**ANTIOXIDANT PROFILING OF *MORINGA OLIEFERA* FLOWER EXTRACT**SAYYED U<sup>1</sup>, SINGH A<sup>1</sup>, PANDEY P<sup>2</sup>, TIWARI RK<sup>1</sup>, BAJPAI P<sup>1\*</sup> AND PATHAK N<sup>1</sup><sup>1</sup>Department of Bioscience, Integral University, Lucknow, Uttar Pradesh, India<sup>2</sup>Department of Bioengineering, Integral University, Lucknow, Uttar Pradesh, India**\*Corresponding Author: Preeti Bajpai** Associate Professor: Department of Biosciences Integral university, India, [pbajpai@iul.ac.in](mailto:pbajpai@iul.ac.in), Phone number: 09918040777Received 4<sup>th</sup> Nov. 2016; Revised 14<sup>th</sup> Jan. 2017; Accepted 27<sup>th</sup> March 2017; Available online 1<sup>st</sup> Sept. 2017**ABSTRACT**

*Moringa oleifera* Lam. (sahjan) belongs to the monogeneric family, Moringaceae and has an impressive range of medicinal uses with high nutritional value. Various parts such as leaves, flowers and pods possess a number of biological activities that are being employed in the treatment of various ailments for therapeutic purpose. The present study was framed to screen the potential bioactive components and determination of antioxidant activity of different flower extracts of *Moringa oleifera* thereof. The biochemical and bioactive potency of flower extracts of *M. oleifera* was evaluated by using different methods.

The phytochemical analysis of *Moringa oleifera* flower extracts showed the presence of numerous bioactive compounds such as sterols, alkaloids, saponins, phenols, flavonoids, terpenoids and glycosides in appreciable amount. The methanolic extracts of *Moringa* flower had shown the maximum presence of all metabolites followed by aqueous, ethanolic and hexane extracts. Moreover, FRAP assay revealed that methanolic extract had maximum reduction of ferric ion with minimum IC<sub>50</sub> (174.86 µg/ml) value. Likewise, reducing power of DPPH was noticed to be 93.20% at 500 µg/ml (IC<sub>50</sub>-242.29 µg/ml) in the methanolic extract. SOD and catalase activity was observed to be 163.63 U/mg at 500 µg/ml and 6112.45 U/g at 500 µg/ml respectively. Hence, from the study it could be concluded the methanolic extract of *Moringa oleifera* flower possessed potent antioxidant activity in terms of both enzymatic (SOD and catalase) and non-enzymatic antioxidants (DPPH and FRAP) which may be attributed due to the presence of appreciable amount of secondary metabolites in the methanolic flower extract of *Moringa oleifera*.

**Keywords:** *Moringa oleifera*, phytochemicals, antioxidants, DPPH, FRAP, SOD, catalase

## INTRODUCTION

In the present scenario, the modern conventional healthcare is associated with various problems of unsafe medicines, chronic diseases, resistant infections, autoimmune disorders and degenerative disorders of ageing, in spite of the scientific advances. The uses of natural resources are safer, cost effective and having wide range of medical applications. These benefits arouse the need for screening of newer and effective chemotherapeutic agents. In this regard *Moringa oleifera* could be used as a potential source for screening of wide range of pharmacologically active components. *Moringa oleifera* Lam. commonly known as Sahjan, belongs to Moringaceae family is the most widely cultivated species in the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan.[1] The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals [2] such as vitamins, minerals and specific phytochemicals, established for hypotensive, anticancer, antibacterial activities, potent anti-inflammatory agent[3] antitumour agent [4] and hepatoprotective against antitubercular drug such as isoniazid and rifampicin.[5,6] There were several reports delineating the pharmacological potential of various parts of *M. oleifera*. However, there are only few studies reporting the pharmacological and

antioxidant potential of *Moringa oleifera* flowers.

Various studies have reported the presence of bioactive compounds such as 4-(4'-O-acetyl- $\alpha$ -L-rhamnopyranosyloxy) benzyl isothiocy-anate, 4-( $\alpha$ -L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-( $\alpha$ -L-rhamnopyranosyloxy) benzyl glucosinolate in the various parts of *Moringa oleifera*. Hence, the rationale for this study was to determine the bioactive and antioxidant potential of the different flower extract of the *Moringa*.

## MATERIALS AND METHODS

### Collection of plant materials

The fresh flowers of the *Moringa oleifera* were collected in the vicinity of Integral University Lucknow. The flowers were cleaned, dried in shadow and crushed into powder.

### Morphological Characters

The morphology of fresh flower of *Moringa oleifera* was observed for the various parameters including shape, size, color, taste, surface characteristics.

### Preparation of extracts for enzymatic assay

Briefly, 0.5 g of flowers was extracted in 8 ml of buffer solution comprising of potassium phosphate buffer and 1% polyvinylpolypyrrolidon on ice bath. Homogenate were centrifuged at 15000

rpm for 30 min and supernatant was collected and stored in 4°C until used.

### **Preparation of Extracts for Non enzymatic assay**

#### **Hot Extraction (Soxhlet)**

Finely grounded crude flowers powder was extracted in different solvents (i.e, methanol, ethanol, hexane & aqueous) by Soxhlet extraction method and was stored in 4° C until used.

#### **Qualitative study of phytochemicals**

The phytochemical properties (saponin, tannin, alkaloids, polyphenols, sterols, flavonoids) were determined by the different methods. [7, 8]

#### **Test for Sterols**

##### **Salkowaski reaction**

2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of the test-tube in each 0.5 mg of extract residue. After shaken for few min, the appearance of red colour in the chloroform layer indicated the presence of sterols.

#### **Tests for Alkaloids**

##### **Wagner's reagent (Iodine-potassium iodine)**

In 5.0 ml of water, 1.27 g of iodine and 2.0 g of potassium iodide were dissolved and the final volume was made up to 100 ml by adding water. A brown flocculent precipitate was formed indicates the

presence of alkaloids, when a few drops of reagent were added to the test filtrate.

#### **Tests for Tannins**

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.

##### **Ferric chloride reagent**

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green colour was obtained showed the presence of tannins.

##### **Lead acetate test**

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitation was obtained shows the presence of tannins.

#### **Tests for Saponins**

##### **Foam test**

A few mg of test residue with a small amount of sodium bicarbonate and water was shaken vigorously in a test tube. Froth confirmed the presence of saponins.

#### **Tests for Phenolic compounds**

##### **Ferric Chloride test**

Extract was taken in water and warmed then added 2 ml of ferric Chloride (FeCl<sub>3</sub>). The formation green and blue colour solution indicates the presence of phenolic compounds.

### Tests for Flavonoids

5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow colouration observed in each extract indicated the presence of flavanoids. The yellow colouration disappeared on standing.

### Test for Terpenoids

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoid.

### Test for cardiac glycosides

5 ml of each extracts were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates deoxy sugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

### Test for volatile oils

A white precipitate confirmed the presence of volatile oils by the addition of 0.1 ml of dilute NaOH and a few drops of dilute HCl in 2 ml of extract.

### Diphenylpicrylhydrazine (DPPH) Assay

The scavenging ability of the flowers towards the stable free radical DPPH was measured by the method of Mensor *et al.* (2001) [9]. Briefly, 0.2 mM DPPH was prepared in 100% methanol and 1 ml of this solution was added to plant extract of different concentration (50 - 500 µg/ml) and control reaction was carried out without plant extract. The mixture was shaken and allowed to stand in dark at room temperature for 30 min and the absorbance was measured at 517 nm by spectrophotometer. The percentage scavenging inhibition was determined and compared with ascorbic acid, which was used as the standard.

$$\% \text{ DPPH radical-scavenging} = \frac{[(\text{Absorbance of Control} - \text{Absorbance of test Sample}) / (\text{Absorbance of Control})] \times 100}{}$$

### Ferric Reducing Ability of Plasma (FRAP) assay

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. [10] The stock solutions included 300 mM acetate buffer (3.1g CH<sub>3</sub>COONa and 16 ml CH<sub>3</sub>OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl<sub>3</sub>·6H<sub>2</sub>O. The temperature of the solution was raised to

37°C before using. Plant extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Observation of the colored product (ferrous tripyridyltriazine complex) was taken at 593 nm. FeSO<sub>4</sub> is used as a standard.

#### Superoxide dismutase assay

Superoxide scavenging activity of the extract was determined by McCord and Fridovich method (1969) [11], which depends on the light induced superoxide generation by riboflavin and the corresponding reduction of NBT. A final volume of assay mixture was made up to 3 ml by adding 0.3 ml of the extract, 0.2 ml ethylene diamine tetra acetic acid (6µM containing 3 µg NaCN), 0.1ml NBT (50 µM), 0.05 ml riboflavin (2µM) and 2.35 ml phosphate buffer (58 mM, pH 7.8). The tubes were uniformly illuminated with an incandescent light for 15 min and the optical density was measured at 560 nm. The assay of SOD is based on the inhibition of the reduction of NBT to blue formazan by the superoxide. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT. The values were calculated as units/mg protein.

#### Catalase assay

Catalase activity was determined by the method of Luck (1974). [12] H<sub>2</sub>O<sub>2</sub>-

phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer (Genesys 10-S, USA). The enzyme solution containing H<sub>2</sub>O<sub>2</sub>-free phosphate buffer served as control. The amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units is called as one unit of enzyme activity.

#### Statistical analysis

Data were expressed as mean±SEM of three-independent experiments. P < 0.05 was considered to test for a significant difference between control and treated groups.

## RESULTS AND DISCUSSION

The phytochemical analysis of *Moringa oleifera* flower extracts showed the presence of numerous bioactive compounds such as sterols, alkaloids, saponins, phenols, flavonoids, terpenoids and glycosides in appreciable amount (Table 2). Flavonoids, alkaloids, tannins & terpenoids were present almost equally in all the extracts. Methanol & ethanol extracts showed maximum presence of all the metabolites followed by aqueous and hexane extracts. The main secondary metabolites in plants are glycosides, terpenoids, steroids, tannins and phenol

compounds. Secondary metabolites are involved in various important biological and pharmacological activities such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic. Alkaloids are naturally occurring chemical compounds which contain basic nitrogen atoms, which often have pharmacological effects and are used as medications and amusing drugs.[13] Flavonoids are known to be biologically active against liver toxins, tumors, viruses and other microbes and enhance the effects of Vitamin C and therefore, used as strong antioxidants.[14] Plant terpenoids play a pivotal role as traditional and herbal medicines and are under investigation for antibacterial, antineoplastic and other pharmaceutical activities. They are extensively used for their aromatic properties. [15] Tannins show potential antiviral, antibacterial and anti-parasitic effects. Saponins are responsible for the hemolysis of red blood cells. [16] Thus, the preliminary screening tests for bioactive compounds may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Table 1 and Table 2).

### **Enzymatic and Non-enzymatic antioxidant assay**

Free radicals are the major cause for several disorders. Medicinal plants are

known to be a potential source of natural antioxidants all over the world. Of the numerous medicinal plants known from the time immortal, few widespread species are of peculiar interest as they are commonly being exploited for production of medical preparation containing phytochemical with significant antioxidant abilities [17]. *Moringa oleifera* is one of those medicinal plants. Therefore, these observations could help in developing novel natural drugs for the therapeutic use in human. Antioxidant activities of flowers extracts of *M. oleifera* was evaluated by using different assays such as DPPH and FRAP assay for non-enzymatic antioxidant activity and superoxide dismutase assay and catalase assay for enzymatic antioxidant activity. DPPH assay was performed to evaluate the antioxidant potential of different extracts of *Moringa* flower in dose dependent manner (Fig 1). Methanolic extract showed maximum inhibition (minimum IC<sub>50</sub> value at concentration 242.7µg/ml) followed by ethanol extract. A moderate DPPH reducing activity was observed in hexane and aqueous extracts with increasing concentration.

The percentage inhibition capacity of the different flower extract shown in Fig. 2. FRAP assays are widely used to determine the efficiency of the antioxidant compounds in the plants to reduce the ferric

ion to ferrous ion. Antioxidant compounds possessing strong reducing ability are categorized as secondary antioxidants. They neutralize free radical formation and prevent oxidative damage [20]. Interestingly, FRAP assay also showed best results with methanolic extract with minimum IC<sub>50</sub> value as observed in DPPH assay. The IC<sub>50</sub> value of various extract depicted in Fig 2. It has been reported that the reducing power of substances is probably because of their hydrogen-donating ability [18].

The determination of enzymatic antioxidant also portrayed the better efficacy of flower extract of *M. oleifera*. The superoxide activity of flower extract of *M. oleifera* was evaluated, which gets increased with increasing concentration of extract and it was observed to be  $161.11 \pm$

0.27 U/g at 500 µg/mL as depicted in Fig 3. Superoxide dismutase plays an important role in catalyzing the dismutation of the superoxide radicals. SOD enzymes work in conjunction with H<sub>2</sub>O<sub>2</sub> removing enzymes, such as catalase and glutathione peroxidase. Increase in SOD activity should accelerate the removal of the reactive oxygen species. Thereafter, catalase activity was found to be 6112.45 U/g of fresh weight depicted in fig 7. Catalase helps in removing the hydrogen peroxide produced by the action of SOD. The antioxidant potential of the extract of *Moringa oleifera* flowers may be due to the presence of vital phytochemicals like flavonoids and phenolics that act as a free radical scavengers attributing to its strong anticancer potential [19-21].

**Table 1: Observation of Organoleptic Characteristics of *Moringa oleifera* flower**

S. No.	Parameters	Flowers
1.	Colour	White and yellow
2.	Odour	Pleasant
3.	Taste	Pungent
4.	Height	1- 5 cm
5.	Shape	Pentamerous
6.	Touch	Smooth and splintery
7.	Season	Spring

**Table 2: Screening of phytochemicals in different extracts of *Moringa oleifera* flower**

Extracts	Sterol	Alkaloid	Tannin	Saponins	Phenol	Flavonoids	Terpenoid	Glycoside	Volatile Oil
Methanol	++	+++	+++	+	++	+++	++	++	+++
Ethanol	+	++	++	++	++	+++	++	++	+++
Aqueous	-	+	++	-	+	++	+	-	-
Hexane	++	+	+	+++	-	++	+	++	-

\*Data is a mean of three replications. \* '-' symbolizes absence of phytochemical; \* '+' symbolizes presence of phytochemical; \* '++' symbolizes moderate presence of phytochemical; \* '+++ symbolizes good presence of phytochemical

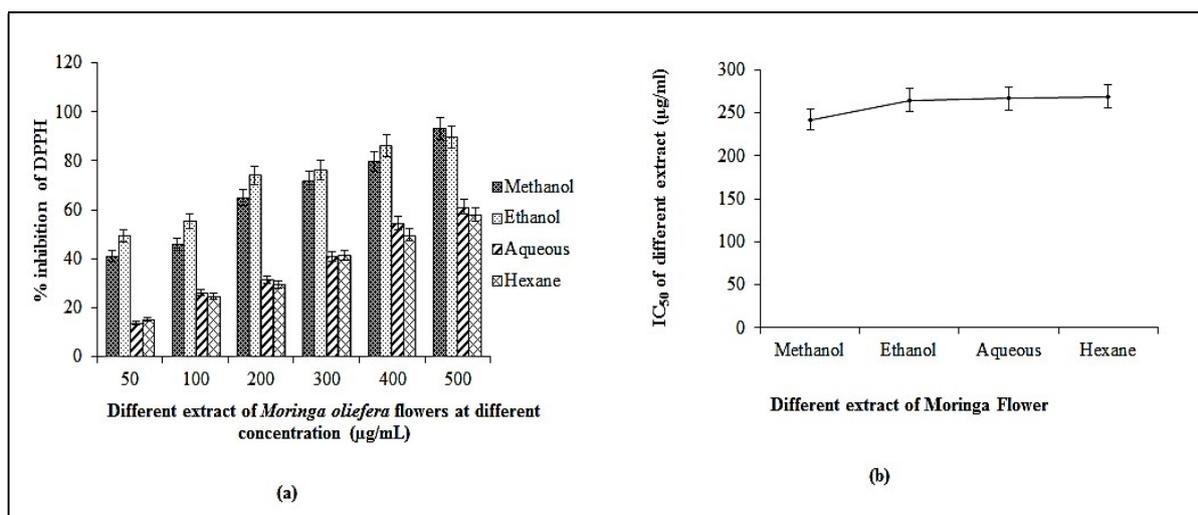


Figure 1: Percentage inhibition of *Moringa oleifera* flower (a) by DPPH assay and (b) determination of IC<sub>50</sub> value. Furthermore, FRAP assay also confirmed the antioxidant potency of *Moringa* flower

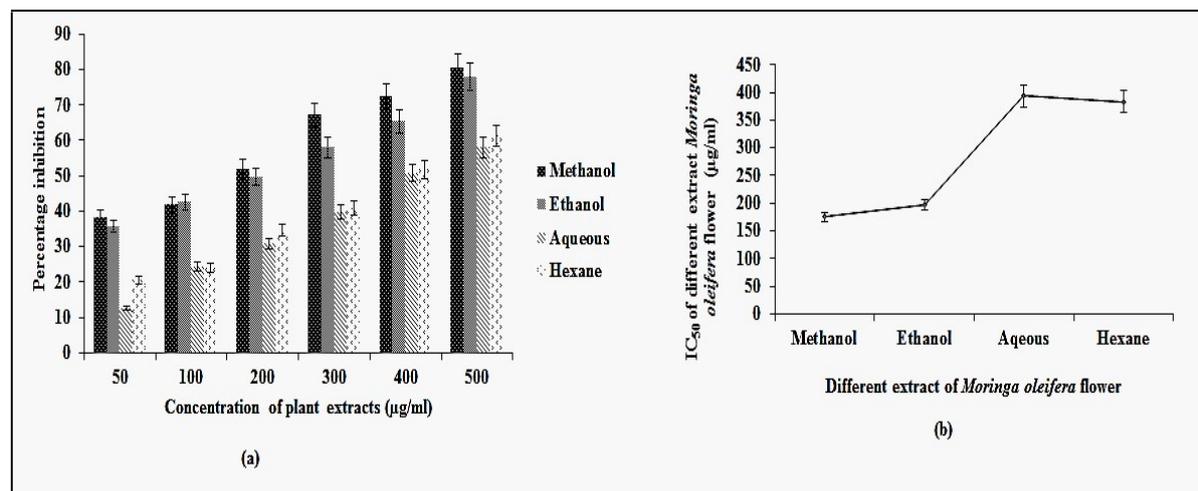


Figure 2: Reducing power assay of different extract of *Moringa oleifera* flower (a) by FRAP assay and (b) determination of IC<sub>50</sub> value

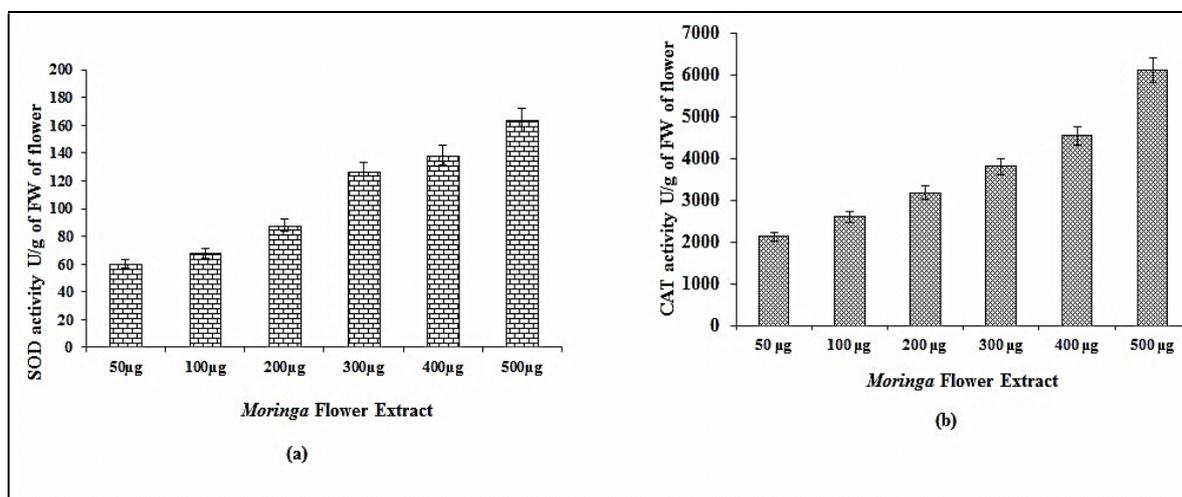


Figure 3: Enzymatic antioxidant assay of different extract of *Moringa oleifera* flower by (a) SOD and (b) CAT

## CONCLUSION

Conclusively, the study revealed that the methanolic extract of *Moringa* flowers possessed appreciable amount of the phytochemicals. Among, the phytochemicals phenol was observed in the maximum amount strongly supporting the antioxidative nature of the *Moringa oleifera* flower. Therefore, the present study proposed that the flower of *Moringa oleifera* could be used as potent source of natural antioxidant that may be advantageous in the prevention of various oxidative stresses induced diseases. However, more studies needed to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of flower extract should be investigated prior to clinical use.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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