



Polyphenolic content, antioxidant activity and organoleptic evaluation of bitter gourd (*Momordica charantia*) L. Tea

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ABSTRACT

Bitter gourd or Karela *Momordica charantia* belongs to the family *cucurbitaceae*. It is high in vitamins and an excellent source of iron and calcium. It is also known to cure diabetes, arthritis, rheumatism, asthma, warts, abscesses and ulcers. In the present study bitter gourd tea was developed and its sensory evaluation was conducted. The bitter gourd tea was screened for possible antioxidant activities by free radical scavenging activity (DPPH) and reducing power assay. The total phenolic content was also determined. The results of the investigations on appearance, color, flavor, taste and overall acceptability values ranged from 6.1 to 8.5. The amount of total phenolic contents found in bitter gourd tea extract was (264 ± 1.63) mg/g gallic acid equivalent (GAE). The bitter gourd tea exhibited antioxidant activity in the range from $18.35 \pm 0.80 - 65.96\% \pm 2.50$ at the concentrations (20-100 μ g/ml) in DPPH radical scavenging method. A concentration dependent reducing potential were exhibited by the bitter gourd tea which was compared with BHA and it was found that its antioxidant activity was higher than standard antioxidant BHA. The bitter gourd tea extract and standard BHA showed antioxidant activity with IC₅₀ value of $72.04 \pm 3.25 \mu$ g/ml and $98.75 \pm 3.92 \mu$ g/ml, respectively. These results indicated that bitter gourd tea may be used as a potential source of natural antioxidant.

Keywords: Polyphenolic content, DPPH, RPA, bitter gourd tea, sensory evaluation.

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INTRODUCTION

Recently, there have been great efforts to find safe and potent natural antioxidants from various plant sources. Numerous crude extracts and pure natural compounds from fruits were reported to have antioxidant and radical-scavenging activities¹. Within the antioxidant compounds, phenolics or polyphenols, including flavonoids have received considerable attention because of their physiological functions such as antioxidant, which may retard or inhibit the oxidation possibly by reactive radicals including ROS in a biological system². Antioxidants have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis and aging process³. Hence, regular consumption of fruits and vegetables is associated with reduced risks of these chronic diseases⁴. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring anti-oxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury⁵. *Momordica charantia* L. or Karala (in Indo-Pakistan) (*cucurbitaceae*) commonly known as 'bitter gourd' is a multi purpose herb cultivated in different parts of the world for its edible fruits⁶. Bitter gourd has also been frequently used as a medicinal herb in Asia, Africa, and South America because of its antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, antimycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties⁷⁻¹⁰. isolated and characterized a novel antihyperglycemic protein from the fruits of *Momordica cymbalaria*.¹¹ found that extracts of wild bitter melon grown in Taiwan, possessed potent antioxidant and free radical-scavenging activities. *Momordica charantia* attenuates high-fat diet-associated oxidative stress and neuro-inflammation¹². Recently¹³ isolated components from Bitter gourd's seeds, conferring its antimicrobial activity. However, there has been little information regarding the development of product from bitter gourd and its pharmacological activities. Therefore, the purpose of present study was to develop the antidiabetic tea from bitter gourd and to evaluate polyphenolic content and antioxidant properties of bitter gourd tea by using two different methods.

MATERIALS AND METHODS

The green tea, bitter gourd and cinnamon used in this study were purchased from local market. The Folin-Ciocalteu reagent and 2,2'-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide [K₃Fe(CN₆)] were obtained from Sigma Chemical Co. (St. Louis, MO, USA), while tri-chloroacetic acid (TCA), ferric chloride (FeCl₃), BHA, sodium carbonate (Na₂CO₃), phosphate buffer and other solvents were procured from Across Chemical and were of analytical grade.

Bitter gourd tea extraction

Bitter gourd (edible portion without seeds) was used. The bitter gourd tea was developed and its extract was prepared by infusing 1 g of tea powder with 100 mL of boiled distilled water. This was allowed to steep for 5 minutes with stirring occurring every minute. The brew was then filtered through filter paper to remove particulate matter and 10 mL of the infusion was placed in a clean vial and labeled for further study.

Total Phenolic Contents

The total content of polyphenols was determined by Folin-Ciocalteu assay¹⁴. Sample of tea solution (200µL) was taken, 1mL of Folin-Ciocalteu reagent and 0.8 mL sodium carbonate (7.5%) reagent were added. The mixture was incubated in the dark for 30 min and the absorbance was measured at 760 nm. Gallic acid was used as the standard for the calibration curve. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 µg/mL. Results are expressed as mg of gallic acid equivalents/g (mg GAE/g)¹⁵.

Antioxidant activity

Antioxidant properties of bitter gourd tea were evaluated by two different methods:

- (i) DPPH assay
- (ii) Reducing power assay

DPPH scavenging assay

The antioxidant activities of the aqueous extracts were measured on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical following the method described by ¹⁶. Aqueous extract (0.1 ml) was added to 3 ml of 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated.

$$\% \text{ DPPH radical-scavenging} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / (Abs_{\text{control}})] \times 100$$

Then % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was calculated. A lower IC₅₀ value indicates greater antioxidant activity ¹⁷.

Reducing power assay

The reducing power of tea extracts was determined according to ¹⁸ with slight modifications. Tea extract (0.5 mL) was mixed with phosphate buffer (1.25 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1.25 mL, 1%) and the mixture was incubated at 50°C in a water bath for 20 min. The sample was then cooled and mixed with 10% TCA (1.25 mL). Afterwards, sample aliquot (1.25 mL) was mixed with distilled water (1.25 mL) and 0.1% ferric chloride (0.25 mL) and then left to react at room temperature for 10 min. Sample absorbance was read at 700 nm. An increase in the absorbance of the reaction mixture indicated an increase in the reducing power ¹⁹.

Organoleptic evaluation

The sensory evaluation of bitter gourd tea was conducted at Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore. The consent was obtained from judges to participate in experiment. The mean score values of two evaluations were reported. The bitter gourd tea samples were rated on a 10 point scale (1, 2: bad; 3, 4: poor; 5, 6: fair; 7, 8: good and 9, 10: excellent. The bitter gourd tea placed randomly in codified cups with three-digit code, were served to each panelist. Judges were placed in different places to avoid communication during the evaluation and asked to score bitter gourd tea for taste, appearance, color, flavor and overall acceptability ²⁰⁻²¹.

Statistics

The data were presented as mean \pm standard deviation of three determinations. Multiple comparisons of means were analyzed by least significant difference. All computations were done by employing the statistical software (SPSS, Version 11.0).

RESULTS AND DISCUSSION

Polyphenolic Content

The Folin-Ciocalteu assay is one of the oldest methods developed to determine the content of total phenols²². In this work the infusion of bitter gourd tea was measured spectrophotometrically in order to be able to evaluate the concentration of phenolic compounds contained in the extract (measured gallic acid equivalents in mg/g). The total polyphenol concentration in bitter gourd tea was found to 264 mg/ g, of gallic acid equivalents (GAE). Plant phenolics present in the fruit and vegetables have received considerable attention because of their potential antioxidant activity²³⁻²⁴. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers²⁵.

Antioxidant activity

Free radicals and ROS are associated with important pathological processes including inflammation, neurodegenerative diseases, cancer and atherosclerosis²⁶. Antioxidants play an important role in the protection of human body against damage by reactive oxygen species. Furthermore, previous epidemiological studies have shown that the intake of natural antioxidants has been associated with reduced risks of cancer and other diseases associated with oxidative damages²⁷. There has been much interest in exploiting antioxidants from natural sources, as there is concern over the toxic effects of synthetic antioxidants. Two different free radical systems were used to evaluate antioxidant activities of bitter gourd tea, stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assay.

DPPH assay

The antioxidant activity of extracts was evaluated by their ability to scavenge free radicals by using DPPH assay. The stable free radical DPPH method is an easy, rapid, reproducible and sensitive way to survey the antioxidant activity of a specific compound or plant extracts²⁸. DPPH produces a violet solution in methanol and it is reduced in the presence of hydrogen donating antioxidant compound due to the formation of a non-radical form (DPPH-H) giving rise to yellow color²⁹. The measured DPPH radical scavenging activity is shown in Figure. 1.

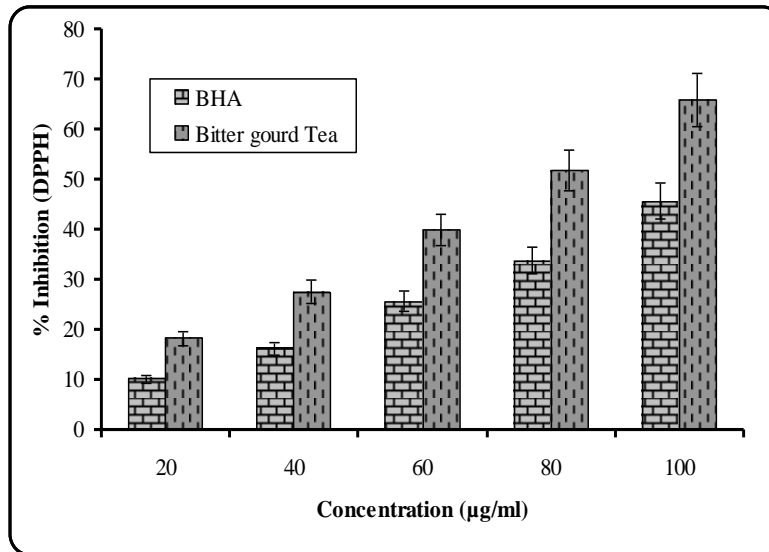


Figure.1: Inhibition% (DPPH) of Bitter gourd tea and BHA.

The bitter gourd tea extract and BHA scavenging antioxidant activities ranged from 18.35 ± 0.80 - $65.96\% \pm 2.50$ and 10.18 ± 0.20 - 45.75 ± 1.30 , respectively at concentrations (20-100µg/ml) in the DPPH radical scavenging method. The bitter tea extract and standard BHA showed antioxidant activity with the IC₅₀ value of 72.04 ± 3.25 µg/ml and 98.75 ± 3.92 µg/ml,

respectively. The results for DPPH are in agreements with the result given by³⁰. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability³⁴. These results indicated that bitter gourd tea extract has a noticeable effect on scavenging free radicals and there was a positive correlation between DPPH and TPC with r^2 0.9938 (Figure. 2). Free radical scavenging activity also increased with increasing concentration. Many researchers have reported positive correlation between free radical scavenging activity and total phenolic compound³¹⁻³⁴ also reported a high correlation between DPPH radical scavenging potential and total phenolic content.

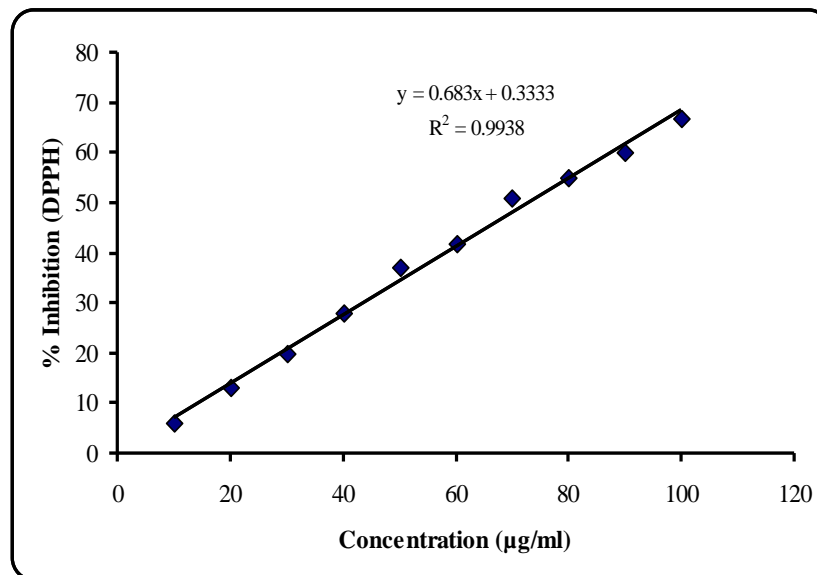


Figure. 2: Correlation between DPPH and TPC of Bitter gourd tea.

Reducing power assay

Reductants provide electrons to the reduced species. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. The yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm³⁵⁻³⁶. Figure. 3 showed Reducing Power Activity of Bitter gourd tea extract. The reducing power of Bitter gourd tea extract increased with increasing dosage. The result shows that Bitter gourd tea extract consist of hydrophilic poly phenolic compounds that cause the greater reducing power. It is believed that antioxidant activity and reducing power are related as reductones inhibit lipid per-oxidation (by donating a hydrogen atom and thereby terminating the free radical chain reaction³⁷⁻³⁸).

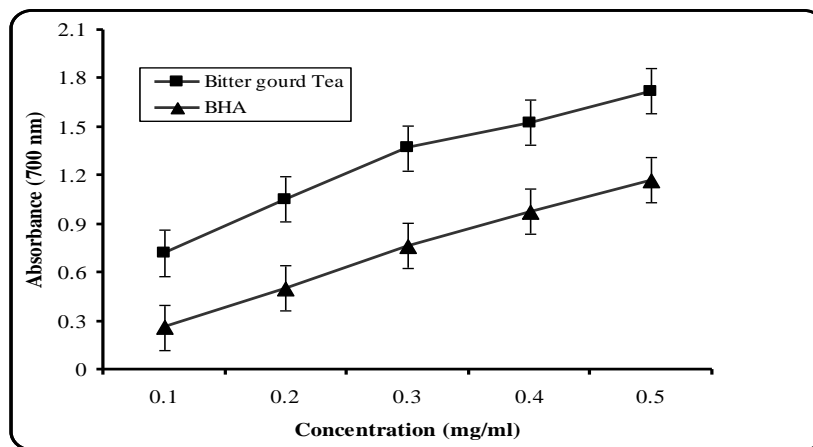


Figure. 3: Reducing Power Activity of Bitter gourd tea and BHA.

Organoleptic Evaluation

Organoleptic Evaluation of food products play an important role in judging the sensory acceptability or rejection of food items in the market ³⁹. Quality is the ultimate criteria for all the food products reeled over by the organoleptic qualities such as appearance, color, texture, flavor and taste. The acceptability trials were carried out for bitter gourd tea by a semi-trained panel of 10 judges. The results of the investigations on appearance, color, flavor, taste and overall acceptability are given in Table 1. The results of the study on appearance, color, flavor, taste and overall acceptability exhibited mean scores 6.31, 7.14, 7.56, 5.95 and 7.35, respectively (Table 1). The flavor of bitter gourd tea got the highest mean score. However, all the judges demonstrated that bitter gourd tea was acceptable regarding organoleptic quality.

Table 1: Organoleptic Evaluation of Bitter gourd Tea.

Judges	Appearance	Color	Flavor	Taste	Overall acceptability
1	7.15	8.10	8.70	7.11	8.30
2	7.20	8.25	8.45	6.20	8.10
3	7.62	7.90	8.80	6.62	8.45
4	6.60	8.50	8.45	6.30	7.90
6	7.25	7.80	8.30	7.05	8.40
7	6.55	8.35	8.40	6.55	8.10
8	7.12	7.25	8.15	6.10	7.80
9	6.75	7.50	8.50	6.75	8.50
10	6.85	7.75	7.85	6.85	8.00
Mean	6.31	7.14	7.56	5.95	7.35

CONCLUSION

Findings of this study revealed that bitter gourd tea had promising sources of natural antioxidants. Further investigations are needed to clarify intracellular pathways involved in the mechanism of the inhibitory activity.

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