PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF MORINGA OLEIFERA LEAVES BASED PRODUCTS

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ABSTRACT

Moringa oleifera is an important food sources in some parts of the world. Because it can be grown cheaply and easily, and the leaves retain lots of vitamins and minerals when dried, *Moringa oleifera* is used in India and Africa in feeding programs to fight Diabetics. Phytochemicals are believed to have benefits over conventional drugs and are regaining interest in current research. Moringa oleifera is a multi-purpose herbal plant used as human food and an alternative for medicinal purposes world wide. It contains essential amino acids, carotenoids in leaves, and components with nutraceutical properties. In this present study *Moringa Oleifera* is nutritionally rich and with a high polyphenol content in the form of phenolic acids,Nutrients such as Protein, Fat, Mineral, Crude fibre, Carbohydrate, Calcium and Iron were analyzed.Quantitative and qualitative phytochemical analysis shows the significance of the *Moringa oleifera* leaves powder. The Product was kept for sensory evaluation . It was carried out by 20 panel members. The keeping quality were also done.

Keywords: *Moringa oleifera*, Polyphenol, Phytochemicals, Nutraceutical
DOI Number: 10.14704/NQ.2022.20.12.NQ77370 **NeuroQuantology2022;20(12): 3623-3632**

INTRODUCTION

The *Moringa oleifera* (MO) tree, Known as “drumstick tree” belongs to Moringaceae family. It is the best known and most widely used of the thirteen species of the *Moringa* genus. It is originally from the southern Himalayas to the north-east of India, Bangladesh,Afghanistan and Pakistan and is nowadays cultivated in tropical and subtrophical areas of Africa, America and Asia.(Gandji et al.; 2018). It is a fast growing perennial tree that can measure up to 12 meters in height and displaying great ecological plasticity since it is able to adapt to the most

dissimilar conditions of the soil, temperature and precipitation, being very resistant to the drought***(Fejer et al.;2019).***Its leaves are commonly consumed as vegetables and nutritional supplements; the seeds are taken fresh, dried or as roasted tea. The leaves and seeds of MO are rich in protein,lipids,vitamins, minerals and phytochemicals.**(Dhakad et al.;2019)**

The leaves have been used as food and traditional medicine ***(Popoola et al.;).***The most (calcium,iron,potassium,magnesium,etc.);
essential and non-essential amino acids;and carbohydrates ***(Falowo et al.;)*.** Moringa oleifera has been used in traditional medicine for the treatment of various conditions and has been proposed to be of benefit in numerous diseases including
cardiovascular,diabetes,cancer,nurological,gastroenterological and inflammatory ***(Anwar et al.;2007)***

Over 50% of plants serve in traditional medicine for their health benefits in combating certain ailments affecting humans such as dysentery, diarrhea, toothache, skin infections and diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Over 400 traditional plant treatments for diabetes have been reported, although only few of the plants have received scientific medical evaluation to assess their efficacy ***(Goji et al.;)***
M.oleifera possesses many therapeutic values. It has analgesic, anti-inflammatory, antipyretic, anticancer, antioxidant, nootropic, hepatoprotective, gastroprotective, anti-ulcer, cardiovascular, anti-obesity, anti-epileptic, anti-urolithiatic, diuretic, local anesthetic, anti-allergic, antihelminthic, wound healing, antimicrobial, immunomodulatory and antidiarrheal properties ***(Bhattacharyaet et al.,2018)***

The leaves are rich in protein, essential amino acids, iron, copper, calcium, Vitamin C and carotenoids ***(Fahey,2005; Fuglie,2002)*.** Due to the presence of both micro-and macro nutrients MO leaves are commonly used as food source in many regions of theworld. The leaves have a high protein content together with high levels of calcium, phosphorous, Iron, and Manganese ***(Olson et al.;2016)*.** The leaves are also rich in several bioactive compounds including beta-carotene, vitamin (B,C and E), polyphenols, phenolic acids, alkaloids, GLSs, ITCs, tannins, saponins, oxalates, phytates and antioxidants***(Kou et al.;2018)*.** In the light of the above mention facts certain objectives were formulated as follows:

1. To find out the Quantitative and Qualitative phytochemical constituents in *Moringa Oleifera* leaves powder.
2. To determine the Nutrient composition of *Moringa oleifera* leaves powder.
3. To formulate *Moringa oleifera*leaves powder soup
4. To evaluate the sensory qualities of the product.
5. To determine its keeping quality of the *Moringa oleifera* leaves powder

Materials and Methods

PROCUREMENT OF THE SAMPLE

The fresh samples of Moringa oleifera leaves were procured in my garden thoongampara, kattakada. The other ingredients were purchased near by Super Market Kattakada, Trivandrum District.

Preparation of the Plant materials

The youngest leaves were (at the tip of the branches) were harvested early in the morning. The leaves were washed with tap water, dried in the shade, then pounded and sifted to give a fine powder. The powder was stored in hermatically sealed glass jars and kept in the dry area.

Formulation of the product

The Moringa oleifera leaves powder was used for the preparation of the Soup powder.

Table– 1 Ingredients used for preparing *Moringa oleifera* Leaves powder Soup

INGREDIENTS	**QUANTITY**
Moringa oleifera leaves powder	20 gm
Onions (small)	6-7 nos
Tomato	5-6 nos
Garlic	2 nos
Cumin Seeds	1tsp
Coriander powder	1 tsp
Turmeric powder	¼ tsp
Black pepper powder	1 tsp
Salt	To taste

It is a flavourful and healthy soup made with Moringa oleifera leaves. Pluck the *Moringa oleifera* leaves from the stem and set aside. If the leaves are tender then the tiny stems can be added too. Rinse it well twice and drain water, set aside. Heat the oil in a pan, add jeera it crackle after that add crushed garlic, Saute for a minute, then add onion and then add the required ingredients. Add salt. Saute till golden then add tomato, saute till tomatoes turn mushy and raw smell leaves. Add the required quantity of water. Now add the Moringa oleifera leaves powder. Boil till the raw smell leaves, when its boiling add little more salt (if needed) and add pepper, cook for a minute and switch off. Serve it hot.

SENSORY EVALUATION

The quality of the prepared products was assessed by the means of human sense of organs is called as " Sensory evaluations or Organoleptic Evaluation". This evaluation is valuable tool in solving problems involving food acceptability useful in production improvement and market research. It depends upon the sources given by different sense organs such as eyes, taste buds of tongue of olfactory of the mouth feel **(ManoranjanKalia,2002)**

Keeping Quality

The Keeping quality was determined by observing the product's storagebehavior. The Soup mix were packed in high density poly ethylene bags and kept at ambient temperature for a period of 2 months. The changes in moisture content, microbial count (total plate count and yeast and mould count) and sensory characters were evaluated periodically at monthly interval.

CONDUCT OF SENSORY ANALYSIS

All the ingredients were mixed according to the Specified ratio for the development of soup mix. In Sensory evaluation, each sample was subjected to five-point hedonic scale test and acceptability of sample was judged by 20 untrained panel members. The panelists judged the sensory characteristics such as appearance, colour, aroma,texture,taste,mouthfeel and over all acceptability of the samples.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phenols

2ml of sample was taken in a test tube and add 1% lead acetate solution. Formation of white precipitate indicate the presence of phenolic compounds.

Flavonoid

2ml of sample was treated with 2ml of 10% Lead acetate solution. Appearance of yellowish green colour indicated the presence of flavonoid.

Alkaloids

(Wagner's reagent): Take 2ml of sample and add 2ml Wagners reagent. Test tubes were observed for the appearance of reddish brown precipitate.

Tannins

Take 1ml of the sample and 5% $FeCl_3$ (1ml) was added to it. Appearance of brownish green coloration showed the presence of tannins

Glycosides

In 5ml sample, 2ml glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added. Brown ring appears, indicates the presence of glycosides.

Saponins

To about 1ml of Sample was added to 2ml of distilled water in a test tube and shaken vigorously with few drops of olive oil. Foam which persisted was taken as an evidence for the presence of saponins.

Steroids

1ml of sample was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Terpenoids

2ml of each sample was mixed with 2ml of chloroform. Then allow to evaporate and add 2ml concentrated sulphuric acid, then heat for 2 minute, Greyish colour indicates the presence of terpenoids.

Quinones

2ml of each sample was mixed with 3 or 4 drops of concentrated HCl. A yellow colour precipitate indicates the presence of quinines.

Fatty Acids

0.5 ml of sample was added to 5ml of ether and allowed it to evaporate on filter paper. Then the filter paper was dried and the appearance of transparency on filter paper is the indication of presence of fatty acids.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Estimation of Phenols

One ml of sample was taken and added 3.0 ml of distilled water. Folin-Ciocalteau reagent (0.5ml) and 2ml 20% Na_2CO_3 were added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 750nm in a spectrophotometer against a reagent blank. Standard gallic solutions (2.5-100ug/ml) were also treated as above.

Estimation of Tannins

Content of tannins in sample was determined by Folin-Ciocalteau method. Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungsto molybdic acid by tannin like compounds in alkaline medium. 1ml of extract and standard solution of tannic acid (20-100ug) was made up to 7.5mL with distilled water. Then 0.5mL of Folin-Ciocalteau reagent and 35% 1mL sodium carbonate solution were added. The volume was made up to 10mL with distilled water and the absorbance was measured at 700nm.

Estimation of Flavonoids

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1mg of extract and 4ml of distilled water was taken in a 10ml volumetric flask. To the flask, 0.30 ml of 5% sodium nitrite was treated and after 5minutes, 0.3 ml of 10% alluminium chloride was mixed. After 5 minutes, 2ml of 1M Sodium hydroxide was treated and diluted to 10ml with distilled water. A set of reference standard solution of Quercetin(20,40,60,80 and 100ug/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solution were determined against the reagent blank at 510 nm with an UV/Visible

Spectrophotometer. The total flavonoid content was expressed as ug of QE/mg of extract.

Estimation of Steroids

1gm of extract was taken in a clean test tube, Cholesterol was used as standard and was taken at varying concentrations of (1-10ug/ml) in test tubes, To the standard and test samples, 5ml of ferric chloride reagent and 4ml of concentrated sulphuric acid were added. The reaction mixtures were incubated at RT for 30 min and OD was read at 540nm. A standard graph was plotted from which the unknown value of steroid in the test samples was determined.

RESULT AND DISCUSSION

Qualitative Phytochemical Analysis

The Phenol, Tannin, flavonoid and Steroid were tabulated and evaluated in phytochemical analysis, which involved the use of several analytical techniques for the secondary metabolites and observation of the qualitative phytochemical analysis

Table – 2
Qualitative Phytochemical Analysis

SI.NO:	Name of secondary metabolites	Name of test	Observation
1.	Phenol	Lead acetate test	Formation of white precipitate
2.	Tannin	Ferric Chloride Test	Formation of brownish precipitate
3.	Flavonoid	Ferric Chloride Test	Formation of yellowish green colour
4.	Steroid	Libermann-Burchard Test	Upper reddish layer and greenish yellow acid layer

Quantitative Phytochemical Analysis

Estimation of Phenols

The phenols were tabulated and evaluated in Quantitative phytochemical Analysis Using Standard – Gallic acid

Table - 3
Estimation of Phenols

Standards	Concentration of gallic acid (ug/ml)	OD at 750 nm
S1	2.5	0.060
S2	5	0.0113
S3	10	0.203
S4	15	0.241
S5	20	0.334
S6	40	0.512
S7	60	0.751
S8	80	0.980
S9	100	1.242

Estimation of Tannins

The phenols were tabulated and evaluated in Quantitative phytochemical Analysis Using Standard – Tannic acid

Table - 4
Estimation of Tannins

Standards Tannic acid (1mg/ml stock)	Concentration of tannic acid (ug/ml)	OD at 700 nm
S1	10	0.476
S2	20	0.659
S3	40	1.002
S4	80	1.494
S5	100	1.914

Estimation of Flavonoids

The Flavonoids were tabulated and evaluated in Quantitative phytochemical Analysis Using Concentration of Quercetin

Table - 5
Estimation of Flavonoid

Standards	Concentration of Quercetin (ug/ml)	OD at 510 nm
S1	20	0.101
S2	40	0.163
S3	60	0.241
S4	80	0.344
S5	100	0.421
S6	120	0.543
S7	140	0.614
S8	160	0.725
S9	180	0.829
S10	200	0.911

Estimation of Steroids

The Steroids were tabulated and evaluated in Quantitative phytochemical Analysis Using Concentration of Quercetin

Table - 6
Estimation of steroids

SI.NO	Concentration (ug)	OD at 540 nm
S1	5	0.011
S2	10	0.032
S3	15	0.072
S4	20	0.101
S5	25	0.148

S6	30	0.187

Total Phenolic and Flavonoid content of M.oleifera leaf extract (mg/100g).

Table – 7

Parameter (unit)	Value
Total phenol (gallic acid equivalent) (mg/100 g)	13.2 ± 1.2
Total flavonoid (quercetin equivalent) (mg/100g)	3.1 ± 0.1

Values represent means + standard deviation of triplicate readings

Chemical composition of Moringa oleifera leaf extract

Table – 8

Component	Mg/g	%
Gallic acid	103.46± 0.01	10.34
Catechin	18.20 ± 0.02	1.82
Chlorogenic acid	60.31± 0.02	6.03
Ellagic acid	48.95± 0.02	4.89
Epicatechin	27.72± 0.01	2.72
Quercetin	134.61±0.01	13.46

Values represent means ± standard deviation of triplicate readings

Nutrient Analysis

The Nutrient Analysis of *Moringa oleifera* Leaves powder

Table-9

NUTRIENTS	VALUE
Moisture	128.93 g
Protein	62.92 g
Fat	6.55 g
Mineral	10.435g
Crude fibre	6.29 g
Carbohydrate	214.875g
Calcium	676 mg
Iron	12.815 mg

Mean score of the Sensory Evaluation of Formulated Soup

The mean score of the Formulated Moringa oleifera soup are shown in

Table -10

S.NO	Product	Appearance	Taste	Colour	Flavour	Texture	Overall Acceptabi -lity
1.	SS	4.9	4.75	4.75	4.8	4.85	4.85
2.	MOS	4.95	4.8	4.95	4.85	4.9	4.9

Keeping Quality of Moringa oleifera Leaves powder

The keeping Quality of Moringa oleifera leaves powder are shown in

Table -11

Days	Room temperature	Refrigerator
1-5	No change	No change
5-10	No change	No change
10-15	No change	No change
15-20	No change	No change
20-25	No change	No change
25-30	No change	No change

DISCUSSION

High antioxidant activity is reported from various medicinal plants**(Lechance et al.;2001)** Phenolics and flavonoids are the common antioxidants in plants **(Sankhalkar.,2014)** The present research work was carried out to know the phytochemical investigation and evaluation of Moringa oleifera based product. The study showed more phenolic content in leaves of Moringa oleifera. However flavonoid content was also more in Moringa oleifera leaves powder. Various reports also exist that indicate Moringa oleifera as a rich source of Phenolic compounds.**(Alhakmani et al.;2013).**Higher phenolic content in Moringa oleifera is also correlated with increased antioxidant activity **(Kostyuk et al.;2001).** In this study Good amount of total flavonoid and phenolic content was presented in methanolic crude extract of Gallic acid. Antioxidant activity of M.oleifera plant extract is correlated with the presence of flavonoid**(Bajpai et al.;2005).**phytochemicals are the chemical constituents in plants with distinct physiological action on the human body**(Vimala et al.,2013).** Alkaloids, flavonoids,phenolics, terpenoids, are some of the bioactive phytochemicals**(Anwar et al.;2006).**A numbe**r** of reports are available shows the presence of phytochemicals such as Quercetin, glycosides and tannins **(Nagaveni et al.;2007).** The chemical components of Moringa oleifera leaves extract analysis as presented in Table – 11 , The presence of phenolic compounds such as Gallic acid (103.46mg/g), Catechin (18.20mg/g), Chlorogenic acid (60.31mg/g), Ellagic acid (48.95mg/g), Epicatechin (27.72mg/g), Quercetin (134.61mg/g).The Formulated Moringa oleifera soup was selected by the panel members when comparing to standard soup.

In this discussion the *Moringa oleifera* contains numerous health benefits, more studies to investigate its potential to treat and manage diabetes using this products in a more extensive study could potentially revolutionize natural products in the treatment and management of diabetes.

CONCLUSION

Diabetes mellitus is becoming a leading cause of death globally in rural and urban areas. It has been used in traditional medicine treat diabetes and various diseases. It can be used as an anti-diabetic, cholesterol lowering, anti –inflammatory, analgesic, anti –oxidant, anticancer, and wound healing agent. The flowers, leaves, bark and seeds of this plant are shown to possess active compounds that can help combat the issue of many diseases condition and promote good health. Hence, more clinical trials should be carried out. Consequently, this will lead to its acceptance as a good therapy in the treatment and management of diabetes, thus potentially revolutionizing many products in the treatment and management of diabetes.

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