

Original Research Article

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Studies on Qualitative and Quantitative Phytochemical Constituents of *Moringa oleifera* leaf Meal

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ABSTRACT

Plants have served animals and humans as a natural source for therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems, thus in the present investigation the phytochemical analysis of *Moringa oleifera* leaf meal was carried out as these plants have been proved to be one of the important nutritional and medicinal plant. The phytochemical analysis was carried out for the *Moringa oleifera* leaf meal extracted with aqueous and alcoholic solvents. The qualitative showed that presence of flavonoids, tannins, total phenols, saponins, and alkaloids were mainly seen in aqueous and alcoholic extract of leaf meal. In quantitative analysis showed that the level of flavonoids, tannins, total phenols, saponin and alkaloids in aqueous extract of *Moringa oleifera* leaf meal were 3.60, 1.25, 2.02, 0.59 and 1.12 per cent respectively. The presences of high amount of phytochemical constituents suggest that the *Moringa oleifera* plant has higher medicinal value and can be extensively studied to extract the natural compounds which are beneficial to animals and humans.

Keywords

Moringa oleifera leaf meal, phytochemical constituents, Extract, Alkaloids, Poly phenols

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Introduction

Medicinal plants and herbs are great importance to the health of the animals and also humans. Plants are composed of complex mixtures of secondary metabolites, which exhibit therapeutic effect. These are called phytochemicals and include alkaloids, glycosides, terpenoids and phenols. *Moringa*

oleifera referred as 'Drum stick tree' belongs to the family of *Moringaceae* is considered as a native plant of India, Pakistan and Africa (Zvinorova *et al.*, 2015). It can survive in harsh climatic condition including destitute soil without being much affected by drought. Every part of the *Moringa oleifera* tree, from the roots to the leaves has beneficial properties. The *Moringa oleifera* leaf contains

various phytochemical constituents, crude protein, amino acids, fatty acids, vitamins, minerals and other nutrients (Fahey, 2005). Many reports described *Moringa oleifera* as having highly potent antioxidant, antibacterial, hypolipidemic, anti-inflammatory and hepatoprotective activity (Divya *et al.*, 2015). The leaf extracts were found to regulate the serum cholesterol level in rats due to its hypocholesterolaemic activity (Ghasi *et al.*, 2000). The leaves, flowers and pods are used as good sources of protein, vitamins A, B, C, and minerals Ca, K, Fe, Zn and amino acids. This makes the plant a natural source of chemical compounds with medicinal value and making it a plant with greater commercial value. In order to contribute to growing body of knowledge on this subject, the present study analysed the phytochemical constituents of aqueous and alcoholic extract of *Moringa oleifera* leaf meal.

Materials and Methods

Collection of sample

Fresh and healthy *Moringa oleifera* leaves were collected from Namakkal district. The plant was authenticated as *Moringa oleifera* Lam by Botanical Survey of India, Coimbatore. Stem and branches were cut from *Moringa* trees and spread out under the shade to dry at room temperature for 7 days. The leaves were removed manually and ground into fine powder and used for extraction.

Preparation of *Moringa oleifera* leaf extract

Ten per cent aqueous and alcoholic extracts of *Moringa oleifera* leaf were prepared by adding ten gram of dry powder in hundred mL of distilled water and 70% alcohol respectively. It was kept in a rotatory shaker for 48hrs, filtered and then incubated at 37°C

for 48hrs to evaporate the solvent. The dried extract was removed and stored in airtight container and used for further evaluation.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of aqueous and alcoholic extracts of *Moringa oleifera* leaf was carried out as per method of Harborne, (1998).

Detection of flavonoids

To 2.0mL of aqueous and alcoholic extracts, few drops of sodium hydroxide solution were added. Formation of intense yellow color, which became colorless on addition of dilute HCl indicated the presence of flavonoids.

Detection of tannins

To 2.0mL of aqueous and alcoholic extracts, 3 drops of 1% ferric chloride was added. Appearance of blue green color indicated the presence of tannins.

Detection of total phenols

Two mL of aqueous and alcoholic extracts were diluted with 2mL of 10% ferric chloride. Formation of bluish color indicated the presence of phenols.

Detection of saponins

Two mL of aqueous and alcoholic extracts were diluted with 10mL of distilled water and mixed for 15min. Formation of layers of foam which remained for 10min indicated the presence of saponins.

Detection of alkaloids

To 2.0mL of aqueous and alcoholic extracts, 2mL of picric acid (Hager's reagent) was added. Formation of an orange or yellow

color precipitate indicated the presence of alkaloids.

Detection of phlobatannin

To 2.0mL of aqueous and alcoholic extracts, 1mL of dilute HCL solution was added. Appearance of red precipitate indicated the presence of phlobatannins.

Detection of hydrolysable tannin

To 2.0mL of aqueous and alcoholic extracts, 2mL of ammonia solution was added. Formation of emulsion indicated the presence of hydrolysable tannin.

Detection of terpenoids

To 2.0 mL of aqueous and alcoholic extracts, an equal amount of chloroform was added followed by addition of 2mL of concentrated H₂SO₄ along the sides of the test tube. Appearance of a brown color ring at the junction of two liquids indicated the presence of terpenoids.

Detection of glycosides

To 2.0mL of aqueous and alcoholic extracts, 2mL of dilute H₂SO₄ was added and heated at 50°C for 2 min. Then 1mL of 10% NAOH was added and 5mL each of Fehling's solution A and B were added. Appearance of brick red precipitate indicated the presence of glycosides.

Detection of cardiac glycosides

To 2.0mL of aqueous and alcoholic extracts, an equal amount of glacial acetic acid was added. Then, one drop of 10% ferric chloride and 2mL of concentrated H₂SO₄ were added. Formation of three layers of colors like upper green, middle brown and lower violet indicated the presence of cardiac glycosides.

Detection of Volatile oil

To 2.0mL of aqueous and alcoholic extracts, 0.1mL of NaOH and a small amount of dilute HCl were added. Formation of white precipitate indicated the presence of Volatile oils.

Quantitative phytochemical analysis

This analysis was carried to determine the amount or concentration of the phytochemical constituents present in the aqueous extract of *Moringa oleifera* leaf.

Flavonoids

Flavonoids in plant extracts were determined by aluminium chloride colorimetric method (Chang *et al.*, 2002). About 0.25 ml of extract (10mg/ml) was mixed with 0.75 ml of ethanol, 0.05 ml of 10 per cent aluminium chloride, 0.02 ml of 1M potassium acetate and 1.4 ml of distilled water. The reaction mixture was incubated at 37°C for 30 minutes. The absorbance of the mixture was measured at 415 nm using UV- VIS spectrophotometer.

Tannins

Tannin content was estimated as per the method of Pearson, (1976). About 1.0 g of the sample was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 min at room temperature and shaken every 5 min. After 30 min, it was centrifuged and the extract gotten. About 2.5 ml of the supernatant extract was dispensed into a separate 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid was dispensed into a separate 50 ml flask. The absorbance was measured at 250 nm.

Total phenols

Total phenol was estimated as per the method of Makkar *et al.*, (1993). Aqueous extract of

MOLM at the rate of 0.1 mg/ml was mixed with 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 dilution with distilled water) and 2.5 ml of 20 per cent sodium carbonate solution. The reaction mixture was allowed to stand for 40 minutes and the absorbance was measured at 725 nm using UV-VIS spectrophotometer.

Saponin

Saponin content was estimated as per the method of Amin *et al.*, (2016). About 2 g of dried plant sample was dissolved in 50 ml of petroleum ether. The suspension was heated over a hot water bath at 55°C for 1hr with continuous stirring. The mixture was filtered and the residue was re-extracted in 50 ml of methanol.

The combined filtrates were reduced to 10 ml by placing over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and acetone was added slowly and shaken well. The aqueous layer was recovered and the purification process was repeated. The remaining solution which contains saponin was heated in a water bath and the sample was dried in oven and weighed.

Alkaloids

The quantity of alkaloids was estimated as per the method of Harborne (1973). About 1 g of powdered sample was mixed with 40 ml of 10% acetic acid and allowed to stand for 4 hrs. It was filtered and concentrated on water bath to one fourth of its original volume then concentrated ammonium hydroxide was added drop by drop to the extracts until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Results and Discussion

Qualitative analysis of *Moringa oleifera* leaf meal

Qualitative phytochemical constituents of aqueous and alcoholic extracts of *Moringa oleifera* leaf meal are present in the Table 1.

Qualitative analysis showed that flavonoids, tannin, total phenols, saponin, alkaloids and phlobatannin were present in both aqueous and alcoholic extracts of *Moringa oleifera* leaf meal. However hydrolysable tannin and terpenoid were present in aqueous extracts of *Moringa oleifera* leaf meal. Similarly, the presence of tannins, flavonoids, saponins, alkaloids, phenols, glucoside in aqueous extract of *Moringa oleifera* leaf meal was observed by Bamishaiye *et al.*, (2011), Ajayi and Fadeyi, (2015). Dzutam *et al.*, (2016) reported that presence of alkaloids, polyphenols, flavonoids, tannins, terpenoids, saponins, coumarins in methanolic extract of *Moringa oleifera* leaf meal.

Quantitative analysis of *Moringa oleifera* leaf meal

Quantitative phytochemical constituents of aqueous extracts of *Moringa oleifera* leaf meal are present in the Table 2. The level of flavonoids, tannins, total phenols, saponin and alkaloids in aqueous extract of *Moringa oleifera* leaf meal were 3.60, 1.25, 2.02, 0.59 and 1.12 per cent respectively.

The levels of flavonoids, tannins, total phenols and alkaloids were comparable to the earlier reports (Moyo *et al.*, 2011; Alikwe and Omotosho, 2013; Kathryn, 2014; Ajayi and Fadeyi, 2015 and Kavoi *et al.*, 2016) and lower level of saponin was reported by (Ogbe and Affiku, 2011; Aye and Adegun, 2013; Kavoi *et al.*, 2016). The medicinal plants are usually linked with the presence of

phytochemicals otherwise called secondary metabolites and these differ from one plant to another, accounting in part for the difference in pharmacological effects of medicinal plants. Flavonoids are potent antioxidant activity and revealed that ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals this may be important function of flavonoids (Bamishaiye *et al.*, 2011). Tannins are proved haemostatic, widely used as mouthwashes, eyewashes and also treat rectal disorders. Total phenols are strong antioxidants which prevent oxidative

damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases (Hollman, 2001). Saponin has possessed cholesterol lower property and stimulation of immune system. Alkaloids have nitrogen containing compound, commonly found to have antimicrobial property due to their ability to intercalate with DNA of the microorganisms (Kasolo *et al.*, 2010). In general, *Moringa oleifera* leaf meal has possess lot of medicinal property might be due to the presence of these phytochemical constituents in *Moringa oleifera* leaf meal.

Table.1 Qualitative phytochemical constituents of aqueous and alcoholic extracts of *Moringa oleifera* leaf meal

Phytochemical constituents	Aqueous extract	Alcoholic extracts
Flavonoids	++	+
Tannin	+	+
Total phenols	++	++
Saponin	+	+
Alkaloids	++	++
Terpenoid	+	-
Phylobatannin	+	+
Hydrolysable tannin	-	+
Volatile oil	-	-
Glycosides	-	-
Cardiac glycosides	-	-

Table.2 Quantitative phytochemical constituents of aqueous extracts of *Moringa oleifera* leaf meal

Phytochemical constituents	Composition (%)
Flavonoids	3.61
Tannins	1.25
Total phenols	2.02
Saponins	0.59
Alkaloids	1.12

Each value is a mean of two observations

In conclusion, the results revealed that *Moringa oleifera* leaf meal has potent antioxidant, antimicrobial, hypocholesterolaemic and growth promoting

properties due to the presence of these phytochemical constituents. The result of this study indicates *Moringa oleifera* leaf meal contains presence of valuable phytochemical

constituents that may offer chemo protective and medicinal value to animals. Viable effects detectable through biological evaluation of animals are necessary to enhance the acceptability and commercialization of this plant.

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