifera* [Dose = 10 µl of 1mg/ml in acetone]

Plant Part	Extracts	Response after acute time								Chronic time		
		30 min	1 h	5 h	6 h	7 h	8 h	9 h	10 h	24h	48 h	72 h
Leaves	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-	-	-
	Distd. Water	-	-	-	-	-	-	-	-	-	-	-
Flowers	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	+	+	+	+	+	+	+	+	-	-
	Distd. Water	-	-	-	-	-	-	-	-	-	-	-

Where - = No reaction, -- = Doubtful reaction, + = Slight reddening of the main vessels ++ =Marked reddening of the main vessels, +++ = Intense reddening to the entire ear

Total antioxidants capacity assay

The Assay was based on deduction of Mo (VI) to Mo (V) by eight extracts of leaves and flowers and subsequent formation of Green phosphate / Mo (V) complex.

The total antioxidants was measured and compared with that of BHT, a standard antioxidant. The absorbance value indicated that the sample possessed significant antioxidant activity. The results represented that extract of leaves in Chloroform had high total antioxidant activity, i.e. 1.614 as compared to 0.905 of standard antioxidant, BHT (Table 2). Furthermore the flower extract in distilled water represented high antioxidant capacity, i.e. 1.241 as compared to 0.676 activities of BHT (Table 3).

Skin irritancy potency

The extracts of leaves and flowers of *M. oleifera* were subjected to test against mammalian skin to record the skin irritation, i.e. dermatological effects by using rabbit's ear, a sensitive skin. The results indicated that extracts of leaves showed no effect on skin while chloroform extract of flower showed slight irritation and redness (+) as documented in the Table 5 and Figures 1 and 2.

DISCUSSION

During the collection of ethnobotanical data about *M. oleifera*, it was revealed that the local people use it as an anticancer plant (Ebrahimzadeh *et al.*, 2010). In order to evaluate or confirm this data, the antioxidant activity of different parts of the plant was investigated by standard techniques. It is generally believed that the total antioxidant activity is directly proportional to the anticancer activity, i.e. higher antioxidant activity means the higher/superior anticancer activity. According to Anwar *et al.* (2007) *M. oleifera* is considered to be a potential source of natural antioxidants due to the marked antioxidant activity as recorded in the present investigation as well as by Siddhuraju and Becker (2003) and Iqbal and Bhanger (2006).

Siddhuraju and Becker (2003) investigated radical scavenging capacity and antioxidant activities of the water, methanol and ethanol extracts of freeze-dried leaves of *M. oleifera* from different agro climatic regions. Among these, the plants of Indian origin showed the highest activity, i.e. from 65.1 to 66.8%. On the other hand Iqbal and

Bhanger (2006) investigated the antioxidant activity of *M. oleifera* from different agro climatic locations and recorded significantly higher activity in plants from Pakistan. As for as the results of the present work are concerned, the total antioxidant activity of flower extracts in distilled water and chloroform extract of leaves were higher than the standard antioxidant, BHT. Overall, total antioxidant activity of *M. oleifera*, as evaluated by two different testing systems indicated that flower and leaf extracts of *M. oleifera* had significant free radical scavenging activities on DPPH radical i.e. 95% of methanolic extract of the leaves and 85% of the aqueous extract of flowers (Table 3). This finding was in line with that of Iqbal and Bhanger (2006) who reported that the antioxidant activity of the plants of *M. oleifera* from Pakistan was higher than the plants from other countries as well as from other potent antioxidants. The free radical scavenging property is one of mechanisms by which an antioxidant, as a herbal medicine exhibits higher antioxidant activity. Thus this study provides a strong instigation for its use in food and medicine industry. Kumar and Pari (2003) investigated the protective effect of *M. oleifera* on hepatic marker enzymes, lipid peroxidation, and antioxidants during anti-tubercular drug (isoniazid, rifampicin, and pyrazinamide)-induced toxicity in rats. Enhanced hepatic marker enzymes and lipid peroxidation of anti-tubercular drug treatment was accompanied by a significant decrease in the levels of vitamin C, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione *S*-transferase. Administration of *M. oleifera* extract significantly decreased hepatic marker enzymes and lipid per-oxidation with a simultaneous increase in the level of antioxidants. It was speculated that *M. oleifera* extract exerted its protective effects by decreasing liver lipid peroxides and enhancing antioxidants. It also provides useful information on pharmacological activities associated with this traditional folk remedy. Antioxidant effect could be higher by combined and synergistic effects of other compounds. Therefore isolation and identification of individual active compounds, their *in vivo* antioxidant activities as well as different antioxidant mechanisms *in vitro* need to be studied.

The ethnobotanical survey of the study area indicated that *M. oleifera* is used both as fodder as well as vegetable. The human skin and that of the ruminant livestock come in contact with the plant material during handling, ingesting or eating processes. Therefore, the evaluation of the possible skin irritant effect of the plant material becomes evident. It was the reason that tests for dermatological effect of *M. oleifera* plant material were carried out as important pharmacological property of the plant. All the extracts of both the shoots and leaves (as a whole) and of flowers were found non-irritant except the chloroform extract of flowers of *M. oleifera* that caused the slight reddening of the blood vessel on the ear's skin of the Rabbit, which is considered to be most sensitive mammalian skin.

Conclusion

The skin irritancy tests showed that chances of skin irritation by the plant material of *M. oleifera* are negligible and it can safely be consumed by the livestock as well as by the local inhabitants of the study area. This conclusion was supported by the fact that no-body reported skin irritation due to *M. oleifera* plant materials during the ethnobotanical survey.

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