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Nutritional assessment of *Moringa oleifera* leaves

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Abstract

Moringa oleifera Lam (Moringaceae) is a very useful tree in tropical countries. In Folklore and Ayurvedic all parts of the tree used in different healing procedure for different diseases. The plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. Various article describes habitat, pharmacognostic features, phytochemistry, nutritive values and pharmacological activities like anticancer, antimicrobial, anti-inflammatory, anti-hyperlipidemic, hypotensive, anti-diabetic, hepatoprotective, anti-asthmatic, anthelmintic, anti-fertility etc. *Moringa oleifera* is the most-rich source of vitamin, protein and enzyme etc. This study aims to determine nutritional properties of *Moringa oleifera* leaves. The possible health benefits of these utilized leaves were explored by determining total protein content by Folin Lowry method. Vitamin-c content by titration method, alpha amylase content by DNSA (Dinitro salicylic acid). Lipase content by titration method. Protease inhibitor content by trypsinolytic assay. *Moringa oleifera* leaves were found to be the presence of high crude protein (733.32 mg/ml) and vitamin-c (297mg/100mg), the leaves also contain appreciable amount of lipase (0.36 milliequivalent) and alpha amylase (0.34% activity) and protease inhibitor (37.62 %).

Keywords: *Moringa oleifera* leaves, protein, protease inhibitor, vitamin C, alpha amylase, lipase

Introduction

Moringa oleifera is a widely cultivated tree considered as a multipurpose plant. It includes its use as functional food cleaning water material oil extraction for biofuel production, and other application. In a traditional way, it is used with medicinal purpose around the world due to empiric observation. (Torres-castillo JA *et al* 2013) [3]. *Moringa oleifera* is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, South Asia, South America and the Pacific and Caribbean Islands because *Moringa oleifera* has been naturalized in many tropic and subtropics region world-wide, the plant is referred to number of names such as Horseradish tree, drumsticks tree, ben oil tree, miracle tree, and "mothers best friend". (Julian Coppin 2008) [4]. Plants parts, including leaves, stem, roots, seeds and flowers have been reported as source of different biochemical compounds with anticarcinogenic, anti-inflammatory, antidiabetic, antioxidant and antimicrobial effects. (Fahey 2005, Anwar *et al.* 2006, Goyal *et al.* 2007, Chumark *et al.* 2008, Peixoto *et al.* 2011) [5]. *Moringa oleifera* Lam is used as a highly nutritive vegetable in many countries. Its young leaves, flowers seeds and tender pods are commonly consumed and they are having some medicinal properties. Traditionally its roots are applied as plaster to reduce the swelling and rheumatism. The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye disease, fever. According to World Health Organisation (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. (Andy I.E *et al.* 2008) [6]. The utilization of leaves of these plants vegetable is very less and mostly discarded or goes waste. The leaves are abundantly available. Hence in present study attempts have been made to determine enzyme, protein and vitamin.

Material and Method

Collection of plant leaves

The leaves of plant *Moringa oleifera* were collected from Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola Maharashtra. The leaves were separated manually and wash with distilled water.

Drying the plant leaves

The plant leaves were separated from each other manually. All separated leaves were air dried under shade for 1 week or longer till a constant weight was achieved. The care was taken to observe the fungal growth on wet parts of the leaves.

Grinding and Sieving of plant leaves

Once dried up to constant weight. The plant leaves were ground in a mixer grinder. Which were easily grinded into the powder form. Such powder was pass into through a test sieve. The remaining coarse powder was again grinded and sieved. The process was continued 4 to 5 time or till the material could not be ground further. The fine powder was immediately stored in an air tight container for further use.

Extraction of trypsin inhibitor

Inhibitor were extracted in six volumes of distilled water containing 1% polyvinylpyrrolidone (PVP) and kept frozen until needed. The suspension was centrifuged at 10,000g for 15minutes at 4 °C; the supernatant was used as a inhibitor source. Protein in the extracts was determined by Lowry's method (Lowry *et al.* 1951)^[8].

Trypsin inhibitory assay

Activity of trypsin inhibitor was assayed according to the procedure described by Kunitz with some modifications. In this method, the TCA soluble fraction formed by action of trypsin on the protein substrate Hammerstein casein was measured by the change in absorbance at 280nm. The residual caseinolytic activity of the trypsin in the presence of inhibitor, at 37 °C, was used as a measure of inhibitory activity; appropriate blanks for enzyme, inhibitor, and substrate were also included in the assay along with the test. The assay procedure included the following steps:

- One ml aliquot of trypsin was preincubated with 1 ml of suitable dilution of inhibitor at 37 °C for 15 minutes.
- To the above mixture 2ml of 1% Hammerstein casein was added and incubated at 37 °C for 30 minutes.
- The reaction was terminated by the addition of 2.5 ml of 0.44 M trichloro acetic acid (TCA) solution.
- The reaction mixture was transferred to centrifuge tube and the precipitated protein was removed by centrifugation, at 10,000 rpm for 15 minutes.
- The absorbance of the clear supernatant was measured at 280nm in UV-Visible spectrophotometer (Shirnadzu, Japan) against appropriate blanks. The TCA soluble peptide fractions of casein formed by the action of trypsin in the presence and absence of inhibitor was quantified.
- The trypsin activity of one unit was defined as the amount of enzyme that liberated 1 ug of tyrosine per milliliter of the reaction mixture per minute under the assay conditions.
- The inhibitor activity of one unit was defined as decrease by one unit of absorbance of TCA soluble casein hydrolysis product liberated by trypsin action at 280nm per minute at 37°C in the given assay volume.

The activity of trypsin inhibitory was expressed in terms of percent inhibition and it was calculated as

$$\text{Inhibitory activity (\%)} = \frac{\text{O.D of Control} - \text{O.D of sample}}{\text{O.D of Control}} \times 100$$

Estimation of protein

According to the method of Lowry *et al.*, (1951)^[8] protein content was determined by using Bovine Serum Albumin (BSA) as the standard and the concentration was expressed in milligram per millilitre (mg/ml).

Reagents:

- Solution I: A stock solution containing 2% (w/v) sodium carbonate (w/v) in 0.1 N sodium hydroxide in distilled water.
- Solution II: A stock solution of 0.5 % (w/v) cupric sulphate III distilled water.
- Solution III: A stock solution of 1% (w/v) sodium potassium tartarate in distilled water.
- Solution IV: Working reagent: To 100 ml of solution (I), 1 ml each of solution (II) and solution (III) was added and mixed well.
- Solution V: 1:1 Folin Ciocalteau's phenol reagent diluted with distilled water was prepared fresh just before use.

Formula

$$\text{Amount of Protein In mg/ml} = \frac{\text{O.D of Test}}{\text{O.D of Sample}} \times 20 \times 25$$

Extraction of Alpha amylase

5 gm powder and extensively defatted with hexane and acetone. The solvent was filtered off and powder was air dried. 5 gm of powdered was homogenised with 5 ml of 0.1 N HCL at 10,000 rpm for 10 minutes. The supernatant was removed; stored in cooling condition and used as a crude enzyme solution.

Measurement of amylase activity

1 mg per ml maltose was used for standard. The reaction mixture comprised of 2.5ml starch and 0.5ml enzyme solution. Immediately after the addition of enzyme solution. Added 0.5ml 2N NaOH to one tube to stop the reaction. This was used as zero time control. Rest of the tubes were incubated at 37°C for 15minutes, at the end of which the reaction was stopped by the addition of 0.5ml 2N NaOH. The concentration of reducing sugar in the reaction mixture was determined by dinitrosalicylic acid reagent. The enzyme activity of one unit was defined as the amount of enzyme that could yield 1µ mol of maltose per minute under these assay condition.

Formula

$$\% \text{ of Reaction} = \frac{\text{Test}}{\text{Control}} \times 100$$

Extraction of lipase

5 gm powder of *Moringa oleifera* leaves were homogenized in chilled acetone at 4°C. The suspension was centrifuge at 3000 rpm and the residue obtained was dissolved in 100 mL distilled water which followed by centrifugation at 7500 rpm. The supernatant was used as source of crude enzyme and was precipitated by ammonium sulphate (80% saturation) according to Michael *et al.* (2001). The precipitate was obtained by centrifugation at 10,000 rpm for 20 min. Precipitate was dissolved in 20 mL Tris-Cl buffer (10 mM, pH 8.5) and dialyzed overnight against the same buffer. The dialyzed enzyme then was used as partially purified enzyme and again used for enzyme characterization.

Lipase assay

The titrimetric method of was used for determination of lipase activity. The Olive oil emulsion was prepared in 180 mL distilled water containing 20 mL olive oil, 0.4g of sodium benzoate and 1g of gum-arabic. Assay mixture contained 5 mL olive oil emulsion, 5 mL 0.1M Tris buffer (pH 8) and 1 mL crude enzyme and incubated at 35°C for 10 min. The reaction was stopped by 10 mL of acetone and methanol mixture (1:1). Each sample was titrated against 0.025 N NaOH using 1% phenolphthalein as indicator. The volume of NaOH used in the titration was noted and then used for enzyme activity calculations. One unit of lipase was defined as the amount of enzyme which required to liberate 1 μ mol of free fatty acid from olive oil per min under the standard assay conditions.

Formula

The milliequivalent of acid split off per hour = (D – C) / 100 X Test

Extraction of vitamin-C

10 gm of *Moringa oleifera* leaves powder was added with 100ml distilled water further the powder was blended in a mortle and pestle. After blending strain the pulp through muslin cloth. And filtrate was use for extraction.

Titration of vitamin-C

1. Pipetted a 20 mL aliquot of the sample solution into a 250 mL conical flask and added about 150 mL of distilled water and 1 mL of starch indicator solution.
2. Titrated the sample with 0.005 mol L⁻¹ iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex.
3. Repeated the titration with further aliquots of sample solution until you obtain concordant results.

Formula

$$\text{Vitamin-c (mg/100g)} = \frac{0.5\text{mg}}{V_2 \text{ ml}} \times \frac{V_1\text{ml}}{5\text{ml}} \times \frac{100\text{ml} \times 10}{\text{wt. of the sample (g)}} \times 100$$

Where,

V1 = Volume of the dye consumed for standard ascorbic acid (ml)

V2 = Volume of dye consumed for the sample (ml)

X10 = Dilution factor

Result and Discussion

Moringa oleifera is an important food source in some parts of the world. Because it can be grown cheaply and easily, and the leaves shows lots of vitamins and minerals when dried. *Moringa oleifera* use in India and Africa in feeding programs to fight malnutrition (10). As an antioxidant it seems to help protect cell from damage (11).

Nutrient	Average \pm std deviation
Alpha amylase	0.34 \pm 0.008 % activity
Lipase	0.36 \pm 0.008 milliequivalent
Protein	733.32 \pm 0.05 mg/ml
Protease Inhibitor	37.62 \pm 0.009 %
Vitamin-C	297 \pm 0.81 mg/100mg

In the present study nutritional assessment of *Moringa oleifera* leaves were done with three independent replicates and the data are represented as mean standard deviation. Which is shows in table 1. Analysis showed that

(733.32 \pm 0.05 mg /ml) protein was present in *Moringa oleifera* leaves. The vitamin-c content was found to be (297 \pm 0.81mg/100mg). While 0.34 \pm 0.008% activity of alpha amylase was present and lipase (0.36 \pm 0.008milliequivalent). In medicine and biotechnology the protease inhibitors have the great demand. We found 37.62 \pm 0.009% protease inhibitor in *Moringa oleifera* leaves.

Conclusion

Moringa oleifera has been given a lot of attention as a nutrient source and has been studied more than many others plants. *Moringa oleifera* leaves could be utilized as a source of feed supplement to improve growth performance and the health status. More work is needed in this direction in order to make sustainable use of indigenouse tree of *Moringa oleifera*.

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