ORIGINAL ARTICLE

Preliminary phytochemical investigation and thin layer chromatography profiling of sequential extracts of *Moringa oleifera* pods

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Context: *Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. **Aim:** The present study, primarily aims to carry out a preliminary phytochemical screening so as to detect the major class of compounds present in *M. oleifera* and to perform thin layer chromatography (TLC) profiling of all sequential extracts. **Materials and Methods:** Phytochemical analysis was performed by various qualitative methods and TLC profiling was carried out using various solvent system of varying polarity. **Results and Conclusions:** Qualitative phytochemical analysis reflects the presence of phenolics, triterpenoids, cardiac glycosides, steroid, alkaloids and saponin in the plant extract. TLC profiling of the *M. oleifera* pods was carried out using sequential extracts of petroleum ether, benzene, petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water respectively. The results obtained in the present investigation indicated *M. oleifera* pods as a rich source of natural antioxidants.

Key words: *Moringa oleifera*, phytochemical screening, retention factor, thin layer chromatography profiling

INTRODUCTION

Nature is the best source of traditional medicinal agents for thousands of years and some modern drugs.[1] Medicinal plants have been subjected to detailed study since ancient time and practically no much difference is seen in value of medicinal herbs or plants and method of treatment used in ayurvedic, the allopathic and the homeopathic literature.[2] Among myriad of natural plants, *Moringa oleifera* Lam (Syn. *Moringa pterygosperma* Gaertn) belongs to an onogeneric family of shrubs and tree, Moringaceae and is considered to have its origin in Agra and Oudh, in the northwest region of India, south of the Himalayan Mountains. Moringa is an important tropical crop that is used as human food, medicine and in oil production.[3] Different parts of this plant are being employed for the treatment of various ailments in the indigenous system of medicine.[4-6] It possesses antitumor, antipyretic, analgesic[7] antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, renal,[5,8] and hepatoprotective activities.[6,9] An examination of the phytochemicals of Moringa species affords the opportunity to examine a range of fairly unique compounds. In particular, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates.[10,11]

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Phytochemicals are in the strictest sense of the word, chemicals produced by plants. The plants generally contain 10 phytoconstituents namely anthraglycosides, arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes and valepotriates. These phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents in plants would help in determining various biological activities of plants.[12] Although, much has been learned about the biological aspects of *M. oleifera* additional knowledge remains to be secured. Therefore, in recent years; considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption. Thus, the aims of present study are to investigate the phytochemical profile and thin layer chromatography (TLC) studies of pods of *M. oleifera* so as to know various components present in it and hence to assess the medicinal potential of the plant and justify its folklore use.

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MATERIALS AND METHODS

Chemicals

All chemicals used in the study were of analytical reagent grade and of highest quality available and were purchased from reliable firms and institutes.

Procurement of Experimental Plant

The experimental plant *M. oleifera* was collected from Krishi Vigyan Kendra, Banasthali University, Banasthali, India, in the month of October 2009. The plant material was taxonomically identified by Botanist of Krishi Vigyan Kendra, Banasthali, Tonk district.

Sequential Extraction of *M. oleifera* Pods

The dried *M. oleifera* pods were powdered with the help of mixer grinder. The powdered pods were then extracted with soxhlet apparatus using sequential solvents that were pet ether, benzene, chloroform, ethyl acetate and ethanol for 16 h. Aqueous extract was also obtained by soaking sequential plant part powder in double distilled water for 2-3 days and then filtered with cheese cloth. The extracts were than concentrated on a rotary evaporator below 50°C and were stored in airtight containers at 4°C temperature for further studies.

Preliminary Phytochemical Screening of Successive Extracts of *M. oleifera* Pods

Qualitative phytochemical analysis of *M. oleifera* pods was carried out using standard procedures to identify the constituents as described by Harborne,[13] Trease and Evans[14] and Sofowara.[15]

Test for alkaloids (Mayer's test)

To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicated positive test.[16]

Test for saponins (Froath forming test)

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A thick (2 cm) layer of foam indicated the presence of saponins.[17]

Test for phytosterols (Libermann-Buchard's test)

The extract was mixed with 2 ml of acetic anhydride. To this 1 or 2 drop of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of colour change showed the presence of phytosterols.

Test for phenolic compounds (Ferric chloride test)

The extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.[18]

Test for tannins

About 0.5 mg of dried powdered samples was boiled in 20 ml of water in test tubes then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black coloration.

Test for flavonoids

To 5 ml of dilute ammonia solution a portion of extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow colour indicates the presence of flavonoids.

Test for terpenoids (Salkowski test)

Extract (5 ml) was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown colouration of the interface showed the presence of terpenoids.

Test for phlobatannins

Formation of red precipitate when aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid indicated the presence of phlobatannins.

Test for ascorbic acid

Dilute 2% w/v solution of sample and 2 ml of water was added. Sodium bicarbonate and ferrous sulphate were added and shake well. Appearance of deep violet colour, which disappears on adding 5 ml dilute sulphuric acid, indicated presence of ascorbic acid.

Test for steroids

To 2 ml filtered extract 2 ml acetic anhydride and 1 ml concentrated sulphuric acid was added. Green colour indicated the presence of steroids.

Test for cardiac glycoside

To 2 ml filtered extract 1 ml of glacial acetic acid, 2 ml ferric chloride and 2 ml of conc. sulphuric acid was added. Brown colour indicated the presence of glycosides.

Chromatographic Purification

TLC was carried out to isolate the principle components that were present in most effective extracts of plant. TLC studies were carried out for different extracts on Silica gel (G) 60 F. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

Solvent phase

The different solvent systems used were: Chloroform: Methanol: H_2O (7:3:1), Chloroform: Methanol: H_2O (5:4:2) Butanol: H_2O (1:1), Chloroform: Glacial acetic acid: Methanol: H_2O (5:4:1:2), Chloroform: Glacial acetic acid: Methanol: H_2O (4:5:1:2), Chloroform: Ethanol (1:1)

Method

The above prepared plant extracts were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under ultra violet light UV at both 254 nm and 366 nm. They were later sprayed with different spraying reagents and some were placed in hot air oven for 1 min for the development of color in separated bands. The movement of the analyte was expressed by its retention factor (R_f). Values were calculated for different sample.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

Analyte detection

After drying the plates, they were exposed to Iodine vapours by placing in a chamber that was saturated with iodine vapours and also exposed to different spraying reagents. All plates were visualized directly after drying and with the help of UV at 254 nm and 366 nm in UV TLC viewer. The R_f value of the different spots that were observed was calculated.

RESULTS

Yield of Extracts from Sequential Extraction of *M. oleifera* Pods

The yield of sequential extracts (g) is shown in Table 1. The amount of the pet ether extract obtained from the extraction was 7 g (7% w/w yield), benzene extract was 13.5 g (13.5% w/w yield), chloroform extract 10 g (10% w/w yield), ethyl acetate extract 3.5 g (3.5% w/w yield), ethanol extract 22 g (22% w/w yield) and aqueous extract was 14 g (14% w/w yield).

Phytochemical Screening of Sequential Extracts of *M. oleifera* Pods

Phytochemical screening of the sequential extract of *M. oleifera* pods revealed the presence of various bioactive components of which phenolics, saponins, steroids, alkaloids, flavonols, proanthocyanidins, terpenoids, tannin, and cardiac glycosides are the most prominent components and the result of phytochemical test is presented in Table 2. Among these phytochemical tests, benzene extract was found to contain maximum saponin content along with plant phenolics such as alkaloids, saponins and tannins, which act as primary antioxidants or free radical scavengers show their presence in the extract of *M. oleifera*. All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

Chromatographic Purification: TLC

TLC of all sequential extracts of *M. oleifera* pods obtained by sequential extraction methods was carried out to confirm its nature by analysing TLC chromatograms and to isolate active ingredients from the extracts [Table 3].

Table 1: Yield of extracts from sequential extraction of *Moringa oleifera* pods (100 g)

Name of extracts	Yield (g)	Yield (w/w) (%)
Pet ether	7	7
Benzene	13.5	13.50
Chloroform	10	10
Ethyl acetate	3.5	3.50
Ethanol	22	22
Aqueous	14	14

Table 2: Qualitative phytochemical screening of sequential extracts of *Moringa oleifera* pods

Name of tests	Name of extracts					
	PE	Ben	Chloroform	EA	Et	Aq
Alkaloids	+++	+++	+	++	+	+
Saponins	++	+++	++	–	+	–
Phytosterols	++	+++	++	+	+	–
Tannins	–	+++	–	++	+	++
Phenolics	–	+++	+	+	–	+
Flavanoids	–	–	–	–	+	+
Terpenoids	+++	++	+	+	+	–
Phlobatannins	–	–	–	–	+	++
Cardiac glycosides	+++	+++	+	+	++	+
Polyphenol	+	+++	++	+	+++	++

– – Absent; + – Weak; ++ – Moderate; +++ – Strong; PE – Pet ether; Ben – Benzene; EA – Ethyl acetate; Et – Ethanol; Aq – Aqueous

TLC of pet ether extract of *M. oleifera* pods revealed the presence of 4 compounds having R_f values of 0.39, 0.47, 0.87 and 0.90 respectively when a solvent phase of Chloroform: Methanol: H_2O (7:3:1) was used. In another solvent phase i.e., butanol saturated with water (1:1), two spots were obtained having R_f of 0.90 and 0.94 respectively.

TLC of benzene extract of *M. oleifera* pods revealed the presence of 8 compounds having R_f values of 0.30, 0.47, 0.62, 0.75 0.87, 0.90, 0.95 and 0.98 respectively when a solvent phase of Chloroform: Methanol: H_2O (7:3:1) was used. Compounds having R_f of 0.90 and 0.87 were most prominent and clear spots (green spots).

TLC of chloroform extract of *M. oleifera* pods revealed the presence of 2 compounds having R_f values of 0.39, 0.47 and 0.90 respectively when a solvent phase of Chloroform: Methanol: H_2O (7:3:1) was used. In another solvent phase i.e., Chloroform: Glacial acetic acid: Methanol: H_2O (5:4:1:2) and Chloroform: Ethanol (1:2), 5 and 4 spots were obtained respectively, but the spots were not as prominent and clear as in solvent phase of Chloroform: Methanol: H_2O (7:3:1).

TLC of ethyl acetate extract of *M. oleifera* pods revealed the presence of 5 compounds having R_f values of 0.47, 0.75,

Table 3: R_f values of sequential extracts of *Moringa oleifera* pods

Seq. extracts	Solvent phase	Solvent run (cm)	Peaks obtained (cm)	R_f values	Colors of peaks
Pet ether	Chloroform: Methanol: H_2O (7:3:1)	5.7	5.0	0.87	Yellow
			5.2	0.90	Brown
			2.7	0.47	Brown
			2.2	0.39	Brown
	Butanol: H_2O (1:1)	5.2	4.7	0.90	Yellow
			4.9	0.94	Brown
Benzene	Chloroform: Methanol: H_2O (7:3:1)	6.3	1.9	0.30	Yellow
			2.9	0.47	Yellow
			3.9	0.62	Brown
			4.7	0.75	Brown
			5.5	0.87	Green
			5.7	0.90	Green
			6.0	0.95	Green
			6.2	0.98	Green
Chloroform	Chloroform: Methanol: H_2O (7:3:1)	5.2	4.7	0.90	Green
			2.45	0.47	Green
			1.55	0.30	Green
	Chloroform: Glacial acetic acid: Methanol: H_2O (5:4:1:2)	5.55	5.0	0.90	Green
			4.8	0.87	Green
			4.1	0.74	Green
			2.6	0.47	Yellow
			1.7	0.31	Yellow
	Chloroform: Ethanol (1:1)	5.55	5.0	0.90	Green
			4.8	0.87	Green
			2.6	0.47	Yellow
			1.7	0.31	Yellow
Ethyl acetate	Chloroform: Methandol: H_2O (7:3:1)	5.55	5.0	0.91	Green
			4.8	0.87	Green
			5.4	0.98	Green
			4.1	0.75	Yellow
			2.6	0.47	Yellow
Ethanol	Chloroform: Glacial acetic acid: Methanol: H_2O (4:5:1:2)	5.5	5.0	0.91	Green
			4.8	0.87	Green
			4.1	0.75	Brown
	Chloroform: Methanol: H_2O (5:4:2)	5.5	4.8	0.87	Brown

0.87, 0.90 and 0.98 respectively when a solvent phase of Chloroform: Methanol: H_2O (7:3:1) was used.

TLC of ethanol extract of *M. oleifera* pods revealed the presence of 3 compounds having R_f values of 0.75, 0.87 and 0.91 respectively when a solvent phase of Chloroform: Glacial acetic acid: Methanol: H_2O (4:5:1:2) was used. In another solvent phase i.e., Chloroform: Methanol: H_2O (5:4:2) 1 spot was obtained but the spot was not as prominent and clear as in solvent phase of Chloroform: Methanol: H_2O (7:3:1) of benzene extract.

DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts.[19,20]

In the present study, qualitative tests for all five extracts showed significant indication about the presence of metabolites. Alkaloids, saponin, polyphenols and cardiac glycosides were found to be present in the all the sequential extracts of *M. oleifera* pods, while flavanoids and pholobatannins were present in very low amounts in the extracts. These findings of phytochemicals were good enough to reflect its importance.[21]

TLC profiling of all 5 extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can

be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analysing the R_f values of compounds in different solvent system.[21] Different R_f values of the compound also reflect an idea about their polariy. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

CONCLUSION

The results obtained in the present investigation indicated *M. oleifera* pods as a rich source of secondary metabolites. These findings suggested that *M. oleifera* pods could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases.

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