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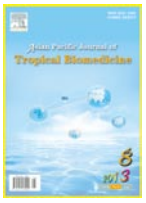

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Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*

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PEER REVIEW

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Comments

The result of this work is a valuable contribution in order to establish the folklore use of this herbal medicine in the treatment of various ailments.
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ABSTRACT

Objective: To evaluate and compare the antioxidant potential and anti-inflammatory activity of ethanolic extract of flowers of *Moringa oleifera* (*M. oleifera*) grown in Oman.
Methods: Flowers of *M. oleifera* were collected in the month of December 2012 and identified by a botanist. Alcoholic extract of the dry pulverized flowers of *M. oleifera* were obtained by cold maceration method. The ethanolic flower extract was subjected to preliminary phytochemical screening as the reported methods. Folin-Ciocalteu reagent was used to estimate total phenolic content. DPPH was used to determine *in-vitro* antioxidant activity and anti-inflammatory activity of flowers was investigated by protein denaturation method.
Results: Phytochemical analysis of extract showed presence of major classes of phytochemicals such as tannins, alkaloids, flavonoids, cardiac glycosides *etc*. *M. oleifera* flowers were found to contain 19.31 mg/g of gallic acid equivalent of total phenolics in dry extract but exhibited moderate antioxidant activity. The anti-inflammatory activity of plant extract was significant and comparable with the standard drug diclofenac sodium.
Conclusions: The results of our study suggest that flowers of *M. oleifera* possess potent anti-inflammatory activity and are also a good source of natural antioxidants. Further study is needed to identify the chemical compounds responsible for their anti-inflammatory activity.

KEYWORDS

Antioxidant, Anti-inflammatory activity, DPPH, Total phenolic content

1. Introduction

Medicinal plants have been known for millennia and are considered as a rich source of pharmaceutical agents for the prevention and treatment of diseases and ailments. According to WHO, more than 80% of the population within developing countries uses herbal and other traditional medicines to treat their common ailments[1]. Nature has bestowed Oman with an enormous wealth of medicinal plants which are widely used in traditional system of medicine[2].

Moringa oleifera Lam. (*M. oleifera*), commonly known as horse-radish or drumstick tree in English, belongs to family Moringaceae. It is a small sized tree, which is native to South Asia, Africa and Arabia and is used as traditional medicine in many tropical and subtropical countries[3]. It is a deciduous tree growing rapidly even in poor soils, well adapted to droughts and able to reach up to 15 m in height. It is one of the 14 species of genus *Moringa*, which is native to India, Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America[4]. The flowers and the fruits appear twice each year, and seeds or cuttings are used to propagate the tree. Almost all the parts of *M. oleifera* are used for various ailments in the indigenous medicine of South Asia, including the treatment of diabetes, hypertension, inflammation and infectious diseases[5]. Its leaves, pods and flowers are generally consumed for nourishment.

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The leaf extracts of *M. oleifera* have been reported to exhibit antioxidant activity both *in vitro* and *in vivo* due to abundant phenolic acids and flavonoids[6]. The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation[7]. Flowers of *M. oleifera* are rich in calcium, potassium and antioxidants (α and γ tocopherol), and are used in human diet, mainly in the Philippines[8].

Pharmacological studies have demonstrated that *M. oleifera* known to possess hypoglycemic, hypotensive, anti-microbial, hepatoprotective, immunomodulatory, antioxidant and antitumor activities[9-11]. These biological activities could be attributed to the presence of secondary plant metabolites present in *M. oleifera* such as carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics[12]. However, flowers of *M. oleifera* variety grown in Oman have never been screened for antioxidant or anti-inflammatory activity. Therefore, the present study was conducted to identify the major classes of phytochemicals present in the flowers of *M. oleifera* variety grown in Oman, to estimate total phenolic content and radical scavenging activity in *M. oleifera* flowers, and to evaluate anti-inflammatory activity of *M. oleifera* flowers against denaturation of protein in search of potent anti-inflammatory agent from natural source.

2. Materials and methods

2.1. Plant material

Flowers of *M. oleifera* were collected from Muscat, Oman in the month of November and December 2012. The plant material was identified and authenticated by a botanist of Department of Natural Science, Oman Medical College. A voucher specimen (PHAR-425-13) was deposited at the herbarium unit of the pharmacy department for future reference. The flowers were detached from the inflorescence rachis at the joint in the pedicel, and dried under shade. The dried samples were powdered and kept in air tight containers until use.

2.2. Drugs and chemicals

Diclofenac sodium was a kind gift from National Pharmaceutical Industries LLC, Muscat, Oman. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich USA. Folin- Ciocalteu reagent was obtained from Merck, Germany. All other chemicals used in the study were of analytical grade.

2.3. Extraction of the plant material

The dried powdered flowers (100 g) were extracted by maceration with 1 000 mL 70% ethanol for 3 d at room temperature with occasional shaking. The extract was filtered and the marc was re-extracted by the same process until plant materials were exhausted. The collected filtrates were pooled and evaporated to dryness under reduced pressure to yield the dry extracts (yield w/w: 8.69 %) and was stored at 4 °C until used.

2.4. Phytochemical screening of ethanolic extracts

The freshly prepared crude extract of *M. oleifera* flowers were subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as tannins, saponins, glycosides, flavonoids, alkaloids, terpenes and steroids *etc.* using standard procedures[13].

2.5. Determination of total phenolic content

The total phenolic content of the *M. oleifera* flower extract was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth[14]. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 μg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

2.6. Determination of antioxidant activity by DPPH-scavenging assay

The free radical scavenging activity of the flower extract of *M. oleifera* and of standard solution (ascorbic acid) were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method as reported in the literature[15]. The assay mixture contained 2 mL of 1.0 mmol/L DPPH radical solution prepared in methanol and 1 mL of standard or extract solution of different concentrations (10-500 μg/mL). The solution was rapidly mixed and incubated in dark at 37 °C for 20 min. The decrease in absorbance of each solution was measured at 517 nm using UV/Vis spectrophotomer. Ascorbic acid, a well known antioxidant was used as positive control while DPPH radical solution with 1 mL ethanol was taken as blank. The percentage of radical scavenging (%) was calculated by the following formula:

$$\% \text{ Free radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c=Absorbance of control at 517 nm; A_s= Absorbance of sample.

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

2.7. Evaluation of in- vitro anti-inflammatory activity

Anti-inflammatory activity of the *M. oleifera* flower extract was evaluated by protein denaturation method as described by Padmanabhan with slight modifications[16]. Diclofenac

sodium, a powerful non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of *M. oleifera* flower extract (100-500 μg/mL) or standard diclofenac sodium (100 and 200 μg/mL) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 2 mL of egg albumin (from fresh hen's egg) and incubated at (27±1) °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{A_t - A_c}{A_c} \times 100$$

Where, A_t=absorbance of test sample; A_b=absorbance of control.

2.8. Statistical analysis

The results are expressed as mean±SD. Student's *t*-test was used to analyze level of statistical significance between groups. $P<0.05$ was considered statistically significant.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical analysis of the extract showed the presence of major classes of phytochemicals such as tannins, alkaloids, flavonoids, cardiac glycosides *etc* (Table 1). Saponins, protein and amino acids were not detected in the extract.

Table 1
Preliminary phytochemical analysis of *M. oleifera* flower extract.

Phytochemical test	Name of the test	Flower extract
Tannins	$FeCl_3$ test, Lead acetate test	+
Steroids	Salkowski test	+
Flavonoids	Shinoda test	+
Saponins	Frothing test	−
Proteins and amino acids	Ninhydrin test	−
Alkaloids	Hager's, Meyer's & Wagner's test	+
Carbohydrates	Molisch's test	+
Glycosides	Nitroprusside test	+
Cardiac glycosides	Keller Killiani test	+
Terpenoids	Salkowski test (modified)	+

+: present, −: absent

3.2. Total phenolic content

The total phenolic content expressed in terms of GAE and yield (%) of flower extract was found to be (19.31±1.79) mg of GA/g and 8.69% (w/w) respectively. The total phenolic contents were calculated using the following linear equation based on the calibration curve of gallic acid;
$A=0.008X+0.0727$, $R^2=0.9967$

Where A is absorbance and *X* is amount of gallic acid in μg.

3.3. DPPH free radical scavenging activity

The scavenging effect of different concentration of *M. oleifera* flower extract on the DPPH free radical was compared with standard anti-oxdiant, ascorbic acid. The results were expressed as inhibition (%) shown in Table 2. Flower extract showed a dose dependent scavenging activity. However, their scavenging ability was found to be non significant ($P>0.05$) in comparison to ascorbic acid.

Table 2
Percentage inhibition of DPPH free radical of *M. oleifera* flower extract/ ascorbic acid at 517nm.

Concentration (μg/mL)	Inhibition of DPPH (%)	
	Flower extract	Ascorbic acid
10	2.84±0.81	21.58±0.12
20	5.79±1.20	89.68±0.35
50	8.42±1.71	90.71±1.23
100	16.95±.0.95	92.32±2.05
200	33.89±.1.36	94.21±1.01
IC_{50} value	−	14.57

Values are mean±SD, $n=3$.

3.4. In-vitro anti-inflammatory activity

The inhibitory effect of different concentrations of *M. oleifera* flower extract on protein denaturation are shown in Table 3. *M. oleifera* flower extract (100-500 μg/mL) showed significant inhibition of denaturation of egg albumin in a dose dependent manner. The *in-vitro* anti-inflammatory activity of the extract was comparable to the diclofenac sodium, a reference drug (100 and 200 μg/mL). A significant difference in the inhibition of thermally induced protein denaturation was observed in case of extract when compared with standard drug at concentration of 100 μg/mL. Though at concentration of 200 μg/mL, inhibition activity of extract and diclofenac sodium were comparable.

Table 3
In-vitro anti-inflammatory effect of *M. oleifera* flower extract.

Treatment	Concentration (μg/mL)	Inhibition of protein denaturation (%)
Flower extract	100	58.16±2.32
	200	88.10±1.80
	500	101.50±2.60
Diclofenac sodium	100	84.95±1.46
	200	120.12±2.76

Values are mean±SD, $n=3$.

4. Discussion

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavonoids, glycosides, tannins, steroids *etc*. Some of these plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart

diseases and stroke by scavenging free radicals which are implicated in the pathogenesis of many diseases[17,18].

The results of preliminary phytochemical screening confirmed the presence of various classes of secondary metabolites in the *M. oleifera* flower extract including poly phenols (tannins and flavonoids). Plant polyphenols, produced either from phenylalanine or from its precursor shikmic acid, are important dietary antioxidants because they possess an ideal structural chemistry for free radical scavenging activity. Numerous *in-vitro* studies have conclusively shown their antioxidant potential in protecting against many diseases[19]. The present study indicated that flowers of *M. oleifera* are rich in polyphenols (19.31 mg/g of GAE of dry extract), but their total phenolic content is found to be lesser than the previously reported result in leaves[5].

DPPH free radical scavenging activity is an easy and widely used method for testing *in-vitro* antioxidant activity of natural compounds or plant extracts[20]. DPPH is a stable free radical at room temperature, purple in color. Its reduction capability to accept an electron or a hydrogen radical from antioxidants is determined by measuring decrease in its absorbance values at 517 nm.

DPPH radical scavenging activity of *M. oleifera* flower extract was compared with standard ascorbic acid in this study. Although standard antioxidant had higher scavenging activity at all tested concentrations than the extract, the extract still showed good free radical scavenging activity. The free radical scavenging property of *M. oleifera* may be one of the mechanisms by which this plant is effective as a traditional medicine. The consumption of the *M. oleifera* flowers can be beneficial in preventing oxidative stress related degenerative diseases.

Inflammation is a very common symptom of many chronic diseases. It is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. Inflammation is a protective attempt by the body to remove injurious stimuli as well as initiate the healing process for the tissue[21]. Non steroidal anti-inflammatory drugs are commonly used for the management of inflammatory conditions, but these are associated with many unwanted side effects such as gastric irritation, ulcer *etc*[22]. Medicinal plants used in traditional medicine to treat anti-inflammatory conditions seem a viable and logical alternative in search of safe and effective anti-inflammatory agents. *M. oleifera* is commonly used traditional medicine in South Asian countries to treat inflammatory conditions; hence, a simple and viable protein denaturation bioassay method was selected to evaluate its potential as anti-inflammatory drug. It is a well known fact that denaturation of tissue proteins lead to inflammatory and arthritic diseases[23]. Natural products that can prevent protein denaturation therefore, would be worthwhile for development of anti-inflammatory drug therapy.

M. oleifera flower extract and reference drug diclofenac sodium exhibited dose dependent percentage inhibition of heat induced protein denaturation in fresh egg albumin. Percent inhibition of protein denaturation with respect to control is a measure of protein stabilization[24]. Although *M. oleifera* flower extract showed a moderate free radical scavenging activity, its effect on inhibition of protein denaturation was found to be comparable with the standard drug diclofenac sodium. Thus it can be concluded that anti-inflammatory activity of *M. oleifera* flowers could be due to their high phenolic content.

The results of our study suggest that *M. oleifera* flowers are rich in phenolic compounds and have a good antioxidant activity. It can be used as a natural source of antioxidants to prevent the progression of many diseases. *M. oleifera* flower extract also produced marked *in-vitro* anti-inflammatory activity that justifies its use in traditional system of medicine in Oman and other Asian countries. However, further detailed investigations are needed to ascertain the mechanisms and constituents behind its anti-inflammatory actions.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

M. oleifera is commonly known as drumstick tree or horse-radish in English which grows and cultivates in different parts of the world such as Malaysia, Africa, Pakistan and Oman. Various parts of *M. oleifera* such as leaves, bark, flowers, fruit, seeds, and root are employed in traditional system of medicine in the treatment of various ailments like hypertension, diabetes, inflammation, hepatic disorders and infectious diseases *etc*. Many research studies have been carried out to validate its traditional uses. Current article aimed to evaluate and compare the antioxidant potential and anti-inflammatory activity of ethanolic extract of *M. oleifera* flowers grown in Oman.

Research frontiers

The present research work described the estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of *M. oleifera* flowers. The study was well performed by the authors and explanation of the medicinal uses of *M. oleifera* is based on the phytochemicals, especially the phenolic compounds.

Related reports

Many researches on *Moringa* revealed the potential properties of its nutrients and phytochemicals. Researchers

found that *Moringa* is a good source for anti-bacterial, beneficial in diabetes, hypertension, and liver ailments.

Innovations and breakthroughs

M. oleifera has been used as folk medicine in different traditional systems of medicines like Siddha medicine and Ayurvedic medicines and in Ayurvedic traditional medicine. The leaves and flowers are reported to affect blood pressure and level of glucose and also used to increase lactation in nursing mothers. In the present study, authors have evaluated and compared the antioxidant potential and anti-inflammatory activity of ethanolic extract of *M. oleifera* flowers grown in Oman in a scientific manner.

Applications

Phenolic compounds are most widely occurring groups of phytochemicals and derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These compounds are secondary metabolites which have vital role in reproduction and growth, giving protection against harmful predators and pathogens. The phenolic compounds act as antioxidants . These compounds are also reported to have anti-cancer, anti-microbial, anti-inflammatory and anti-allergic activities *etc*. *M. oleifera* can be used in the treatment of anemia, arthritis, asthma, cancer, constipation, diabetes, epilepsy, hypertension, kidney stones, thyroid disorders, and infections *etc*.

Peer review

The result of this work is a valuable contribution in order to establish the folklore use of this herbal medicine in the treatment of various ailments. The authors have demonstrated the antioxidant potential and anti-inflammatory activity of ethanolic extract of flowers of *M. oleifera* in Oman. The result of this research revealed that this drug can be used as a natural source of antioxidants to prevent the progression of many diseases.

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