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Phytochemical constituents of *Moringa peregrina* seeds

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ABSTRACT

Moringa peregrina is one of the species of the genus Moringa and is the only genus in Moringaceae family with small dark leaves of flowering plant. It is used in treatment of infectious diseases, inflammation, gastrointestinal, hematological, cardiovascular and hepatorenal disorders. In recent studies anti-fat-like effect of this plant has also been focused by researchers. The aim of this article is to investigate phytochemical properties of Moringa peregrina. So, the plant was prepared by the specified code from Isfahan Center for Research of Agricultural Science and Natural Resources. After getting the required approvals, anthraquinone, saponins, tannins, phenolic groups, flavonols, flavonoids, alkaloids, and cardiac glycosides were tested. Experiments showed that the plant contained alkaloid, tannin, glycoside, flavonoid, flavonol, and phenolic compounds but no antraquinone or saponnin. Based on the results of this research, it is obvious that ethanolic extract of M. peregrina has noticeable antioxidant ability against various oxidative systems. Furthermore, this extract can be used as an accessible source of natural antioxidants in food supplements or in pharmaceutical industries.

Key word: Phytochemical properties, *Moringa peregrina,* Secondary metabolites.

INTRODUCTION

Medicinal plants have been frequently used in traditional and complementary alternative medicine [1-10]. Also the positive effects of more than 1000 medicinal plants in treatment of diseases have been investigated [11-16]. They are continually considered as a source of medicine [17-22]. *Moringa peregrina* features prominently in the indigenous systems of medicine in South Asian countries. These cultures use the flowers, seeds, fruits, and oil from the seeds for treating conditions such as infectious diseases, inflammation, and gastrointestinal, hematological, cardiovascular, and hepatorenal disorders. The isothiocyanate and thiocarbamate components in Moringa confirm antihypertensive properties. The presence of vitamin C and carotenoids also makes *M. peregrina* a valuable source of antioxidants. This plant has been mentioned in traditional medicine as an antimicrobial, lowering blood sugar, lowering blood pressure, anti-parasitic, and anti-tumor resource, protecting liver cells and treating digestive disorders and bowel spasm [23-28]. Previous studies on this plant's effect on hyperlipidemia in rats have been reviewed and approved. The aim of this study is to investigate the phytochemical properties of this plant.

MATERIALS AND METHODS

Preparation and authentication of the plant material:
The leaves of the herb were prepared by means of the specified code in Isfahan Center for Research of Agricultural Science and Natural Resources and physicochemical properties of the plant in terms of both macro- and microscopic study was then confirmed by Professor Mahmoud Rafieian, Ph.D., Head of Medical Plants Research Center of Shahrekord University of Medical Sciences on 10/11/2014.

Determining of total phenolic compounds:
The amount of phenolic compounds was measured based on folin-ciocalteu colorimetric method and in terms of gallic acid [29-31]. Standard solutions consisting of 12.5, 25, 50, 62.5, 100, and 125 PPM concentrations were produced from gallic acid in a 60%-methanol solution. Then, 0.1 ml of each one was transferred to laboratory tubes and 0.5 ml of 10% folin-ciocalteu reactor solution was added to the tubes and after 3-8 minutes, 4 ml of 7.5% carbonate sodium solution was introduced. The tubes were kept at the laboratory temperature for 30 minutes and the amount of optical absorption at a 756-nm wavelength was measured by a spectrophotometer and the standard diagram was drawn accordingly. Then, 0.01 to 0.02 gram of the dried extract was dissolved in 60% methanol and reached a volume of 10 ml and the total phenol was determined based on folin-ciocalteu method. Only instead of a 0.1-ml standard, solution extract solution was added. The read absorption was plotted on the standard graph. In this way, the amount of the extract's total phenol was measured in mg/g gallic acid [32].

Determining of the amount of total flavonoids and flavonols:
The amount of flavonoids and flavonols was measured using aluminum chloride colorimetric method and in terms of rutin standard. At first 25, 50, 100, 250 and 500 PPM solutions were produced from rutin in 60% methanol solution and 1 ml of this solution was transferred to the test tube. Then, 1 ml of 2% aluminum chloride solution was added to it and 6 ml of 5% acetate potassium solution was introduced. The amount of optical absorbance was read at 415-nm wavelength for flavonoid standard after 45 minutes and at 440-nm wavelength for flavonol standard after 2.5 hours and the diagram was drawn based on the standard graph. Then, 0.01 to 0.02 of the dried extract samples were dissolved in 60% methanol, reaching to 10-ml volume and the amounts of flavonoids and flavonols of the total extract were determined based on chloride aluminum colorimetric method in terms of mg/g [33]. All experiments were done three times and the average values obtained from 3 times were calculated using the mean and standard deviation [34].

Extracting and identifying alkaloids by using chemical method:
1. **Producing solution sample**:
Half a gram of tested herbal powder or condensed remnant of 20 ml of the extract or tincture was taken and 1 ml of 2 normal hydrochloric acid and 9 ml of distilled water were heated on Water Bath for 2 to 5 minutes. After cooling, it was strained for the next experiments [35].

2. Preliminary experiment:
Two or three drops of the prepared solution sample were added on one part of the two plates. Iodine reagent and Maier's reagent were added by two drops on the other part of the glass and mixed together. Producing turbidity or sedimentation indicated the presence of alkaloids. The results of creating precipitate by these two reagents are as follows:

Iodine reagent + alkaloids: dark brown precipitate
Maier's reagent + alkaloids: yellowish white precipitate
Otherwise, the tested solution lacks such compounds or its value cannot be measured by the primarily experiment (Datura leaves were used as control) [36].

Extraction and identification of alkaloids by using chemical methods:
1. Separating tropane alkaloid and preparing solution sample:
One gram of herbal powder or 10 ml of condensed tincture was mixed well with 10 ml of 0.1 normal sulfuric acid and stirred for 5 minutes. Then it was filtered and 1 ml of concentrated ammonia and 5 ml of water were added to the produced solution. The product was moved to a decanter and settled well by chloroform twice, each time by 10 ml. It should be noted that this procedure should be done slowly to avoid causing any emulsion. Then, chloroformed phase was filtered by sodium sulfide and divided into two parts for the next experiments [37].

2. Vitali-reaction:
The produced solution was poured into a crucible and concentrated to dry up (on a Bon Mary). After the dried solution cooled, 10 drops of nitric acid was added to it and it was concentrated for the second time. 10 ml of acetone was added to crucible after getting cold. A few drops of 3% Potash in ethanol proved the presence of tropane alkaloids after a bluish-purple color was produced (Datura leaves were used as control) [26].

Identification of purine alkaloids:
10 mg of caffeine was mixed with 2-3 drops of hydrochloric acid (6 normal) and then dried up on a steam bath to produce a reddish-yellow precipitate. 0.5 ml of 6 normal ammonia was also added. The reddish-purple color of the product proved the presence of purine alkaloids.

Identification of cardiac glycosides:
1. Extracting cardiac glycosides:
One gram of the plant powder was mixed with 10 ml of a solution containing ethanol and water (3:7). Then it was refluxed for 5 minutes. After cooling, it was strained and 30 ml of water and 15 ml of 0.15 lead acetate solution were added and mixed well (in the case of hydraulic solutions, this action continued by adding water and lead acetate solution). The product was kept in laboratory temperature for 5 minutes and filtered. The strained product was transferred to decanter and settled three times with 10 ml of a solution containing chloroform and isopropanol (2:3). The organic phases were mixed together and drained with anhydrous sodium sulfate and divided into three parts for the next experiments (Digitalis leaf was used as control) [25].

2. Identification of cardiac glycosides belonging to the category of cardenolids by chemical method: Baljet reaction:
The first part of the produced solution was concentrated and the rest was dissolved in 3 ml of methanol. 3 ml of Baljet reagent was added to it and the obtained solution was mixed well and the product was stirred. For a closer observation of the experiment, a sample containing 3 ml of methanol and 3 ml of Baljet reagent was prepared to be compared.

Due to the presence of cardiac glycosides belonging to the category of cardioids, the color of solution turned into orange that was stable for several hours.

3. Producing Baljet reagent:
9.5 ml of 0.1% Acetic acid and 0.5 ml of 0.10% NAOH solutions were mixed together and then it was used. This reagent should be always fresh.

4. Kedde – reaction:
The second part of the prepared solution was completely condensed and then 2 ml of solution 2% of 3-5di nitro benzoic acid was added to methanol and the mixture was stirred well. Then 2 ml of 1 normal Potash solution was added to the solution. Due to the presence of cardiac glycosides belonging to the category of cardenolids the purple color was produced that was not stable and disappeared quickly.

Identification of saponins:
1. Producing foam experiment:
0.5 gram of herbal powder was poured into a test tube and 10 ml of hot water was added to it. After cooling, the mixture was stirred well for 10 seconds (in the case of the extracts and herbal tincture, 1 ml of them were diluted and stirred). If being present, saponins produced foam from 1-10 cm in height and were stable at least for 10 minutes. Its stability should be kept by adding several drops of 2 normal hydrochloric acid (suds is not stable in this case). For being certain about the experiment, we should do hemolysis experiment too (The stem of liquorice was used as control) [38].

2. Hemolysis experiment:
0.1 gram of herbal powder was mixed with 0.10 ml of isotonic phosphate buffer solution of blood with a pH of 7.4. The mixture was boiled for a short time and filtered. Then, 1 ml of the mixture was mixed with 1 ml of 0.2% blood. It is necessary for samples containing tannin (like quillaja ticture) to have a more diluted solution. For doing this, 0.2 ml of the filtered product was diluted by 0.8 ml of the above buffer and 1 ml of 0.2 blood solution was added to it. The hemolytic action for samples containing saponin compounds was done at least within 30 minutes and proved

their presence. This experiment could be done by a blood agar plate. Three equal holes were made in a plate by a small test tube. Then the ends of the holes were welded to prevent the tested solution from penetrating into the plate's surface. The tested solutions, including water, control, and sample solutions, were poured into the holes by a dropper and left alone for half an hour and then the hemolytic action was observed around the hole and the calculation was done [39].

Identification of anthraquinones:
0.2 gram of the herbal powder was added to 5 ml 2normal sulfuric acid and the solution was boiled for a short time (for sugar hydrolysis). After cooling by 10 ml of toluene in a decanter resulting in dead decant, it was separated from toluene phase and filtered. It must have turned into yellow due to the presence of anthraquinone compounds. Then this phase was settled by 2 ml of 2 normal sud. After 2 phases were separated, a completely red phase was seen while the other was colorless.

This process/reaction could be done easily in the case of rhubarb and the skin of black dogwood, frangula, In the case of *Aloe vera*, the color is lighter. In the case of the leaf and fruit of *Cassia angustifolia*, the color is orange-red, and hence another experiment was done to have a better result and also some changes were done in Borntrager's method (rhubarb's stem was used as control) [40].

Identification of tannins:
0.2 gram of herbal powder was mixed with 10 ml of ethanol and then filtered. The solution produced a dark color. Then, a piece of filtered product was taken and diluted by ethanol to make it bright brownish-yellow [41].

For extraction by hexane, chloroform, ethyl acetate, aqueous and hydroalcoholic solutions, firstly hydroalcolic extraction was condensed. 15 ml of water was added to the extract. Then, 15 mL of hexane was added three times, Dkanth for 3 min and hexane phase was separated and kept in a another container. The remaining 3 times and each time 15 ml of chloroform was added and 3 min Dkanth. The chloroform phase was separated. The remaining was added 15 ml of ethyl acetate three times, and each time was Dkanth additional 2-3 minutes and the phase was separated. Finally, the aqueous phase remained in container [42].

RESULTS AND DISCUSSION

The present study on the plant samples revealed the presence of medicinally active constituents. The phytochemical characteristics of *M. peregrina* seeds are summarized in Tables 1.

Table 1: The results of phytochemical tests of *M. peregrina* seeds based on the reagents

Effective compounds	Control	Positive observations	Method or the agent used	Result
Alkaloid	*Datura stramonium.* L	turbidity and sedimentation turbidity and sedimentation	Wagner's test Mayer's test	+ +
Anthraquinone	*Aloe vera*	Red color	*Bontrager's tes*	-
Tannins	*Punica granatum*	Yellowish white precipitate clear, blue or green	lead acetate test chloroferriciTest	+ +
Saponins	*Glycyrrhiza glabra* L	hemolytic action: sustainable flooring	foam test (hemolysis experiment)	- -
Flavonoid	*Berberis vulgaris*	greenish yellow fluorescence	Wilson Tabuk test	+
Glycoside	*Digitalis purpurea*	orange color	Baljet-reaction	+

Also, some of secondary metabolites based on non-polar to polar solvents were qualitatively evaluated and the results are shown in Table 2.

Table 2. Qualitative phytochemical analysis of extract of *Moringa peregrina* seeds based on the selected solvents

Solvents used for extraction	Alkaloid	Flavonoid	Glycoside	Saponin	Tannin	Terpenoids	Volatile oil
Water	-	+	+	-	+	+	-
Ethanol	-	+	+	-	+	+	-
Ethyl acetate	-	-	-	-	+	-	-
Hexan	+	-	-	-	+	-	-
Chloroform	+	-	-	-	+	-	-

Appearance of the extracts based on solvents (hexane, chloroform, ethyl acetate, ethanol and water) of Moringa seeds were examined and are listed in Table 3

Table3: Qualitative and quantitative evaluation of the extracts

Type of extract	The value obtained mg/100g	Color	Extracts appeared
Total extract	12000	Yellow	Oily
Hexane	4650	Yellow	Oily
Chloroform	423	Yellow	Oily
Ethyl acetate	164	Pale yellow	Oily
Watery extract	6810	Pale yellow	Orange-yellow color

Percentage of some secondary methabolites were examined and are shown in Table 4.

Table 4: Percentage of crude alkaloids, phenols, tannins, flavonoids and saponins in the plant

Alkaloids (%)	Flavonoid (%)	Phenols (%)	Saponin (%)	Tannins (%)
0.31	0.71	0.87	0.00	7.90

Amounts of phenolic, flavonoid and flavonol compouds and antioxidant capacity of *M.pergrina* were identified and are shown in Table 5.

Table 5: Antioxidant capacity and Flavonoid, Flavonol and phenolic compounds in *M. peregrina* seeds

DPPH scavenging activity (µg/ml)	Flavonoid (mg/g)	Flavonol (mg/g)	phenolic compounds(mg/g)
105.34	226.3	168.0	465.8

The presence of alkaloids, tannins, glycosides and flavonoids in this plant indicates its importance for medical purposes. Alkaloids could explain anti-inflammatory, antibacterial, antineoplastic, and palliative effects of this plant [43]. Phenolic compounds such as flavonoids are among the most important secondary compounds which have antioxidant activity via protective mechanisms against oxidative stress [44]. Furthermore, due to active hydroxyl groups in these compounds, they are more effective in deactivating free radicals and there were considerable amounts of these substances in the seeds of this plant. Moreover, these compounds play an important role in optimal human nutrition [45]. Glycosidic compounds also indicate favorable cardiovascular effects of this plant [46]. Tannins such as the above-mentioned antioxidant compounds have also had an important role in cancer prevention [47]. The synergistic effects of the flavonoids and other phenolic compounds of the plant could be effective on reducing cancer incidence. Moringa species oil and olive oil have been found to be similar in therapeutic effects [48]. According to Alkhatani and colleagues studies on Olifera species and determination of the antioxidant capacity of mentioned species and determination of flavonols and flavonoids and phenolic compounds in peregrina species, it seems *M. peregrina* has higher antioxidant cutie, compared to *Moringa oliefera* [24].

CONCLUSION

Based on the results of this research, it is obvious that ethanolic extract of has noticeable antioxidant ability against various oxidative systems in vitro. Furthermore, this extract can be used as an accessible source of natural antioxidants in possible food supplements or in pharmaceutical industries.

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