

NUTRITIONAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF BITTER GOURD (*MOMORDICA CHARANTIA*) FROM PAKISTAN

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Summary

Recently antioxidants and secondary metabolites have attracted a great deal of attention for their effect in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases. *Momordica charantia* (*Cucurbitaceae*), also known as “Karela”, is a wild variety of bitter gourd in Pakistan. It is commonly consumed as vegetable and also used as a popular folk medicine. In this study, the nutrients and antioxidant of bitter gourd’s seed, peel and flakes were evaluated. The results from nutritional analysis showed that it is good source of micronutrients (ash), and macronutrients (carbohydrate, protein, fiber contents). The results obtained from the DPPH assay and reducing power activity indicated that bitter gourd extracts exhibits potent antioxidant activity. Bitter gourd’s flakes extract possess potent free radical scavenging activities (63.20%) followed by seed (33.05%), DPPH (%Inhibition) at 2mg/mL concentration, which was compared with standard antioxidant BHT. These antioxidant activities could have contributed, at least partly, to the therapeutic benefits of the certain traditional claims of bitter gourd.

Key words: Bitter gourd, nutritional analysis, DPPH, RPA.

Introduction

The increasing populations of the world food demands have overwhelmed the available land resources. Along with other food alternatives, vegetables are considered the cheap source of energy. Vegetables are very rich sources of essential biochemicals and nutrients such as carbohydrates, carotene, protein, vitamins, calcium, iron, ascorbic acid and palpable concentration of trace minerals. These vegetables will continue to remain the basic source of energy for the developing countries ⁽¹⁾.

Bitter gourd (*Momordica charantia*) is one of the most popular vegetables in Southeast Asia. It is a member of the cucurbit family along with cucumber, squash, watermelon, and muskmelon. Native to China or India and Pakistan, the fast-growing vine is grown throughout Asia and is becoming popular worldwide.

It is regarded as one of the world's major vegetable crops and has great economic importance. Depending on location, bitter gourd is also known as bitter melon, Karela, or balsam pear ⁽²⁾.

The immature fruits and tender vine tips are used in a variety of culinary preparations. It has a number of biological activities like: anthelmintic, antibacterial, antibiotic, anti-diabetic, anti-inflammatory, anti-leukemic, antimicrobial, anti-mutagenic, anti-mycobacterial, antioxidant, anti-tumor, anti-ulcer, antiviral, aperitive, aphrodisiac, astringent, carminative, cytostatic, cytotoxic, depurative, hormonal, hypocholesterolemic, hypertensive, hypotriglyceridemic, hypoglycemic, immunostimulant, insecticidal, lactagogue, laxative, purgative, refrigerant, stomachic, styptic, tonic and vermifuge etc.

The main constituents of bitter melon which are responsible for these effects are such as triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds. The protein in bitter melon including protein MAP-30, alpha-momorcharin, and beta-momorcharin were shown to have the ability for fighting against HIV. A steroid, charantin, contained mainly in the aerial parts, has been proven for its anti-diabetic activity. The phenolic compounds from bitter melon extracted by solvent extraction were reported to exhibit antioxidant activity ⁽³⁾.

The reactive oxygen species produced in cells include hydrogen peroxide (H₂O₂), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (\cdot OH) and the superoxide anion (O₂⁻). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules ⁽⁴⁾. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein degradation ^(5, 6). Oxidative stress thought to contribute to the development of a wide range of diseases including Alzheimer's disease ⁽⁷⁾, Parkinson's disease ⁽⁸⁾, the pathologies caused by diabetes, rheumatoid arthritis, and neurodegeneration in motor neuron diseases. The antioxidants and secondary metabolites have attracted a great deal of attention for their effect in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases ⁽⁹⁾. Moreover recent investigations have shown that the antioxidants with free-radical scavenging properties of plant origins could have great importance as therapeutic agents in aging process and free radical mediated diseases ⁽¹⁰⁾.

Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant ⁽¹¹⁾. In view of its wide use and its chemical composition, the nutritional evaluation and free radical scavenging activity of bitter gourd's seed, peel and flakes was determined.

Materials and Methods

Plant material

Fresh fruits of *Momordica charantia* were procured from the market. Authentication of the plant was carried out by Botanist and voucher specimens of the plants have been retained in the department herbarium.

Chemicals

2,2'-diphenylpicryl-1-hydrazyl (DPPH) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals like potassium hexacyanoferrate, trichloroacetic acid, ferric chloride, phosphate buffer, sodium hydroxide, BHT and other solvents were procured from local market and were of analytical grade.

Sample preparation

The fruits were sliced into two halves and the seeds were selectively collected manually, washed with fresh water and dried at 60°C in cabinet dryer their recovery of yield were measured. The dried seeds, peel and flakes were grounded into fine powder by an electrical mill and mesh (mesh number 50). The powdered were kept in airtight containers at 4°C until the time of further use. For antioxidant activity the extracts of bitter gourd seeds, peel and flakes were prepared by dissolving a known amount of powders in distilled water using sonicator. It was then filtered under reduced pressure.

Nutritional analysis

The nutritional analysis of bitter gourd's seeds, peel and flakes were carried out for moisture content, total ash, crude fat, crude fiber and crude protein according to the method of AOAC⁽¹²⁾.

Free radical scavenging activity

The free radical scavenging activity of bitter gourd seeds, peel and flakes were determined by the DPPH assay described by Brand-William *et al.*,⁽¹³⁾. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.004% solution of DPPH in methanol was prepared, and 3 ml of this solution was added to 100 µl of sample solution in methanol at different concentration 0.5-2.0 mg/ml. 30 minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenging Effect (\% Inhibition)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

Where

A_{blank} is the absorbance of DPPH radical + methanol

A_{sample} is the absorbance of DPPH radical + sample extract/ standard.

The purple colored DPPH is a stable free radical, which is reduced to 2', 2'-diphenyl-1-picrylhydrazine (yellow colored) by reacting with an antioxidant. The decrease in absorbance at 517 nm was being recorded in a digital spectrophotometer.

Total reduction capability by Fe³⁺ to Fe²⁺ transformation

The Fe³⁺ reducing power of the extract was determined by the method of Oyaizu⁽¹⁴⁾. The extract (0.75mL) at various concentrations was mixed with 0.75mL of phosphate buffer (0.2 M, pH 6.6) and 0.75mL of potassium hexacyanoferrate [K₃Fe(CN)₆] (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5mL of the supernatant was mixed with 1.5mL of distilled water and 0.1mL of ferric chloride (FeCl₃) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured using a UV-Vis spectrophotometer (Nicolet, Evlution-300, Germany) as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis

All the grouped data were statistically evaluated with SPSS/ 7.5 software. All the results were expressed as mean ± SD.

Results and Discussion

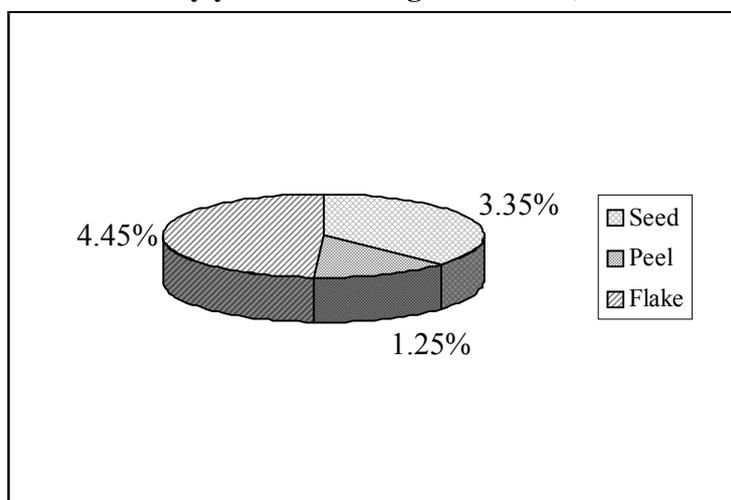
Bitter gourd is a powerful nutrient-dense plant composed of a complex array of beneficial compounds. These include bioactive chemicals, vitamins, minerals and antioxidants which all contribute to its remarkable versatility in treating a wide range of illnesses. The fruits contain high amounts of vitamin C, vitamin A, vitamin E, vitamins B1, B2 and B3, as well as vitamin B9 (folate). The fruit is also rich in minerals including potassium, calcium, zinc, magnesium, phosphorus and iron, and is a good source of dietary fiber⁽¹⁵⁾. Researchers have found that bitter gourd is full of antioxidants such as carotenoids, including alpha and beta-carotene, lycopene and zeaxanthin⁽¹⁶⁾.

Recovery of yield

The recovery of yield of bitter gourd seeds, peel and flakes were measured. It was found that the recovery of yield of flakes were higher than seed followed by recovery yield of peel as depicted in Figure 1. Other 90.95% was moisture in bitter gourd.

Nutritional analysis

Proximate and nutrient analysis of edible fruit and vegetables plays a crucial role in assessing their nutritional significance⁽¹⁷⁾. The considerable use of vegetable species by the local people in their diet motivated us to carry out the present proximate and nutrient analysis.

Figure 1: Recovery yield of Bitter gourd's Peel, Seed and Flakes

The result of nutritional analysis shows variant concentration/proportions of biochemical and other contents. The moisture contents of each part were different. Considering the overall percentage of moisture composition, it was highest in flakes followed by peel and seed (Table 1). Considering the results obtained from carbohydrate flakes and seed had prominent levels compared to peel. From the results established after fat analysis, seed had significant level of fat content while the flakes and peel had lesser values. While analyzing the protein contents in the three part of bitter gourd, the results showed that flakes and peel had highest concentration of protein as compared to seed. Considering the resulted achieved from fiber analysis, it was high in seed than peel and flakes. According to the results revealed, Seed had highest and significant level of energy values (Figure 2) than flakes and peel. All the results of proximate analysis were comparable as described by Hussain *et al.*,⁽¹⁸⁾.

Table 1: Nutritional Evaluation of Bitter gourd's Peel, Seed and Flakes

| Sr # | Parameters | Peel (%) | Seed (%) | Flakes (%) |
|------|--------------------|--------------|--------------|----------------|
| 1 | Moisture | 4.15 ± 0.9 | 4.09 ± 0.8 | 4.72 ± 1.1 |
| 2 | Ash | 14.99 ± 1.8 | 4.56 ± 0.9 | 6.43 ± 1.4 |
| 3 | Fat | 0.18 ± 0.2 | 5.24 ± 1.2 | 0.25 ± 0.2 |
| 4 | Fiber | 17.77 ± 1.8 | 22.46 ± 2.3 | 17.08 ± 1.9 |
| 5 | Protein | 20.37 ± 1.9 | 19.01 ± 1.8 | 20.66 ± 2.0 |
| 6 | Carbohydrates | 42.54 ± 2.7 | 44.64 ± 2.8 | 50.86.54 ± 2.9 |
| 7 | Energy (Kcal/100g) | 253.26 ± 4.3 | 301.76 ± 4.9 | 283.33 ± 4.5 |

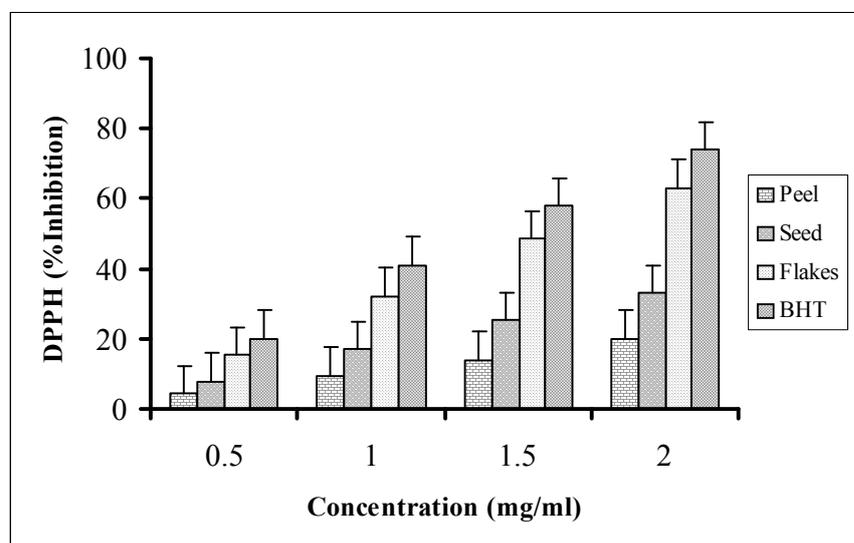
Data represented as ± SD (on dry basis)

DPPH• radical scavenging activity

Compelling evidence indicates that increased consumption of dietary antioxidants or fruits and vegetables with antioxidant properties may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging. The stable free radical DPPH method is an easy, rapid, and sensitive way to survey the antioxidant activity of a specific compound or plant extracts⁽⁵⁾. Antioxidants react with DPPH•, which is a stable free radical, and convert it to 2,2-diphenyl-1-picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant⁽¹⁹⁾.

In this study, antioxidant activities of bitter gourd seeds, peel and flakes exhibited marked DPPH free radical scavenging activity in a concentration-dependent manner. Figure 2 illustrates a significant decrease ($p < 0.05$) in the concentration of DPPH radical due to the scavenging ability of bitter gourd seeds, peel, flakes and standards. The scavenging effect of bitter gourd's flakes, seeds, peel and standard on the DPPH radical decreased in that order: BHT > bitter gourd flakes > seed > peel, which were 74.15, 63.20, 33.05 and 20.10%, at the 2mg/mL concentration, respectively. Many researchers have reported positive correlation between free radical scavenging activity and total phenolic compound^(20, 21). The DPPH scavenging ability of these extracts may be attributed to its hydrogen donating ability.

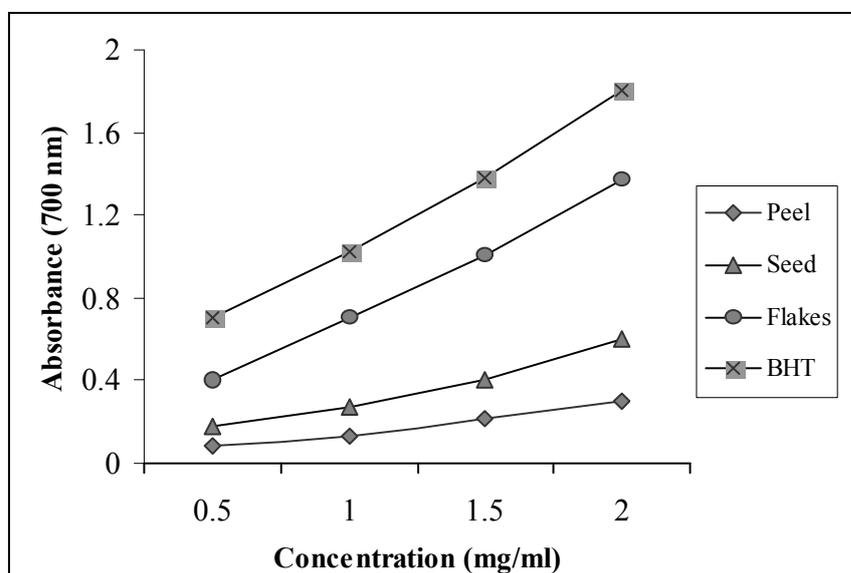
Figure 2: DPPH (%Inhibition) of aqueous extract of bitter gourd's peel, seed, flakes and standard BHT

**Reducing power**

The reducing capacity of compound Fe^{3+} /ferrocyanide complex to the ferrous form may serve as significant indicator of its antioxidant capacity and it is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action⁽²²⁾. The reducing power was determined according to the method of Oyaizu⁽¹⁴⁾.

In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in the reductive ability. Figure 4 shows the dose response curves for the reducing power of the extracts from *M. charantia*. The extracts exhibited a good reducing power at 1.5 and 2.0 mg ml^{-1} that was comparable with BHT, it was evident that the extracts showed reductive potential and could serve as electron donor, terminating the radical chain reaction. It is believed that antioxidant activity and reducing power are related as reductones inhibit LPO by donating a hydrogen atom and thereby terminating the free radical chain reaction⁽²³⁾.

Figure 3: Reducing power activity of aqueous extract of bitter gourd's peel, seed, flakes and standard BHT



Conclusion

The present investigation was undertaken to assess the nutritional evaluation and antioxidant activity of an aqueous extract of *Momordica charantia* seeds, peel and flakes. The results from nutritional analysis showed that it is good source of micronutrients (ash), and macronutrients (carbohydrate, protein, fiber contents). The results obtained from the DPPH assay and reducing power activity indicated that bitter gourd extracts exhibits potent antioxidant activity. The findings of the present study suggest that bitter gourd extracts could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. However the further studies are necessary to establish this activity in vivo and to identify the agents responsible for free antioxidant activity.

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