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Research Article

Preliminary, phytochemical analysis and minerals present in Moringa Oleifera seed oil

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Abstract

Moringa Oleifera seed is one of the plants that is rapidly gaining popularity because of the numerous benefits associated with it. This study aims to determine the phytochemical analysis and mineral content using standard methods. The qualitative screening of phytochemicals in ethanol seed extracts of Moringa Oleifera showed the presenceAlkaloid, Saponins, Flavonoid, Terpenoid, Steroids, Amino acids. , the seed can be used as a food supplement to increase the nutritional composition of foods lacking protein, carbohydrate and lipid.

Keywords: Moringa Oleifera seed, Phytochemicals, minerals, ethanol.

Introduction

Moringa is a plant that is commonly found in India, Pakistan, Bangladesh and some parts of Africa. The bark, fruit, root, flowers leaves and seeds are used in making herbal medicine. Moringa is potentially one of the planet's most valuable plants, at least in humanitarian terms. Perhaps the fastest growing useful tree, it commonly tops 3m or even 5m within a year of the seed being placed in the ground. Some people actually grow it as an annual. It yields at least four different edibles: pods, leaves, seeds and roots. Beyond edibles, it provides products that make village life more self-sufficient: Lubricating oil, Lamp oil, Wood, Paper, Liquid fuel, Skin treatment, to purify water. This tree ranges in height from 5 to12 m with an open, umbrella-shaped crown, straight trunk and corky; whitish bark, the tree produces a tuberous tap root. The evergreen or deciduous foliage (depending on climate) has leaflets 1 to 2cm in diameter, the flowers are white or cream coloured. The fruits (pods) are initially light green, slim and tender, eventually becoming dark green, firm and up to 120 cm long, depending on the variety. Fully mature, dried seeds are round or triangular, the kernel being surrounded by a lightly wooded shell with three papery wings. It was shown that the average relative masses of shells and kernel in M. Oleifera were 27 % and 73 %, respectively.

Plant Classification	**(Taxonomy)**
Kingdom	**Plantae**
(Unranked)	**Angiosperms**
(Unranked)	**Eudicots**
Order	**Brassicales**
Family	**Moringaceae**
Genus	**Moringa**
Species	**M. Oleifera Lam**

Varieties

Thirteen Moringa species are known:

- M. oleifera
- M. arborea
- M. borziana
- M. concanensis
- M. drouhardii
- M. hildebrandtii
- M. longituba
- M. ovalifolia
- M. peregrina
- M. pygmaea
- M. rivae
- M. ruspoliana
- M. stenopetala

Botanical Description

Moringa Oleifera is a small, graceful, deciduous tree with sparse foliage, often resembling a leguminous species at a distance, especially when in

flower, but immediately recognized when in fruit. The tree grows to 8 m high and 60 cm. Bole crooked, often forked from near the base. Bark smooth, dark grey; slash thin, yellowish. Twigs and shoots shortly but densely hairy. Crown wide, open, typically umbrella shaped and usually a single stem; often deep rooted. The wood is soft.

Leaves alternate, the old ones soon falling off; each leaf large (up to about 90 cm long), with opposite pinnae, spaced about 5 cm apart up the central stalk, usually with a 2nd lot of pinnae, also opposite, bearing leaflets in opposite pairs, with a slightly larger terminal leaflet. Leaflets dark green above and pale on the under surface; variable in size and shape, but often rounded-elliptic, seldom as much as 2.5 cm long.

Flowers produced throughout the year, in loose axillary panicles up to 15 cm long; individual flower stalks up to 12 mm long and very slender; 5 pale green sepals 12 mm long, finely hairy, 5 white petals, unequal, a little longer than the sepals; 5 stamens with anthers, 5 without; style slender, flowers very sweet smelling.

Fruit large and distinctive, up to 90 cm long and 12 mm broad, slightly constricted at intervals, gradually tapering to a point, 3- (4-) angled, with 2 grooves on each face, light brown. It splits along each angle to expose the rows of rounded blackish oily seeds; each with 3 papery wings. The generic name comes from the Sinhalese name 'morunga'.

Biology

The bisexual, oblique, stalked, axillary and heteromorphic flowers are highly cross-pollinated due to heteromorphic. The carpenter bees (Xylocopalatipes and X. pubescens) have been found the most reliable and appropriate pollinators. Sunbirds Nectariazeylanica and N. Asiatic

Uses and Benefits [1-3]

Root – Antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation.

Leave – Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling.

Stem bark - Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevent enlargement of the spleen and formation of tuberculosis glands of the neck, to destroy tumours and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti-tubercular activity

Gum –It is mixed with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, and asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism.

Flower – High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumours, enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolemia rabbits and increased the excretion of faecal cholesterol.

Seed – Seed extract its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosides have been isolated from the acetate phase of the ethanoic extract of Moringa pods.

Phytochemical Screening [4]

The phytochemical analyses of the oil samples were determined as described in. The presence or absence of the following plant secondary metabolites were determined alkaloids, phenols, sterols, terpenes, tannins, flavonoids, cardiac glycosides, saponins.

1. Phenols

Equal volumes of each extract and ferric chloride solution (which is prepared by dissolving 135.2g of $FeCl_3.6H_2O$ in distilled water containing 20 ml of concentrated HCl dilute to 1 litre) are added together. A deep bluish green precipitate indicates the presence of phenol.

2. Alkaloids

Each extract was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with (2g of Iodine in 6g of Potassium iodide in 100 ml of distilled water) .Formation brown or reddish brown precipitate indicates presence of alkaloids.

3. Steroids

Each Extract was added to 2ml acetic anhydride and 2ml H_2SO_4. Colour change from violet to blue or green indicates the presence of steroids.

4. Terpenes

Each Extract was added to 0.5ml acetic anhydride and few drops of concentrated H_2SO_4. A bluish green precipitate indicates the presence of terpenes.

5. Tannins

Each Extract was boiled in 20ml water and filtered. A few drops of 0.1% Ferric Chloride solution were added. Brownish green or blue-black colour indicates the presence of Tannins.

6. Flavonoids

5ml Ammonium solution was added to aqueous filtrate of each extract and then few drops of concentrated H_2SO_4. Yellow coloration indicates the presence of Flavonoids.

7. Saponins

1g each extract was boiled with 5ml distilled water and filtered. 3ml distilled water was added to the filtrate and shaken vigorously for 5 minutes. Persistent frothing on warming indicates the presence of Saponins.

8. Glycosides

5ml H_2SO_4 was added to each of the test extract in a boiling tube. The mixture was heated in boiling water for 15minutes. Fehling's solution A and B was added and the resulting mixture was heated to boiling. A brick red precipitates indicates the presence of glycosides.

9. Carbohydrates

5ml of the equal mixture of both Fehling's solutions A and B was added to 2ml of test extract in a boiling tube, this was heated for 2 minutes. A brick red precipitates indicates a positive result.

10. Amino acids

A small quantity of the extract was dissolved in a few ml of distilled water and 1ml of ninhydrin reagent was added to it. Development of a blue colour indicates the presence of amino acids.

Determination of Mineral Elements

Quantitative Analysis of Elementals [5-10]

Sample was prepared according to the following procedures

Ash was estimated using Gravimetric method by ASTM (1988). Total Nitrogen estimated by the method of Micro Kjeldhal (Bremner, 1965). Sulphur was measured with Gravimetric method (Ali Ehyaeei, 1997). Total Phosphorous by Pemberton (1927) method. Total Potassium, Total Sodium, Total Calcium, Total Magnesium were analysed with the method of Flame Photometry (Systronics mid flame 127) by the method of Stanford and English method (1949). Total Zinc, Total Copper, Total Iron, Total Manganese, Total Boron, Total Molybdenum are analysed by using Atomic Absorption Spectroscopy (Solar-AAS2-UK made).

AtomicAbsorption Spectrophotometer

200 ppm stock solution of the Zinc, Copper, Iron, Manganese, Boron and Molybdenum were formedby mixing required quantity of salts in distilled water for elemental analysis ofplant powder. Perchloric-aciddigestion method was used for elemental analysis (Allen, 1974). 0.25 g powder wasimmersed in 6.5 ml of mixed acid solution i.e. nitric acid, sulphuric acid and Perchloricacid (5:1:0.1) and digested in a flask (50 ml) in fume hood on hot plate till thedigestion was completed which was indicated by white fumes coming out from theflasks. Digested samples were allowed to cool and transferred in 50 ml volumetricflask, by rising volume with distilled water. Filtrate (Whatmann No. 42) was collectedand concentration of each element was determined on Solar-AAS2-(UK made) atomicabsorption spectrophotometer. Quantity of each element was calculated by usingformula:

Nutrient caution in plants= (ppm in extract – blank) × A/W × dilution factor

A = Total volume of extract (ml)

W = Weight of dry plant

Flame Photometry

The estimation for sodium, calcium, Magnesium and potassium ions was carried out using Systronics med flame 127 – flame photometer.

Preparation of stock solutions

Sodium stock solution was prepared by dissolving 2.542g NaCl in 1 litre of distilled water. It contains 1mg Na per ml (i.e. 1000 ppm). Stock solution was diluted to give four solutions containing 10, 5, 2.5 and 1 ppm of sodium ions.

Potassium stock solution was prepared by dissolving 1.909g KCl in 1 litre of distilled water. It contains 1mg potassium per ml (i.e. 1000 ppm). Stock solution was diluted to give four solutions containing 20, 10, 5 and 2 ppm of potassium ions.

Calcium stock solution. 2.497 g $CaCO_3$ (1000-ppm) (AR grade) into a 100-ml beaker and then transfer to a 1-liter volumetric flask. Make up to volume with deionized-distilled water. Diluted standards of 100, 75, 50 and 25 ppm using deionised water as diluent.

Result and Discussion

Table 1: Phytochemical Analysis of Oil

S. NO	SAMPLES	MORINGA OLEIFERA SEED OIL
1	Alkaloid	+
2	Carbohydrate	-
3	Saponins	+
4	Flavonoid	+
5	Tannin	-
6	Terpenoid	+
7	Steroids	+
8	Glycoside	-
9	Phenol	-
10	Amino acids	+

The Phytochemical results of Moringa Oleifera oils are presented in Table (1). There are at least fourteen classes of secondary metabolites (chemical compounds) from fruits and vegetables that exert biological activities and can potentially be used to promote human health. These include alkaloids, amines, cyanogenic glycosides, diterpenes, flavonoids, glucosinolates, monoterpenes, non –protein amino acids, phenylpropanes, polyacetylenes, polyketides, sesquiterpenes, tetraterpenes, triterpenes, saponins and steroids. Flavonoids, terpenoid, steroid were detected in oil samples. The biological functions of flavonoids include protection against allergies, inflammation, free radicals scavenging, platelets, aggregation, microbes, ulcers, hepatoxins, viruses and tumours. The presence of steroids in the oil is of importance and interest in medicine due to their relationship with compounds as sex hormones. Alkaloids and Saponins which are known to exhibit medicinal, physiological activity and can be seen as a potential source of useful drugs. Many pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. The presence of alkaloid in the oil suggests a possible medicinal use of the oil.

TABLE 2: Mineral Compositions (mg/100g) of Moringa Oleifera Seeds

S. NO	MINERALS	*Moringa Oleifera* SEEDS
	Macro Elements	
1	Sodium	11.06
2	Potassium	5.54
3	Calcium	39.9
4	Phosphorous	9.7
5	Magnesium	5.24
	Micro - Elements	
6	Iron	129.51
7	Copper	0.10
8	Manganese	10.41
9	Zinc	1.02

The result of mineral element composition of the seeds of Moringa Oleifera in mg/g. The value obtained for calcium was 39.9 mg/g. The RDA for calcium is 600-1400mg. Calcium is essential for bone and teeth formation and development, blood clothing and for normal functioning of heart, nervous system and muscles. Calcium deficiency can lead to rickets, osteomalacia and tooth decay. Calcium may in the soil interfere with phosphorus nutrient and may encourage chlorosis because of reduction of soil manganese, iron and zinc. The manganese content of the seed of Moringa Oleifera

seed was 10.41 mg/g. The RDA for manganese varies between 2mg/kg to 8mg/kg. Certain trace elements such as copper, iron, manganese, constitute essential part of any balanced diet. Some of them are micro nutrients, if not present in the right proportion may have adverse effect on human and plant. The Copper content was 0.10 mg/g. Copper is very vital in diet because it is involved in the proper usage of iron and especially for the synthesis of cytochrome oxidase, which contains both iron and copper. Excess copper can lead to jaundice. The Zinc content was 1.02 mg/g. Zinc is essential in the activation of certain enzymes, these include: dehydrogenase, alkaline phosphatase and carboxy peptides. Zinc containing organic compounds is employed as astringent and antifungal agents. It aids wound healing and metabolism of nucleic acid and insulin. Zinc in excess causes anemia and if deficient in the body can lead to dermatitis.

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