

Antioxidant Properties of Bitter Gourd (*Momordica Charantia* L.)

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Abstract— Bitter gourd is regarded as an antioxidant rich vegetable with beneficial properties for the circulatory, respiratory, digestive and nervous systems according to the Indian indigenous system of medicine. Several methods have been used to determine antioxidant activity of plants. The present study, therefore, involved four various established methods to evaluate anti oxidative activity of bitter gourd fruit, namely, total antioxidant capacity, DPPH radical scavenging activity, hydroxyl radical scavenging activity and super oxide anion radical scavenging activity by using different types of solvents like petroleum ether, acetone, ethanol and methanol.

The present study revealed that light green big sample had the highest DPPH activity with an IC_{50} value of 50.88 $\mu\text{g/ml}$ in methanol solvent. In the case of bitter gourd dried samples, highest DPPH activity with an IC_{50} value of 50.10 $\mu\text{g/ml}$ was reported in light green big type. The hydroxyl radical scavenging activity of light green big was found to be highest both in the case of fresh and dried bitter gourd samples with an IC_{50} values of 50.95 $\mu\text{g/ml}$ and 50.10 $\mu\text{g/ml}$ respectively.

Light green small sample showed higher superoxide anion radical scavenging activity with an IC_{50} value of 50.36 $\mu\text{g/ml}$ in fresh samples and 49.76 $\mu\text{g/ml}$ in dried samples, in solvents like petroleum ether and acetone respectively. Antioxidant activity ranged with an IC_{50} value of 50.09 $\mu\text{g/ml}$ to 61.90 $\mu\text{g/ml}$ in fresh bitter gourd samples and maximum antioxidant capacity was observed in light green big (50.09 $\mu\text{g/ml}$) whereas dried samples, the highest antioxidant activity was observed in light green dried (50.07 $\mu\text{g/ml}$) in acetone solvent.

Keyword— *Momordica charantia*, Bitter gourd, DPPH, Hydroxyl radical, Superoxide radical, Antioxidant activity.

I. INTRODUCTION

Bitter gourd (*Momordica charantia*) is one of the most popular vegetable in South Asia, which belongs to the family *cucurbitaceae*. The Latin name *Momordica* means “to bite” referring to the jagged edges of the leaves, which appear as if they have been bitten. It is regarded as

one of the world’s major vegetable crops and has great economic importance. Bitter gourd grows in tropical and subtropical areas, including parts of East Africa, Asia, Caribbean, and South America, where it is used not only as a food but also as a medicine. Furthermore, Indians have traditionally used the leaves and fruits as a medicine to treat diabetes, colic and to heal skin sores and wounds (Paul et al., 2009). Bitter gourd also known as balsam pear, karela, or bitter melon is a fast growing tropical vegetable crop. All parts of plant, especially roots, leaves, fruits and seeds are widely used in traditional medicine throughout Asia, East Africa and South America (Gbeassor et al., 2006).

Recently antioxidants and secondary metabolites are attracting a great deal of attention for their effects in preventing diseases due to oxidative stress, which leads to degeneration of cell membranes and leads to many pathological diseases. Antioxidants and secondary metabolites play a major role in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases (Ahmed and Beigh, 2009). Moreover, recent investigations have shown that the antioxidants with free radical scavenging properties of plant origin could have great importance as therapeutic agents in ageing process and free radical mediated diseases (Zhang et al., 2009). The herbal plants are considered as useful means to prevent or ameliorate certain disorders such as diabetes, atherosclerosis and other complication (Scartezini and Speroni, 2000). The present investigation was carried out to quantify the different antioxidant activities present in the fresh and dried fruit extract of bitter gourd (*Momordica charantia*) by chemical analysis.

II. MATERIALS AND METHODS

Four types of commercially cultivated bitter gourd viz., light green small, light green big, dark green small, dark green big along with *Nei paval* were selected for the study. First two types were collected from VFPCCK, Kalliyoor and the second two types were collected from local market in Trivandrum and *Nei paval* was collected from Madurai, Tamil Nadu (Plates 1 to 5).



Plate 1. Light green big



Plate 2. Light green small



Plate 3. Dark green big



Plate 4. Dark green small



Plate 5. Nei paval

The fresh bitter gourd fruits were cut in to thin slices and dried in an electric drier below 55° C and were processed into powder form with the help of mixer. The powdered bitter gourd of the five types was stored at ambient condition in zip lock pouches. The antioxidant activities of the selected bitter gourd types were carried out both in fresh and processed form using different types of solvents like petroleum ether, acetone, methanol and ethanol.

Antioxidant activity

The antioxidant activity was determined according to the thiocyanate method with slight modifications (Oliveri, 2000). For the stock solution, 10 mg of ascorbic acid was dissolved in 10 ml water. Then the solution of standards samples (100mg/l) in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6) was added to 2.5 ml of linoleic acid emulsion. Fifty ml linoleic acid emulsion contained Tween-20, linoleic acid and potassium phosphate buffer (0.04 M, pH 7.6). On the other hand, 5.0 ml of control contained 2.5 ml of linoleic acid emulsion and 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6). Each solution was then incubated at 37°C in a glass flask in the dark. At 24 h intervals during incubation, 0.1 ml of this incubation solution was added to 4.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate.

Precisely 3 min after 0.1 ml of 0.02 M FeCl₂ in 3.5% (w/v) HCl was added to the reaction mixture and the absorbance of the red colour was measured at 500 nm in a spectrophotometer. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Inhibition \%} = [(A_0 - A_1)/A_0 \times 100],$$

Where A₀ is the absorbance of the control reaction and A₁ is the absorbance in the presence of standards.

Diphenyl Picryl Hydrazyl (DPPH) radical scavenging activity

Free radical scavenging activity of sample to characterize antioxidant activity was estimated as suggested by Blois (1958). Different amount of the methanolic, ethanolic, acetate, petroleum ether extracts of the sample was taken and DPPH (0.1 mM dissolved in methanol) was mixed together and the reaction mixture was left in dark room for 20 minutes. The absorbance was measured at 517 nm against the blank prepared by mixing DPPH and methanol. The antioxidant activity was expressed in terms of per cent inhibition of DPPH free radicals using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, Abs_{control} = absorbance of DPPH solution (blank) and Abs_{sample} = absorbance of sample. The IC₅₀ of each sample (concentration in µg/ml required to inhibit DPPH radical formation by 50 per cent) has also been calculated.

Hydroxyl radical scavenging activity

In order to assess the hydroxyl free radical scavenging activity of the methanolic, ethanolic, acetate and petroleum ether extracts of the bitter gourd samples, the deoxyribose method was used, as described by Halliwell et al. (1996), with some slight modifications. The reaction mixture contained phosphate buffer (20 mM, pH 7.4), (60 mM) deoxyribose, (10mM) hydrogen peroxide, (1 mM) ferric chloride, (1.04 mM) EDTA, different amount of powered samples and (2mM) ascorbic acid. The reaction mixtures were incubated for 1hr at 37°C, after which 17 mM trichloro acetic acid (TCA) was added. The mixture was then boiled for 15 minutes, ice cooled and measured for absorbance at 532 nm. Distilled water in lieu of extract was utilized as blank and the sample solution without added deoxyribose was used as a sample blank.

Superoxide anion radical scavenging activity

Superoxide anion scavenging activity was measured based on the method described by Robak and Gryglewski (1988). Superoxide radicals were generated in a PMS-NADH system via the oxidation of NADH and then assayed by the reduction of nitro blue tetrazolium (NBT). The superoxide radicals were generated in reaction mixture containing sodium phosphate buffer (100 mM,

pH 7.4) containing 150µM NBT, 468 µM NADH solution in sodium phosphate buffer and different concentrations of methanolic, ethanolic, acetate and petroleum ether extracts of the samples. To this 60 µM phenazine metho sulphite (PMS) solution was added. The reaction mixture was incubated for 5 minutes at 25°C and the absorbance was measured at 560 nm.

III. RESULTS AND DISCUSSION

Total antioxidant activity

Antioxidants can protect the human body from free radical and reactive oxygen species (ROS) effects. They retard the progress of many chronic diseases as well as lipid peroxidation. Being enzymatic or non enzymatic species, antioxidant molecules are classified in different categories. Antioxidants are major compounds that protect the quality of life by retarding the oxidation process through scavenging free radical produced during many natural events. Although their ultimate aim is removal of ROS, they may use different mechanism depending on their structure and site of action. Antioxidants are also able to act by up regulating the expression of the genes encoding the antioxidant enzymes, repairing oxidative damage caused by radical and increasing elimination of damaged molecules (Wood et al., 2006).

The total antioxidant activity of fresh and dried bitter gourd samples is depicted in Table 1.

Table.1: Total antioxidant activity of fresh and dried bitter gourd types

| Types | IC ₅₀ Values (µg/ml) | | | | | | | |
|-------------------------|---------------------------------|---------|---------|----------|-----------------|---------|---------|----------|
| | Fresh samples | | | | Dried samples | | | |
| | Petroleum ether | Acetone | Ethanol | Methanol | Petroleum ether | Acetone | Ethanol | Methanol |
| Light green big (LGB) | 54.17 | 51.22 | 50.09 | 54.23 | 52.33 | 50.78 | 50.07 | 50.13 |
| Light green small (LGS) | 55.67 | 53.34 | 52.34 | 56.76 | 53.45 | 50.90 | 51.34 | 51.23 |
| Dark green big (DGB) | 61.90 | 60.98 | 56.88 | 59.76 | 58.90 | 54.12 | 55.67 | 52.37 |
| Dark green small (DGS) | 60.98 | 59.77 | 54.76 | 54.38 | 57.77 | 53.21 | 53.21 | 51.98 |
| Nei paval (NP) | 58.90 | 58.30 | 50.65 | 55.00 | 54.22 | 52.22 | 50.90 | 52.13 |

The results of above table revealed that antioxidant activity ranged with an IC₅₀ values of 50.09 µg/ml to

61.90 µg/ml in fresh bitter gourd samples and maximum antioxidant capacity was observed in Light green big

(50.09 µg/ml) and minimum antioxidant capacity was observed in dark green big fresh samples (61.90 µg/ml). In the case of bitter gourd dried samples, the highest antioxidant activity was observed in light green big type (50.07 µg/ml) in acetone solvent and lowest antioxidant capacity was noticed in dark green big (58.90 µg/ml) sample in petroleum ether as solvent obtained with the help of linear regression equations.

The study is in accordance with the findings of Leelaprakash et al. (2011) who had reported that IC₅₀ value of antioxidants activity varies from 53.75 to 56.25. A study conducted by Islam et al. (2011) on bio active compounds of bitter melon genotypes in relation to their physiological functions reported that antioxidant activities of Indian green, Indian white, China green and China white ranged from 79-88, 79-87, 80-86, and 79-87 per cent inhibition, respectively. The antioxidant activities of oven dried samples and freeze dried samples were 79-88 and 79-86 per cent respectively. A study conducted by Asan and Karacoka, (2013) reported that antioxidant

activity of bitter gourd was 39.92 mg. Bitter gourd dried at 55 °C retained the highest antioxidant activities compared to samples dried at 50 or 60 °C. Thus, the best drying temperature to retain antioxidant properties in bitter gourd is at 40 °C (Aminah and Permatasari, 2013).

DPPH radical scavenging activity

Free radical scavenging is one of the known mechanism by which antioxidants inhibit lipid peroxidation (Blokhina et al., 2003). The DPPH radical scavenging activity has been extensively used for screening antioxidants from fruits and vegetable juices or extracts (Sanchez, 2002). Free radical scavenging activities of the bitter gourd types were studied by the DPPH assay in different types of solvents. Table 2 illustrates the results of the DPPH activity among the bitter gourd fresh and dried samples. The IC₅₀ value was calculated from the graph (it was noted as the concentration of sample needed to scavenge the free radicals at 50 per cent inhibition).

Table.2: DPPH radical scavenging activity of fresh and dried bitter gourd types

| Types | IC ₅₀ Values (µg/ml) | | | | | | | |
|-------------------------|---------------------------------|---------|---------|----------|-----------------|---------|---------|----------|
| | Fresh samples | | | | Dried samples | | | |
| | Petroleum ether | Acetone | Ethanol | Methanol | Petroleum ether | Acetone | Ethanol | Methanol |
| Light green big (LGB) | 53.66 | 52.98 | 52.33 | 50.88 | 50.79 | 50.10 | 50.22 | 50.98 |
| Light green small (LGS) | 53.97 | 53.09 | 55.34 | 52.33 | 50.90 | 51.23 | 50.31 | 53.12 |
| Dark green big (DGB) | 55.98 | 53.76 | 58.90 | 59.09 | 53.21 | 53.21 | 55.76 | 57.88 |
| Dark green small (DGS) | 54.90 | 52.15 | 57.89 | 55.22 | 52.78 | 52.15 | 53.22 | 56.22 |
| Nei paval (NP) | 54.77 | 53.09 | 55.65 | 53.33 | 50.98 | 51.34 | 52.45 | 54.20 |

The findings revealed that light green big sample had the highest DPPH activity with an IC₅₀ value of 50.88 µg/ml in methanol solvent, followed by light green small (52.15 µg/ml) in acetone media. The lowest DPPH radical scavenging activity was found in dark green big (59.09 µg/ml) in methanol solvent.

In the case of bitter gourd dried samples, highest DPPH activity with an IC₅₀ value of 50.10 µg/ml was reported in light green big and lowest activity (57.88 µg/ml) was observed in dark green big types with the help of linear regression equation (Table 2). A study by Kubola and Siriamornapun (2008) on antioxidant activity of various boiled water extracts from unripened bitter melon fruit revealed 53.9 ± 0.73% reduction in the DPPH radical at a

concentration of 0.2 mg/g. Hamissou et al. (2013) conducted a study on antioxidant properties of bitter gourd and zucchini and reported that bitter gourd was 82.05 per cent as effective as ascorbic acid in inhibiting the free radical DPPH.

Aminah and Anna (2011) observed that scavenging activity ranges between 37 per cent to 64.48 per cent. The DPPH of wild bitter gourd ranges between 36.60 per cent to 75.8 per cent (Wu and Ng, 2008). Microwave cooked samples had significantly (*p* < 0.05) higher percentage of DPPH radical scavenging activity (88.54%) (Aminah and Permatasari, 2013) compared to oven drying, frying and boiling methods.

Hydroxyl radical scavenging activity

The scavenging of H₂O₂ by extracts may attribute to their phenolics, which can donate electrons to H₂O₂, thus neutralizing it to water (Nabavi et al., 2008). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells. Addition of H₂O₂ to cells in culture

can lead to transition metal ion dependent OH radical mediated oxidative DNA damage (Aljudi and Kamaruddin, 2004). Thus, removing hydrogen peroxide as well as superoxide anion is very important for protection of pharmaceuticals and food products (Gulcin et al., 2007).

Table.3: Hydroxyl radical scavenging activity of fresh and dried bitter gourd types

| Types | IC ₅₀ Values (µg/ml) | | | | | | | |
|-------------------------|---------------------------------|---------|---------|----------|-----------------|---------|---------|----------|
| | Fresh samples | | | | Dried samples | | | |
| | Petroleum ether | Acetone | Ethanol | Methanol | Petroleum ether | Acetone | Ethanol | Methanol |
| Light green big (LGB) | 56.02 | 52.32 | 50.95 | 52.98 | 50.68 | 50.10 | 50.66 | 55.77 |
| Light green small (LGS) | 56.78 | 52.98 | 53.22 | 58.90 | 51.34 | 51.64 | 50.98 | 53.44 |
| Dark green big (DGB) | 58.76 | 55.67 | 58.89 | 65.33 | 52.84 | 53.45 | 55.34 | 59.56 |
| Dark green small (DGS) | 57.82 | 54.87 | 56.34 | 64.12 | 52.02 | 52.34 | 53.22 | 57.62 |
| <i>Nei paval</i> (NP) | 55.32 | 53.97 | 54.22 | 62.55 | 52.13 | 52.89 | 51.76 | 56.14 |

The hydroxyl radical scavenging activities of fresh and dried bitter gourd samples were shown in the Table 3. In this study, the hydroxyl radical scavenging activity of light green was found to be highest in fresh bitter gourd samples with an IC₅₀ value of 50.95 µg/ml, whereas it was found to be minimum with an IC₅₀ value of 65.33 µg/ml. Ascorbic acid was used as reference compound and its IC₅₀ value was 3.90 µg/ml.

The findings revealed that light green big sample had the highest hydroxyl radical scavenging activity with an IC₅₀ value of 50.10 µg/ml in acetone solvent followed by light green small (50.98 µg/ml) in ethanol solvent media.

Leaf extract of Thai bitter melon possesses hydroxyl-radical scavenging activity with an IC₅₀ value of 167 ± 0.96 mg/mL (Kubola and Siriamornpun 2008). Liu et al. (2004) demonstrated fruit extract of the most effective white bitter melon cultivar in Taiwan exerted potent

hydroxyl-radical scavenging activity with IC₅₀ value of 37 µg/mL). These results further supported that leaf and fruit of white bitter melon possess strong hydroxyl-radical scavenging activity.

Super oxide anion-radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reaction such as autoxidation by catecholamines. In the present study, superoxide radical reduces NBT to a blue coloured formazan that is measured at 560 nm (Panda and Kar, 2000). Superoxide anions indirectly initiated lipid peroxidation as a result of superoxide and hydrogen peroxide serving as precursors of single O₂ and hydroxyl radicals.

The super oxide radical scavenging activity of fresh and dried bitter gourd types is presented in Table 4.

Table.4: Super oxide radical scavenging activity of fresh and dried bitter gourd types

| Types | IC ₅₀ Values (µg/ml) | | | | | | | |
|-----------------------|---------------------------------|---------|---------|----------|-----------------|---------|---------|----------|
| | Fresh samples | | | | Dried samples | | | |
| | Petroleum ether | Acetone | Ethanol | Methanol | Petroleum ether | Acetone | Ethanol | Methanol |
| Light green big (LGB) | 54.12 | 52.76 | 52.33 | 54.31 | 52.34 | 50.12 | 49.65 | 51.61 |

| | | | | | | | | |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Light green small (LGS) | 50.36 | 58.65 | 50.67 | 54.60 | 50.89 | 49.76 | 45.23 | 50.71 |
| Dark green big (DGB) | 57.82 | 61.22 | 58.90 | 59.11 | 56.90 | 57.23 | 53.99 | 53.61 |
| Dark green small (DGS) | 56.87 | 60.88 | 54.10 | 55.17 | 54.76 | 52.33 | 50.87 | 50.94 |
| <i>Nei paval</i> (NP) | 55.89 | 60.12 | 52.99 | 55.27 | 52.90 | 51.21 | 49.99 | 51.98 |

Light green small sample showed higher superoxide anion radical scavenging activity with an IC₅₀ value of 50.36 µg/ml and 50.67 µg/ml in fresh samples, in solvents like petroleum ether and ethanol respectively. The results of above table revealed that dark green big possessed lowest superoxide anion radical scavenging activity in fresh sample with an IC₅₀ value of 61.22 µg/ml.

Light green small dried sample showed higher superoxide anion radical scavenging activity with an IC₅₀ value of 45.23 µg/ml and 49.76 µg/ml in dried samples, in solvents ethanol and acetone respectively. The results of above table revealed that dark green big possessed lowest superoxide anion radical scavenging activity with an IC₅₀ value of 57.23 µg/ml.

According to Hamissou et al. (2013) an average of 1.55 units of super oxide dismutase activity per µg total proteins was recorded for bitter gourd fruits. A study conducted by Tsai et al. (2014) on antioxidant, cell protective and anti melanogenic activities of leaf extracts from wild bitter melon cultivars reported an activity of 9.12 mg/ml in leaf extracts.

IV. CONCLUSION

Antioxidant activity in the present study revealed that light green big fresh sample had the highest DPPH activity with an IC₅₀ value of 50.88 µg/ml in methanol solvent followed by dark green small fresh (52.15 µg/ml) in acetone media. The hydroxyl radical scavenging activity of light green big was found to be highest both in the case of fresh and dried bitter gourd samples.

Light green small fresh sample showed higher superoxide anion radical scavenging activity with an IC₅₀ value of 50.36 µg/ml in fresh samples and 49.76 µg/ml in dried samples, in solvents like petroleum ether and acetone respectively. In the case of bitter gourd dried samples, the highest antioxidant activity was observed in light green big dried type (50.07 µg/ml) in acetone solvent and lowest antioxidant capacity was noticed in dark green big dried (58.90 µg/ml) sample in petroleum ether as solvent.

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