

Original Research Article

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Qualitative and Quantitative Phytochemical Analysis of *Moringa oleifera* (Lam) Pods

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ABSTRACT

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Moringa oleifera (Lam.) has remarkable medicinal properties and nutritional value possessed by various parts of the tree Viz. leaves, seeds, pods, roots and bark. The therapeutic effects of *M. oleifera* are due to the combined actions or effects of various bioactive components or phytochemical constituents such as alkaloids, flavonoids, saponins, sterols and tannins. The present study was carried out to find the availability of these five important phyto-constituents in aqueous, ethanol, methanol and chloroform extracts. Quantitative analysis was also carried out for the same metabolites in Moringa pods' crude extract.

Introduction

Plants are unique source of food, medicinal artifacts, energy and shelter for both human and animal., many useful harvests obtained from plants directly or indirectly validate their importance to the human and other living organisms (Kawo, 2007). *Moringa oleifera* (Lam.) has many common names such as ben oil, drumstick, horseradish, and miracle tree. *M. oleifera* is a widely distributed species of the family Moringaceae. It is a small graceful, deciduous plant with thin foliage and can grow up to 8 m height (Keay, 1989). Moringa is native to Western and sub Himalayan regions, India, Pakistan, Asia and Africa and it is distributed throughout the world in arid and semi-arid climate (Makkar and Becker, 1997).

Moringa trees are having a remarkable range of medicinal properties as high as nutritional values. Most parts of the plant., leaves, seeds, fruit or pods, roots, stem, and bark are used as medicines or foods in various countries with especial references to the traditional communities. The leaves are rich source of both macro- and micronutrients, such as protein and many vitamins (Siddhuraju and Becker, 2003). Fresh leaf juice inhibits the growth of human pathogens (Das *et al.*, 1957). The seeds also show antimicrobial activity (Oliveira *et al.*, 1999). Fruits or pods have wide spectrum of antimicrobial and antifungal activities against common pathogens (Sayeed *et al.*, 2012). The roots have been reported to have antispasmodic and antimicrobial activity and used for diarrhea treatment (Caceres *et al.*,

1992). Moringa contains specific plant pigments., alpha-carotene and beta-carotene, lutein and phytochemical constituents such as alkaloids, flavonoids, saponins, sterols, phenols and tannins. The therapeutic effects of *M. oleifera* could be due to the combined actions of various bioactive components found in the plant, including trace metal ions, vitamins, alkaloids, polyphenols and other elements (Coppin, 2008) and they collectively act on broad physiological processes including metabolism and immunity (Amaglo *et al.*, 2010).

Materials and Methods

Study area

The research work was carried out at Division of Aquaculture, ICAR- Central Institute of Fisheries Education, Mumbai, Maharashtra, India. Moringa pods were collected from different trees around the CIFE campus in Versova area suburb of western Mumbai city.

Plant materials

Fresh dry samples from Moringa pods were submitted to Blatter Herbarium of ST. Xavier's College in Mumbai, India. The samples were identified and authenticated as *Moringa oleifera* (lam). The samples matched with the herbarium specimen number NI. 4891 of N. A. Irani. For the research work, fresh and dry pods were collected and seed were removed from dry pods. The pods were blended as fine powder using, normal electrical grinder and kept in air tight container for further analysis.

Plant extraction

Aqueous extract was prepared using method described by Awodele *et al.*, (2012). 100 g of sample powder was macerated in 2 liters of distilled water (MILLPORE, Q-gard1) for 24

hours. The solution was decanted and filtered using Whatman filter paper No.1 (QUALIGEN- Germany). The filtrate was dried in oven (Hexatec, Model HIPL03A) at 40°C for 4days. The dry extract again was reconstituted in distilled water and used for the qualitative analysis. Chloroform extract was prepared using method described by Sathishkumar and Baskar, (2014).

100 g of sample powder was added to 400 ml chloroform and was shacked in water bath for ten hours. The solution then filtered using Whatman filter paper No. 1. The filtrate was dried using Rotary evaporator (IKA, model RV10B) to get the filtrate which again was dissolved in same amount of the solvent and used for further analysis. Ethanol extract was prepared using method described by Olatunde and Dikwa, (2014). 80 g of the sample powder was added to 400 ml ethanol (98%) for 72hours.

The solution was filtered using Whatman filter paper No. 1. The solvent was evaporated using rotary evaporator. The filtrate was again dissolved in same amount of the solvent and was used for further analysis. Methanol extract was prepared using method described by Pavithra, *et al.*, (2009). 50 g of the sample powder was added into a round beaker containing 500 ml of Methanol and kept for 24 hours and the solution then filtered using Whatman filter paper No. 1. The filtrate was evaporated at room temperature to get the dry extract which was dissolved in same amount of methanol and stored for further analysis.

Qualitative screening of bioactive components

Each extract was tested for presence of alkaloids, flavonoids, saponines, sterols and tannins using different methods. The tests were performed in triplicates to ensure the accurate results.

Detection of alkaloids

The occurrence of alkaloids was tested using method reported by Sabri *et al.*, (2012). 10 ml of the extract were evaporated to dryness. Two ml of 2% HCL acid were added to the dry residue.

Few drops of Wagner's reagent were added to the solution. The presence of alkaloids was confirmed when reddish brown precipitate occurred.

Detection of flavonoids

The test was based on method described by Pamar *et al.*, (2012). Few drops of NaOH were added to two ml of the extract and intense yellow color appeared. Few drops of dilute HCL were added and the solution turned to colorless as indicator of presence of flavonoids.

Detection of saponins

Precipitation and foam test method was used as described by Devmurari, (2010). Few drops of (1%) lead acetate solution were added to one ml of the extracts. Intense white precipitate appeared due to presence of Saponins.

Foam test also confirmed presence of saponins in the extract, in which 20 ml of distilled water were added to one ml of the extract in graduated cylinder. The solution was shacked for 5 to 15 minutes and formation of stable foam indicated the presence of saponins.

Detection of sterols

The test was performed based on method described by Solihah *et al.*, (2012). Two ml concentrated sulphuric acid were added to two ml of the extract. Formation of red precipitate indicated presence of sterols.

Detection of tannins

The test method was described by Ugochuhwu *et al.*, (2013). One ml of 3% of Ferric chloride was added to one ml of the extract. Brownish green color development indicated presence of tannins.

Quantitative analysis of the bioactive constituents

Different methods were used to determine the amount of the required constituents in the pods based on gravimetric tests. Three replicates were used for each test to ensure the accuracy.

Estimation of alkaloids

Alkaloids were quantified by the method described by Agoreyo *et al.*, (2012). Five grams sample was weighed into beaker and 200 ml of 10 % acetic acid in ethanol were added. The mixture was covered and allowed to stand for four hours. After that the mixture was filtered and the filtrate was concentrated on water bath (i-therm, model AI7482) at 100°C, to one-quarter of the original volume.

Concentrated ammonium hydroxide was added to the extract in the form of drops until precipitation completed. After settlement, the extract was filtered and the precipitate washed with dilute ammonium hydroxide and then dried and weighed (Citizen sensitive balance, CY510 C).

Estimation of flavonoids

The method was described by Bharathidasan *et al.*, (2013). 100 ml of 80% aqueous methanol was used to extract ten grams of the sample. The mixture was filtered by using (QUALIGEN- Germany) Whatman filter paper No. 42 (125 mm). The filtrate was evaporated until dried, and then weighed until constant weight was achieved.

Estimation of saponins

The method was described by Gupta (2013). 20 grams of plant sample was put into a conical flask and 100 ml of 20% ethanol was added. The solution was heated over water bath at 100°C for 4 hours with continuous stirring at 55°C. The solution was then filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath.

The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and was vigorously shaken. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated. 60 ml of n-butanol was added, the combined extract was washed twice with 10 ml of 5% NaCl. The remaining solution was heated in a water bath and after evaporation., the sample was dried in the oven at 105°C to a constant weight.

Estimation of sterols

Method described by Araujo *et al.*, (2013), was used with some modification to get the gravimetric weight. The extract was cooled to room temperature 25°C, filtered on cotton, and the residue (cotton and plant materials) re-extracted twice, using 30 ml of chloroform for 15 min. The two filtrates were collected and were dried under reduced pressure at 40°C, rotary evaporator. The residue was then placed to an oven at 80 °C until reached constant weigh.

Estimation of tannins

As method was described by Vetter and Barbosa, (1995), 100 ml of distilled water was added to two grams of the sample. The solution was kept in water bath at 90°C for one hour. The mixture was filtered by using Whatman's paper No. 1 and the residue was

re-extracted again. The two filtrates were collected together and allowed to cooldown. Distilled water was added to the filtrates up to 500 ml. One hundred ml of the solution transferred to a beaker, and then 10 ml of 40% formaldehyde and 5ml of concentrated sulphuric acid were added respectively. The whole mixture was refluxed for 30 minutes and was left to cool down. The mixture was filtered and the precipitate dried and weighed.

Results and Discussion

Qualitative analysis of phytochemical constituents for *Moringa* pods

Basically phytochemical screening or qualitative analysis is used to reveal the chemical constituents or the secondary metabolites of the plant extract or tissue in different plant parts. In the present study, preliminary phytochemical screening for pods' extract of *M. oleifera* revealed the presence of alkaloids, flavonoids, saponins, sterols and tannin (Table 1). Alkaloids were present in the aqueous, ethanol, methanol and chloroform extracts. Flavonoids were present in aqueous, ethanol, methanol extracts and were absent in the chloroform extract. Saponins were present in chloroform and ethanol extracts and were absent in aqueous and methanol extracts. Sterols were revealed in aqueous, ethanol, methanol and chloroform extracts. The phytochemical screening of *Moringa oleifera* (Patel *et al.*, 2014) exhibited presence of alkaloids, flavonoids, saponins, sterols and tannins in both aqueous and ethanolic extracts of leaves. Presence of alkaloids, flavonoids, saponins, sterols and tannins in aqueous, ethanol, ether and chloroform extracts in leaves of Guava *Psidium guajava* L. was reported by Arya *et al.*, (2012). Gupta *et al.*, (2013) reported that in pods of *Acacia concina*, alkaloids were presented in aqueous and methanol extracts but absent in chloroform extract (Fig. 1-5).

Fig.1 *Moringa oleifera* tree with pods



Fig.1 Alkaloids crude extract



Fig.3 Flavonoids crude extract



Fig.4 Saponins crude extract



Fig.5 Tannins crude extract



Table.1 Phytochemical screening of *Moringa* pod extracts

Presence(+) /Absence (-) of secondary metabolites				
Plant part	Pods			
	Solvent			
Constituent	Aqueous	Ethanoic	Methanolic	Chloroform
Alkaloids	+	+	+	+
Flavonoids	+	+	+	-
Saponines	-	+	-	+
Sterols	+	+	+	+
Tannins	+	-	+	-

Table.2 Quantitative analysis of secondary metabolites in crude extracts of *Moringa* pods (Mean \pm SE)

S. No	Phytoconstituents	Quantity (%)
1	Alkaloids	3.1 \pm 0.004
2	Flavonoids	5.2 \pm 0.003
3	Saponins	6.5 \pm 0.1
4	Sterol	4.7 \pm 0.002
5	Tannins	22.9 \pm 0.01

Flavonoids appeared in aqueous and methanolic extracts but absent in chloroform extract. Saponins, sterols and tannins, were absent in aqueous, methanol and chloroform extract. In the same study, screening of pods of *Emblica officinalis* revealed the presence of alkaloids in aqueous and methanolic extracts and absence in chloroform extract. Flavonoids appeared in methanol extract and were absent in aqueous and chloroform extract. Saponins and tannins were absent in

aqueous, methanol and chloroform extracts. Sterols were presented in aqueous and methanol and were absent in chloroform extracts.

Quantitative analysis of phytochemical

Constitutes for pods

Quantitative analysis of secondary metabolites benefits to disclose the chemical

constituents of the plant extract and to know which phytochemical conquered over the other. It can also be used to search for bioactive components for developed products that may contain medicinal values and can assist in synthesis of useful drugs (Harbone, 1998). The quantitative analysis result of secondary metabolites in *Moringa* pods shown in (Table 2). The amount of alkaloids in crude extract of pods was (3.1%), amount of flavonoids was (5.2 %), amount of saponins was (6.5%), amount of sterols was (4.7%) and amount of tannins was (22.9%). In *Acacia coccina* pods extract, the amount of alkaloids was (10.2%), amount of flavonoids was (7.2%), amount of saponins was (0.51%) and amount of tannins was (2.3%), while in *Acacia catechu*, amount of alkaloids was (11.3%), amount of flavonoids was (0.72%), amount of saponins was (0.53%) and amount of tannins was (2.1%). In pod extract of *Emblica officinalis*, quantity of alkaloids was (11.2%), quantity of flavonoids was (0.04%), quantity of saponins was (0.55%), and quantity of tannins was (1.10%), (Gupta *et al.*, 2013). Fruit extract of *Tribulus terrestris* the quantity of alkaloids was (0.75%), quantity of flavonoids was (1.10%), amount of saponins was (0.85%), and amount of tannins was (1.25%), (Kumar ashwani *et al.*, 2013). In some plant parts the amount of these phytoconstituents was comparatively higher as reported in a study for flower crude extract of *Bauhinia tomentosa L.* which showed that amount of alkaloids was (5.6%), amount of flavonoids was (15.8%) and amount of saponins was (2.1%), (Sathya *et al.*, 2013). General plant secondary metabolites or phytoconstituents are considered as good and unique source for food supplements and pharmaceuticals., their various roles were reported in many studies. The importance of alkaloids, sterols, saponins and tannins comes from their uses as antimicrobial for treating many pathogens (Kubmarawa, 2007) and (Mensah, 2008). Phyto-sterols and flavonoids

have a role as immunomodulatory, anti-cancer, antioxidants, and anti-inflammatory agents (Laparra and Sanz, 2010). Alkaloids have anti-microbial and anti-fungal activities or properties, flavonoids have role in preventing liver peroxidation and anti-cancer properties, and sterols have anti-metastatic activity (Saxena *et al.*, 2013). Alkaloids are also effective in reducing headaches resulting from hypertension (Ayitey and Addae, 1977).

Declaration of interest

Authors report no conflict of interest regarding present work.

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