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**Amee Ravani**
College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

**RV Prasad**
College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

**Kedar Damle**
College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

**DC Joshi**
College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

**Correspondence**
**Amee Ravani**
College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

# Profiling of polyphenols, antioxidants potential, nutrients and flavor compounds of *Moringa oleifera* pods (var. PKM - 1)

**Amee Ravani, RV Prasad, Kedar Damle and DC Joshi**

**Abstract**
The aim of the present investigation is to study qualitative and quantitative values of polyphenols present in moringa pods. The antioxidant activity of methanolic extract of pods were evaluated by free radical scavenging activity using 1, 1-diphenyl-2-picryl-hydrazil (DPPH). The natural falvour compounds present in moringa pods were identified. The nutrients as well as fatty acids present in moringa pods were evaluated. It was found that moringa pods exhibited source of polyphenols, free radical scavenging activity and nutrients. Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, antiaging and anti-inflammatory properties for nutrition and healing.

**Keywords:** polyphenols, antioxidants, fatty acids, flavor compounds, *Moringa oleifera* pods

## Introduction
*Moringa oleifera* (Drumstick) is called miracle vegetable and is valued as medicinal and functional food. Moringa is an underutilized vegetable crop in India and almost all parts of the tree are edible. In literature, moringa is often called the *Power house of minerals* and *Mother's best friend*. Traditionally, besides being a daily used vegetable, it is also widely known for its health benefits. Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, antiaging and anti-inflammatory properties for nutrition and healing. Since 1998, the World Health Organization has promoted moringa as an alternative to imported food supplies to treat malnutrition (Johnson 2005) [13].
*Moringa oleifera* also consists of anti-inflammatory, anti-spasmodic, anti-hypertensive, anti-tumor, anti-oxidant, anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering, renal, anti-diabetic, and hepatoprotective activities. It has also long been labeled for its great cosmetic value in recent years. Several investigations were carried out to isolate, identify and characterize bioactive compounds from parts of the plant. Research has shown that biologically active components present in this plant contributing to its health benefits are compounds called glucosinolates and isothiocyanates, including 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate, niaziridin, niazirin as well as carotenoids (Fahey 2005; Shanker *et al*. 2007) [10, 18]. Searching for the herbs that have therapeutic potential for the prevention and scientifically proven to be useful as an alternative treatment a systematic approach is required. Thus the aim of the present study is to investigate the therapeutic values of *Moringa oleifera* pods.

## Materials and Methods
### Sample
Tender moringa pods (var. PKM-1) were procured from the horticultural farm of the University. The pods were sorted for diseased, damaged and broken pieces. The pods were washed thoroughly under tap water and used for experiments.

### Extraction
The edible portion of moringa pod was extracted. The pod pulp was dried using cabinet dryer at 50ºC. The powder was prepared using electric grinder. The powder samples were extracted with methanol using reflux extractor at 95 ºC for 5 h.

The methanol was evaporated using rotary vacuum evaporator (Heidolph, Germany). The percent yield of extract was estimated. The extracts were used for further analysis.

**Polyphenols screening**
The phytochemical screening of moringa pulp for the presence of steroids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, and cardio glycosides, quinone, terpenoid and coumarin, were performed by the standard methods (Ojiako 2014) [15]. Solvents used were ethanol, acetone, chloroform and petroleum ether.

**Preparation of extracts**
Extracts were obtained by following methods. About 15 g of pod pulp of moringa was mixed with 150 ml of ethanol (75 %), acetone, chloroform, petroleum ether and the aqueous samples were soaked overnight at room temperature. The samples were then filtered through Whatman no.1 paper in buchner funnel. The filtered solution was concentrated in a rotavator at 40°C, then the pulp extract was dissolved using different solvents and these samples were stored in a freezer below 10°C till further analysis.

**Test for saponins:** 2 ml distilled water was added to 1 ml of pulp extract in a test tube. The solution was shaked for 15 min. The formation of about 0.5 to 1 cm layer of stable mass of bubbles observed is the indication for the presence of saponin.

**Test for flavonoids:** 1 ml of 2N NaOH was added to 1 ml of pulp extract. Formation of yellow colour was taken as indication for the presence of flavonoids.

**Test for cardio glycosides (Keller killani test):** To 1 ml of pulp extract, 2 ml of glacial acetic acid and 0.5 ml of 5 % ferric chloride were added and then 1.5 ml of concentrated sulfuric acid was added and observed for the formation of brown colour.

**Test for terpenoids (Salkowski test):** 1 ml of chloroform was added to 1 ml of pulp extract and 1.5 ml of concentrated sulfuric acid was added to it. Formation of reddish brown colour indicates the presence of terpenoids.

**Test for phenols:** To 1 ml of pulp extract, 1 ml of sodium carbonate was added. To that 1 ml of folin was added. Formation of blue or green colour indicates the presence of phenols.

**Test for coumarins:** 1 ml of 10 % Sodium hydroxide was added to 1 ml of pulp extract and observed for the appearance of yellow colour.

**Test for steroids:** To 1 ml of pulp extract, 1 ml of chloroform was added and then 1.5 ml of concentrated sulfuric acid was added. The appearance of reddish brown colour at the inter phase indicates a positive reaction.

**Test for alkaloids:** To 1 ml of pulp extract, 1ml of concentrated sulfuric acid was added. To that 1 ml of mayer's reagent was added. The formation of green or white precipitate was regarded as positive for the presence of alkaloids.

**Identification of phytochemicals**
Moringa pod pulp characterization for phytochemicals carried out on Q-TOF.

**Preparation of extract:** Moringa pulp sample was dried using liquid nitrogen. 50 mg dried sample was dissolved in 3 ml of extraction buffer (Chloroform: Methanol: Water 1:1.2:1 v/v). The mixture was centrifuged at 9000 rpm for 15 minutes at 4ºC. The supernatant was collected and dried in liquid nitrogen. The reconstitute was dissolved in 1 ml solvent (methanol: water 1:1 v/v) and was stored at -20 ºC until use.

**Total phenolics**
Total phenolics were estimated as per the methods described in Singleton *et al*. (1999) [20]. Total phenolics in the extracts were quantified using Folin-Ciocalteu reagent and expressed as gallic acid equivalent. Extract (0.5 ml) was mixed with Folin-Ciocalteu reagent (0.5 ml) and distilled water (7.5 ml). The resulting mixture was incubated at room temperature for 10 min. Thereafter, sodium carbonate solution (1.5 ml, 20 %) was added to the mixture and sample was further incubated in a water bath for 20 min at 40 ºC. The absorbance of the solution was measured using a UV-visible spectrophotometer at 755 nm. A calibration curve was prepared by plotting concentration vs absorbance using gallic acid as standard. Total phenolics in the extract samples were quantified from the calibration curve and were expressed as gallic acid equivalent (GAE) per g of dry extract.

**Total antioxidant capacity**
The antioxidant activity of the moringa extracts was determined using the DPPH free radical scavenging method with some modifications (Brand Williams *et al*. 1995) [6]. DPPH solution (0.1 mM litre-1) was prepared in methanol. Working concentration of the blank and extracts were prepared using appropriate dilutions. Freshly prepared DPPH solution (1 ml) was added to blank and extracts solution (3 ml). The mixture was incubated in the dark for 30 min and thereafter, absorbance was recorded at 517 nm against the blank. For control, DPPH solution (1 ml) was mixed with methanol (3 ml) for measuring the absorbance value. The decrease in absorbance of DPPH solution on addition of test samples in relation to the control was used to calculate the antioxidant activity in terms of percentage inhibition of DPPH radical. The capability of scavenging DPPH radical (% RSA) was calculated using the following equation as described by Lee *et al*. (2003) [14].

$$\text{Radical scavenging acticity (\%)} = \frac{(\text{A control} - \text{A test})}{\text{A control}} \times 100$$

The $IC_{50}$ values of the standard antioxidants as well as the plant extracts corresponded to concentration inhibiting fifty percent of initial concentration of DPPH values were obtained from the graph.

**Chemical composition**
Composition of pods were analyzed as per AOAC (2012) [3] and other standard methods for moisture, protein, fat, crude fiber, ash, carbohydrate, β-carotene, vitamin C, calcium, phosphorous, iron

**Identification of fatty acids**
Fatty acids were analyzed using a GC-QTOF MS (Agilent Technology, USA). The compounds were separated on 30 m

× 0.25 mm × 0.25 µm HP-5MS column (Perkin Elmer, USA). The column temperature was increased from 70 ºC to 280 ºC at a rate of 5 ºC/min in a total run time of 60 min and the conditions were injector temperature, 250 ºC; injection volume, 0.5 µl; transfer temperature, 280 ºC. MS parameters were: EI mode, ionization voltage 70 eV, ion source temperature, 250 ºC; scan range, 50-800 Da. The peaks were tentatively identified using NIST library.

**Identification of flavor compounds**

GCMSHS analysis was carried out using Perkin-Elmer Turbo Matrix 40 headspace analyzer, (lans 680 Perkin Elmer gas Chromatograph) coupled to a mass detector (claws SQ8C). Elite 5 MS, 30 m * 0.25 mm * 0.25 µm capillary column was used. The headspace conditions were: oven temperature 90ºC, needle temperature 100 ºC and transfer line temperature 110ºC. Thermostat time was 30 min, pressure time was 3 min, inject time was 0.1 min and withdrawal time was 0.3 min. In GC Injection, temperature was maintained at 250 ºC, helium flow rate was 1.0 ml/ min and ion source temperature was at 230 ºC. Injection was performed in split mode with ratio 5:1. The oven was set to initial temperature of 45 ºC and maintained at 5 min. The temperature was raised to 170 ºC at the rate of 3 ºC/ min and then to 250 ºC with the ratio of 7 ºC/min and maintained at 2 min.

## Results and Discussion

**Qualitative tests for phytochemicals in moringa pod pulp**

Qualitative tests for phytochemicals were performed as described in previous section. The present study revealed that moringa showed the presence of phytochemicals namely flavonoids, phenolic compounds, cardio glycosides, terpenoids, steroid and coumarin in different solvent extracts as shown in Table 1. The extract obtained by aqueous methanol showed the highest quantity of phytochemicals compared to other solvents. Solvent polarity played a key role in increasing phenolic solubility. This suggests that choice of solvent will definitely influences the extraction of phenolics/ flavonoids content and subsequently antioxidant activity. In the present investigation, tannins and quinine were not observed in moringa pod. Similar observations were recorded by Sharma and Paliwal (2013) [19], where, phytochemical screening of the sequential extraction of moringa pods showed the presence of various bioactive components of which phenolics, saponins, steroids, alkaloids, flavonols, proanthocyanidins, terpenoids, tannin, and cardiac glycosides.

**Table 1:** Qualitative tests for phytochemicals in moringa pod

| Type of solvent | Saponins | Flavonoids | Cardiac Glycosides | Terpenoids | Phenols | Coumarins | Steroids | Alkaloids |
|---|---|---|---|---|---|---|---|---|
| Aqueous extract | ND | ++ | ND | + | + | + | ND | ND |
| Methanol | ND | +++ | + | ++ | +++ | ++ | +++ | ++ |
| Acetone | ND | ++ | + | +++ | ND | +++ | ND | ND |
| Petroleum ether | ++ | ND | + | ND | ND | ND | ND | ND |
| Chloroform | ++ | +++ | ND | ND | ++ | +++ | + | + |

− Absent; + Weak; ++ Moderate; +++ Strong; ND: not detected

**Quantitative estimation of phytochemicals and antioxidant properties of moringa pod**

The moringa pod pulp was dried and extracted in methanol. The extract yield, total phenol, flavonoids and antioxidant potential of moringa pod pulp is shown in Table 2. Results revealed that pulp yields 33.09 %. The extract of moringa pod pulp contained high level of phenolics which was 11.02 mg GAE/g and 3.42 mg QAE/g of flavonoid compounds. Phenolic compounds are important plant antioxidants which exhibit considerable scavenging activity against free radicals. Scavenging activity of free radicals by DPPH activity has been widely used to evaluate the antioxidant activity of natural products from plant and natural sources. The $IC_{50}$ value of moringa pod pulp was 228.62 µg/ml. Moringa pod pulp with the low $IC_{50}$ proves high antioxidant potential. Raghavendra *et al*. (2015) [16] reported the $IC_{50}$ values for DPPH radical scavenging activity was 240µg/ml for moringa pod. Free radicals have a broad range of effects in biological systems. It has been proved that these mechanisms may be important in controlling certain diseases and ageing.

**Table 2:** Moringa yield, Total phenolics, DPPH and $IC_{50}$ activity moinga pod

| Parameters | Values |
|---|---|
| Moringa extract yield (%) | 33.09 ± 0.44 |
| Total phenols (mg GAE/g extract) | 11.02 ± 0.45 |
| Total Flavonoids (mg QAE/g extract) | 3.42 ± 0.12 |
| DPPH radical inhibition (%) | 45.10 ± 0.51 |
| $IC_{50}$ (µg/ml) | 228.62 ± 0.49 |

**Identification of phytochemicals in moringa pulp**

The pod pulp of moringa was analyzed to identify phytochemicals present as described previous section. The list of identified phytochemicals in moringa pod pulp is tabulated in Table 3. The LC-MS chromatogram showing separation of phenolics and flavonoids of moringa pod pulp is depicted in Figure 1. It can be noticed that moringa pulp is a rich source of phenolic and flavonoid compounds.

It was found that moringa contain flavonoids, phenolic compounds, steroid conjugates, glucoside, alkaloids and steroid saponins. The identified compounds of moringa pod pulp are caohuoside D, ambofuracin, evasterioside D, (+)-syringaresinol O-beta-D-glucoside, trypanothione disulfide, (+)-mayurone, kaempferol 3-lathyroside-7-rhamnoside, mesquitol-4beta-ol 3,8-dimethyl ether, paeonoside, his-tyrme-oh, avenanthramide 1s, 4-(hydroxymethyl) benzenediazonium (1+), convallasaponin A and phenylgalactoside. Similar compounds were also identified by Anwar *et al*. (2007) [2], Bennett *et al.* (2003) [4], Cheenpracha *et al*. (2010) [7], Faizi *et al*. (1994) [9], Faizi *et al*. (1995) [8] which confirms the presence of flavonoids, phenolic compounds, steroid conjugates, glucoside, alkaloids and steroid saponins.

**Table 3:** Phenolic acids and flavonoids in moringa pod pulp

| Name | Formula | Mass | RT | Area | Height | Nature of the compound |
|---|---|---|---|---|---|---|
| Caohuoside D | $C_{28}H_{34}O_{12}$ | 562.207 | 11.061 | 74174 | 11436 | Flavones and Flavonols |
| Ambofuracin | $C_{27}H_{32}O_{12}$ | 548.1916 | 10.668 | 80639 | 14339 | Flavonoids |
| Evasterioside D | $C_{33}H_{58}O_{10}$ | 614.402 | 13.815 | 112219 | 14479 | Steroid conjugates |
| (+)-Syringaresinol O-beta-D-glucoside | $C_{28}H_{36}O_{13}$ | 580.2137 | 11.666 | 106258 | 22181 | ß-D-glucoside |
| Trypanothione disulfide | $C_{27}H_{47}N_9O_{10}S_2$ | 721.287 | 13.379 | 50780 | 6143 | organic disulfide |
| Theobromine | $C_7H_8N_4O_2$ | 180.065 | 1.828 | 25960 | 5649 | naturally occurring alkaloid metabolite of caffeine |
| (+)-Mayurone | $C_{14}H_{22}O$ | 206.1672 | 6.735 | 42642 | 5488 | C14-sesquiterpene ketone |

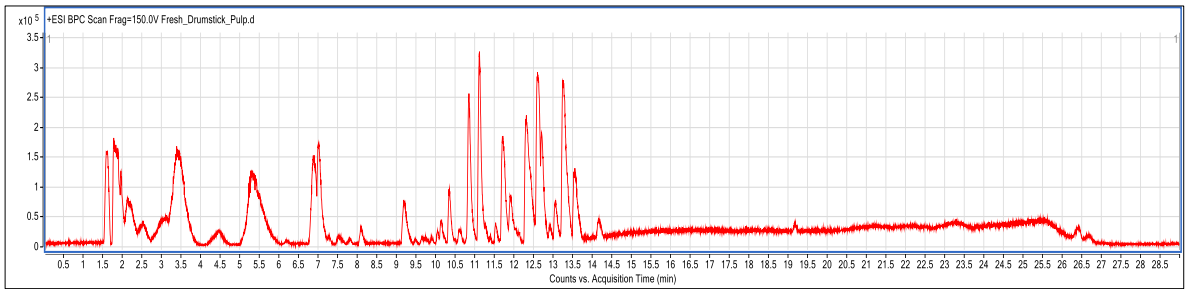


**Fig 1:** LC-MS chromatogram showing separation of phenolics and flavonoids of moringa pod

## Chemical characterization of moringa pod pulp

The data obtained on the chemical composition of edible portion of pod i.e. moisture, protein, fat, carbohydrates, crude fiber, ash, iron, calcium, phosphorous, beta-carotene, ascorbic acid, oxalates are presented in Table 4. Experiments were carried out in triplicate and the average values were reported. Moringa pulp had moisture content of 85.60 % (wb). The fat and ash were 1.39 and 1.74 %, respectively. Protein, crude fiber and carbohydrate were found to be moderately high i.e. 4.03, 2.31 and 4.92 %, respectively. The micronutrients in the moringa pulp, beta carotene (119.95 µg /100 g) and vitamin C (118 mg/100 g) were found to be good amount and minerals such as Ca, P and Fe were also present in considerable amounts. Moringa pulp contained anti nutritional component oxalates in moderate amount i.e. 11.28 mg/100 g.

Present results are in good accordance with the results reported by Gopalan *et al*. (2004) [12]. The chemical composition per 100 g of edible portion of moringa pod reported was 86.9 g moisture, 2.5 g protein, 1.0 g fat, 2.0 g minerals, 4.8 g fiber, 3.7 g carbohydrates, 30 mg calcium, 110 mg phosphorous, 110 µg carotene and 120 mg vitamin C. The marginal differences in the composition of moringa may be due to variety, environmental, geographical and cultivation practices.

**Table 4:** Chemical composition of moringa pod (per 100 g of edible portion)

| Parameter | Mean values |
|---|---|
| Moisture (%) | 85.60 ± 1.06 |
| Protein (%) | 4.03 ± 0.57 |
| Fat (%) | 1.39 ± 0.66 |
| Ash (%) | 1.74 ± 0.11 |
| Crude fiber (%) | 2.31 ± 0.57 |
| Carbohydrate, by difference (%) | 4.93 ± 1.60 |
| β-Carotene (µg/ 100g) | 119.95 ± 1.62 |
| Vitamin C (mg/ 100g) | 118.00 ± 6.24 |
| Calcium (mg/ 100g) | 37.96 ± 2.46 |
| Phosphorous(mg/ 100g) | 136.16 ± 1.61 |
| Iron (mg/ 100g) | 3.25 ± 0.62 |
| Oxalates (mg/ 100g) | 11.28 ± 0.69 |

## Identification of fatty acids in moringa pulp

The moringa pulp when analyzed for fatty acid profile by GCMSQTOF found fifteen major components. The retention time varied from 18.248 to 37.096. Compounds hexadecanol, dodecanoic acid, glycidyl palmitate, glycidyl oleate 1, 2, 3, 4-Tetrahydro-1-naphthylamine, N-heptafluorobutyryl- etc have been identified. Similar compounds were identified by Godinez-Oviedo *et al*. (2016) [11] from seeds of moringa pods. The lipid content of moringa seeds consists of saturated palmitic, stearic, arachidic and behenic fatty acids, while oleic acid was the major unsaturated fatty acid (Abdulkarim *et al*. 2005; Rashid *et al*. 2008) [1, 7]. The high level of oleic acid in moringa seed oil enhances its nutritional value. Other lipids found in the moringa seed oils were "tocopherol, sitosterol and stigmasterol". The major flavor compounds identified at different RT are; D-limonene (14.26), alpha.-pinene (18.575), 3-(prop-2-enyl)-1, 3-diaminopropane (18.756) and 2, 4-dihydroxy-2, 5-dimethyl-3(2h)-furan-3-one (21.262). Bhattacharya *et al*. (2014) [5] identified similar compounds through GC-MS profiling of ethanolic extract of *Moringa oleifera* leaf.

## Conclusion

From the comprehensive study of it was found that moringa pods are rich source of polyphenols, antioxidants potential as well as nutrients. Aqueous methanol extract showed better retention of polyphenols as well as antioxidant activity. Moringa pod is a rich source of micronutrients as well as natural flavour compounds. Traditionally moringa pods are utilized in curries, *Dals*, *Sambhar*, soups as flavoring and thickening agent. These data indicated the potential of pods of selected cultivar of moringa to be use as valuable source of polyphenols for development of functional food and nutraceuticals. Looking to the health prospect of the moringa pulp its market demand, there is a great scope for the value addition of moringa pulp in terms of development of value added products.

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