The study was undertaken to explore the nutritional and antioxidant property of *Cucumis dipsaceus*. The results revealed significant amount starch (1.07 mg/g), proteins (85.9 mg/g), essential amino acids and some most important minerals like calcium (14820 ppm) and nitrogen (6300 ppm). The phenolic (3.04 g GAE/100 g extract) tannin (1.66 g GAE/100 g extract) and flavonoid content (11.26 g RE/100 g extract) was found to be high in ethyl acetate, chloroform and methanol extract of fruit. Response of *Cucumis dipsaceus* fractional extracts towards various antioxidant assays was appreciable especially in ABTS', metal chelating, nitric oxide and DPPH assays. Methanol extract of *Cucumis dipsaceus* fruit showed the highest activity (4907.22 µg TE/g) to stabilize ABTS radical. Metal chelating activity was efficiently exhibited by *Cucumis dipsaceus* fruit methanol extract (12.4 g EDTA equi/100g extract). DPPH (IC$_{50}$ = 10.37 µg/mL) assay also revealed higher free radical inhibition of fruit. This study has clearly pointed out the nutritional and antioxidant properties of *Cucumis dipsaceus* which could support its use as a nutraceutical supplement in health promoting diets.
been undertaken to evaluate the nutritional, anti-nutritional and antioxidant properties of fruit.

**Materials and Methods**

**Collection of plant materials**

The fruits were collected during the month of November 2011. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical survey of India (BSI), Southern circle Coimbatore, Tamil Nadu (No. BSI/SRC/5/23/2011-12/Tech-1466). The Plant was certified by Dr. P. Satyanarayana, Scientist ‘D’, BSI, Coimbatore. Freshly collected fruits were cleaned to remove adhering dust and then dried under shade. The dried sample was powdered and used for further studies.

**Successive solvent extraction**

Fresh (1 kg) fruits were taken and air dried under room temperature. The air dried, powdered fruit (100 g) was extracted in Soxhlet extractor successively with petroleum ether and methanol. Finally, the material was macerated using hot water (80ºC) with occasional stirring for 24 hr and the water extract was filtered. The methanol extract alone was subjected to fractional extraction using chloroform, ethyl acetate and methanol (Raaman, 2006). The period of extraction may range from 24 to 48 hr for each solvent depending on the extractability of the solvents. Each time before extracting with the next solvent, the material was dried in hot air oven below 40°C. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The extracts were freeze dried (1-12 g) and stored in desiccators until further analysis.

**Nutritional analysis**

**Proximate composition**

The moisture content of the fruit was estimated by taking plant samples and the weight was taken before and after incubation in a hot-air-oven at 50°C for 24 h, followed by cooling in a desiccator. The recommended methods of Association of Official Analytical Chemists (1990) were used for the determination of ash. Ash content was determined by incineration of 2 g of sample in a muffle furnace kept at 600°C for 6 h. The protein was estimated as described by Lowry et al. (1990). The starch content was estimated as described by Sadasivam and Manickam (1992) using glucose as a standard. The samples were analyzed in aliquots and the results expressed on dry weight basis.

**Estimation of amino acids**

Amino acids in leaves were determined according to the procedure of Ishida et al. (1981). Extracted samples (1 mg/ml) were filtered through a 0.45 µm membrane filter and 20 µL of the filtrate was injected in to a HPLC (model LC 10 AS, Shimadzu, Mount holly, New Jersey) equipped with a cation exchange column packed with a strongly acidic cation exchange resin, i.e., styrene divinyl benzene copolymer with sulphonic group. The temperature was maintained at 40°C and the run time was 15 min. The amino acid analysis was with the non-switching flow method and fluorescence detection after post-column derivatization with o-phthaldehyde. Identification was based on the comparison between the retention time of the standards of the amino acids and those in fruit and was confirmed and quantified by a fortification technique (spiking).

**Mineral quantification**

For the sample digestion, 0.5 g of dried sample was mixed with 5 ml digestion mixture and kept in digestion unit at 300°C. The process was allowed to continue till the mixture turns colourless. Desired volume of distilled water is added to the digested and cooled samples. Solution was filtered and mixed well till all sediments got dissolved. Subsequently minerals were determined as follows: nitrogen (N) through micro Kjeldahl method; phosphorus (P) by treating the digested samples with ammonium molybdate and freshly prepared ascorbic acid and analyzed by spectrophotometer (Hitachi U-2001 Japan); potassium (K), sodium (Na), and calcium (Ca) were determined by Flame Photometer by the method of Allen (1989). The microelements (Fe, CO, Cu, Mg, and Zn) were determined through Atomic Absorption Spectrophotometer.

**Analysis of anti-nutritional factors**

Trypsin inhibition ability was evaluated in powdered fruit samples on a synthetic substrate BAPNA. The degree of inhibition is expressed in TIU/mg protein by the method of Sadasivam and Manikam (1992).

**In vitro antioxidant studies**

**Quantification of total phenolics, tannins and flavonoids**

The total phenol content was determined according to the method described by Siddhuraju and Becker (2003). Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) Sidduraju and Manian,
The tannin content of the sample was calculated as follows:

\[ \text{Tannin (\%)} = \text{Total phenolics (\%)} - \text{Non tannin phenolics (\%)} \]

The analysis was performed in triplicate and the results were expressed as gallic acid equivalents. The flavonoid contents of all the extracts were quantified as it act as a major antioxidants in plants reducing oxidative stress. Estimated as per described by Zhishen et al. (1999). The amount of flavonoid was calculated in rutin equivalents.

**Total antioxidant activity assay by radical cation 2, 2'-azinobis (3-ethylebenzothiozoline-6-sulphonic acid) (ABTS⁺⁻)**  
The total antioxidant activity of the extracts were measured by ABTS radical cation decolorization assay according to the method of Re et al. (1999) described by Siddhuraju and Manian (2007). The unit of total antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µMol/g extract.

**Radical scavenging activity using DPPH· method**  
The antioxidant activity of the extracts were determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 2,2-diphenyle-1-picrylhydrazyl (DPPH·), according to the method of Blois, (1958). The mixture of methanol, DPPH and standard (BHT, BHA, quercetin and rutin) served as positive control. The IC₅₀ of the extracts were also calculated.

**Ferric reducing antioxidant power (FRAP) assay**  
The antioxidant capacities of phenolic extracts of samples were estimated according to the procedure described by Pulido et al. (2000). Results were calculated in ascorbic acid equivalents.

**Metal chelating activity**  
The chelating of ferrous ions by *Cucumis dipsaceus* fruit extracts was estimated by the method of Dinis et al. (1994). All the reagents without addition of sample extract were used as negative control. Metal chelating activity was determined in EDTA equivalence.

**Nitric oxide radical scavenging activity**  
The nitric oxide scavenging activity of *Cucumis dipsaceus* fruit extracts on nitric oxide radical was measured according to the method of Sreejayan and Rao (1997). BHT and rutin and the same mixture of the reaction without *Cucumis dipsaceus* extracts were employed as positive and negative control respectively. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula

\[ \text{% radical scavenging activity} = \left( \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \right) \times 100 \]

**Phosphomolybdenum assay**  
The antioxidant property of samples was evaluated by the phosphomolybdenum method (Prieto et al., 1999). The results reported are mean values expressed as grams of ascorbic acid equivalents per gram extract (AEAC).

**Statistical analysis**  
The results were statistically analyzed and expressed as mean (n = 3) ± standard deviation. Values are analyzed by Duncan’s multiple test range (Statistical package for Social Sciences (SPSS), Analysis of Variance (ANNOVA) statistical software, TULSA, USA version 20.0).

**Results and Discussion**

**Nutritional evaluation**

The moisture content of fruit was determined by calculating its initial and final weights. After 2 days of hot air oven treatment under 60°C, moisture content of fruit is 89.1%. The ash content of fruit was determined by calculating its initial crucible weight and final crucible weights. The ash content of fruit is 17.8% (table 1c). The investigation of amino acids and minerals in the fruit resulted the presence of almost all essential amino acids are present in an appreciable amount in the fruit sample. In fruit, the amount of alanine is higher (32.33%) whereas the amount of phenylalanine is found to be the lowest (2.44%). The results are compared with recommended levels of amino acids by FAO/WHO/UNU and shown in table 1 a and b respectively. It was reported that for a healthy human diet, a normal man should take 15 mg of threonine, 4 mg of cysteine, 10 mg of methionine, 26 mg of valine, 20 mg of isoleucine, 39 mg of leucine, 15 mg of tyrosine, 10 mg of histidine, 25 mg of phenylalanine and 30 mg of lysine per kg/day of body weight (WHO/FAO, 2007). The amino acid profile of this fruit has proved its efficiency to provide rich nutritious supply as a food. Moreover these results could combat the demand of plant based food which has such amino acid strength. Leusine, isoleusine, alanine, and valine enhance muscular energy production, stimulate metabolic signals, and are precursors of several other amino acids. Amino acids are also reported to have the property of quench
the deleterious 2,2-diphenyle-1-picyrylhydrazyl (DPPH) radical also (Yokozawa et al., 1998; Wu and Meininger, 2000). The amino acid deficiency can be met by consuming large amounts of legumes, by employing the complimentary that exists between high sulphur amino acid substitutes (Arunachalam and Parimelazhagan, 2012). Since the plant Cucumis dipsaceus is observed to be having those amino acids in sufficient quantities, it can be justified as a promising amino acid source. In addition, the Macro-nutrients are essential in this plant will promote proper functioning of cell and cellular organs and they act mainly as electrolytes (Nelson, 2000).

Based on the evidences for the edible property (Verdcourt and Trump, 1969), fruit of Cucumis dipsaceus, were quantified for the presence of important macro and micro nutrients and the results are presented in table 1 b. The fruit sample found to have N, K, Ca, P, Si, and Fe in a well appreciable amount. In fruit, the calcium content (14820 ppm) is estimated to be higher than all the other macro and micro-nutrients. Nitrogen content was also found to be higher. Hence this fruit could be suggested for use as a source of good calcium and nitrogen intake. Agrahar-Murugkar and Subbulakshmi (2005) analysed the nutritive value of wild edible fruits, berries, nuts, roots and spices consumed by the khasi tribes of India. They observed that calcium is rich in Solanum indicum, phosphorus and magnesium in Solanum gilo, iron in Prunus nepalensis, manganese in Viburnum corylifolia, sodium and copper in Solanum xanthocarpum, zinc in Vangeria spinosa and potassium in Gomphogyne cissiformis. This study supports the use of wild fruits with some important minerals in diet. It was also reported that the trace elements are mainly involved in catalytic activity (Lippard and Jeremy, 1994). Magnesium is required by many enzymes, in particular the sugar and protein kinase families of enzymes that catalyze ATP-dependent phosphorylation reactions (Rahul and Parimelazhagan, 2012). Manganese is an essential trace metal found in all tissues and is required for normal amino acid, lipid, protein, and carbohydrate metabolism (Aschner and Aschner, 2005). Cu, Fe, Zn and Mn are characterised by the presence of unpaired electrons which allow them to participate in redox reactions. Hence these elements are believed to have good antioxidant potential (Brígida et al., 2011). This would also support the free radical scavenging ability of fruit. Since the calcium and iron forms the important part of our daily diet as they play a major role in strengthening of bones and haemoglobin formation, fruits of the plant Cucumis dipsaceus can be recommended to have as a dietary supplement. Beside Cu and Fe as main minerals it can also serve as an accessory source of other minerals.

Apart from the minerals and amino acids, the fruit of Cucumis dipsaceus shows commendable presence of starch and proteins which are essential in daily human diet. The fruit is found to have 1.07 mg of starch and 85.9 mg of proteins.

### Analysis of anti-nutritional factors

Table 2 shows the anti-nutritional factors of the Fruit. It showed 9.38% of phenolics and 4.99% of tannins. 3.2 TIU/mg protein was determined from the fruit sample. From the nutritional studies, it can be established that Cucumis dipsaceus can form a promising source of both minerals and amino acids. However, the negative nutritional effects of tannins are diverse and incompletely understood, but the major effect is to cause growth depression by decreasing the digestibility of protein and carbohydrate. This is most likely the consequence of the interaction of tannins with either protein or starch to form enzyme-resistant substances (Liener, 1994). Trypsin inhibitors ingested in significant amounts disrupt the digestive process and may lead to undesirable physiological reactions. Trypsin inhibitor is thermolabile and its inhibitory activity can be reduced considerably by thermal treatment (Liener, 1994). The negligible presence of antinutritional factors should not pose a problem.
to human health if leaves and flowers are properly processed. Levels of phenolics and tannin can be reduced by simple processing methods including soaking, roasting, and autoclaving.

Quantification of total phenolics, tannins and flavonoids

Table 3 shows the total phenolics, tannins and flavonoid content of *Cucumis dipsaceus* fruit. The ethyl acetate extract of fruit showed maximum phenolic content (3.04 g GAE/100 g extract) and the least amount was detected in hot water extract (1.30 g GAE/100 g extract). On the other hand, higher tannin content was observed in chloroform extract (1.66 g GAE/100 g extract) and higher flavonoid content methanol extract has (11.26 g RE/100 g extract) of fruit. Compared to other extracts methanol extract showed significant (P < 0.05) result for tannins and flavonoids. Fu et al. (2011) studied the phenolic content of 62 fruits which include *Psidium guajava* (194.11 mg GAE/100 g), *Vitis vinifera* (80.28 mg GAE/100 g), *Artocarpus heterophyllus* (60.35 mg GAE/100 g). this study strongly support that the Phenolic content present in this fruit could also be significant candidate for its antioxidant activity. There are many other classes of phenolic compounds from plants which can act as good antioxidant agents. The antioxidant activity of plant materials was well correlated by Velioglu et al. (1998) with the content of their phenolic compounds. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as potential antioxidants have been reported (Arumugham, 2005). It was also demonstrated that in Olea europaea, there is a significant antioxidant activity, which is higher than vitamin C and E, due to the synergy between flavonoids, substituted phenols etc (Benavente-García et al., 2000). These studies strongly support that Cucumis dipsaceus undoubtedly have antioxidant and other medicinal property.

Total antioxidant activity assay by radical cation

Table 2. Anti-nutritional properties in *Cucumis dipsaceus* fruit

<table>
<thead>
<tr>
<th>Phenolics (%)</th>
<th>Tannins (%)</th>
<th>Trypsin (TIU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.38</td>
<td>4.99</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table 3. Total Phenols, flavonoids and tannin content of *Cucumis dipsaceus* fruit

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Samples</th>
<th>Total phenolics (g GAE/100 g)</th>
<th>Tannins (g GAE/100 g)</th>
<th>Flavonoids (g RE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>Chloroform</td>
<td>2.43±0.42 b</td>
<td>1.66±0.28 a</td>
<td>4.0±1.99 b</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>2.04±0.18 b</td>
<td>1.34±0.27 b</td>
<td>4.69±0.15 b</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>2.61±0.012 b</td>
<td>1.48±0.97 b</td>
<td>11.26±0.18 a</td>
</tr>
<tr>
<td></td>
<td>Hot water</td>
<td>1.30±0.27 b</td>
<td>0.51±0.37 b</td>
<td>10.10±0.18 b</td>
</tr>
</tbody>
</table>

Values are mean of triplicate determination (n=3) ± standard deviation
GAE - Gallic acid equivalents, RE - Rutin equivalents.

Values followed by superscript indicates statistical significance p < 0.05, where a>b>c>d in columns

Figure 1. DPPH radical scavenging activity of fruit extract of *Cucumis dipsaceus*

2,2’-azinobis (3-ethylenbenzothiazoline-6-sulphonic acid) (ABTS+). In fruit the maximum activity was observed in methanol extract (4907.22 µg TE/g extract) and the least but noticeable activity of chloroform extract was found to be 1889.99 µg TE/g extract. In ABTS radical scavenging, fruit samples were expressed as trolox equivalent. The results of the total antioxidant activity of different extracts are given in Table 4. The activity was observed as follows: Methanol> hot water> ethyl acetate> chloroform. Hagerman et al. (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS+). As the total phenolics and tannins in *Cucumis dipsaceus* have been proved, the plant can be suggested for the use in various nutraceuticals.

Radical scavenging activity using DPPH- method

The free radical scavenging activity of the fruit extracts of *Cucumis dipsaceus* were estimated by comparing with standards such as BHT, BHA, quercetin and rutin and the result is shown in Figure 1. Importantly IC50 value of the extracts was also calculated to determine the amount of extract needed to quench 50% of radicals. A lower value of IC50 indicates a higher antioxidant activity. Chloroform extract of fruit (10.373 µg/mL) registered higher DPPH radical scavenging activity respectively compared to other extracts. Even though the radical scavenging activity shown by the extracts were low when compared to synthetic antioxidants like BHT and BHA, it can be prescribed as a safe antioxidant source, as the synthetic antioxidant are reported to

Table 4. ABTS, FRAP, Metal chelating and Phosphomolybdenum radical scavenging Activity of *Cucumis dipsaceus* fruit

<table>
<thead>
<tr>
<th>Sample Extracts</th>
<th>ABTS (µm trolox equiv/g extract)</th>
<th>FRAP (mM Fe(II)/mg extract)</th>
<th>Metal chelating (mg EDTA equivalents/100 g)</th>
<th>Phosphomolybdenum radical scavenging Activity (mg AAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>1889.99±17.86 a</td>
<td>155.16±6.13 b</td>
<td>3.07±0.07 b</td>
<td>547.2±5.9 c</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3239.98±20.25 b</td>
<td>106.96±6.08 d</td>
<td>1.84±0.08 b</td>
<td>174.3±4.2 b</td>
</tr>
<tr>
<td>Methanol</td>
<td>4007.22±30.93 c</td>
<td>276.58±35.08 e</td>
<td>12.44±0.03 b</td>
<td>262.3±4.3 b</td>
</tr>
<tr>
<td>Hot Water</td>
<td>4808.22±40.50 d</td>
<td>136.96±8.14 d</td>
<td>8.6±0.14 b</td>
<td>67.8±4.1 b</td>
</tr>
</tbody>
</table>

Values are mean of triplicate determination (n=3) ± standard deviation
ABTS - 2,2'-azinobis (3-ethylenbenzothiazoline-6-sulphonic acid)
FRAP - Ferric reducing antioxidant potential
Metal chelating - Trolox equivalent
Phosphomolybdenum radical scavenging Activity - Ascorbic acid equivalence
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pose certain side-effects. A related species *Cucumis melo* was reported to possess highest DPPH radical scavenging activity in methanolic seed extract which was found to be 75.59% at concentration of 300 µg Ml⁻¹ (Arora et al., 2011). As the inhibition percentage of *Cucumis dipsaceus* leaves extract also showed appreciable activity against DPPH, it can be prescribed as a safe and economical antioxidant source.

**Ferric reducing antioxidant power (FRAP) assay**

The results presented in Table 4, shows that methanolic extract of fruit can be appreciated for significantly (p < 0.05) higher (276.58 µM Fe (II)/mg) activity over the least reducing activity of hot water (136.96 µM Fe (II)/mg). The FRAP assay measures the antioxidant effect of any substances in the reaction medium as reducing ability. The efficiency of antioxidant property depends on the redox potentials of the compound under study (Pulido et al., 2000). The FRAP assay measures the antioxidant effect of any substances in the reaction medium as reducing ability. Iron is an essential element which is necessary for transport of oxygen molecule through blood. But under certain stress conditions these Iron act as harmful free radical which will catalyze oxidative change in lipid, protein and other cellular components (Decker and Hultin, 1992) which are needed to be scavenged using efficient antioxidants.

**Metal chelating activity**

The Fe²⁺ chelating activity of extracts are shown in Table 4. The maximum chelation was observed in fruit methanolic extract (12.4 g EDTA equi/100 g extract). Ethyl acetate extract (1.84 g EDTA equi/100 g extract) showed least chelation. Metal chelating ability was significant as they reduce the concentration of catalyzing transition metal in lipid peroxidation (Duh et al., 1999). Quantification of EDTA equivalent metal chelator has given a clear indication that these extracts can effectively chelate metal ions thereby reducing the harm of such metal radicals.

**Nitric oxide radical scavenging activity**

It was observed that the scavenging percentage of nitric oxide was higher in the ethyl acetate extract of fruit (61.60%) and lower in hot water (35.02 %) extract. The results are shown in Figure 2. The nitric oxide scavenging activity was in the order ethyl acetate>methanol>chloroform>hot water. So it can be interpreted that the plant has the property to counteract the harmful effects of NO and other reactive nitrogen species (RNS). Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985). All these investigations about this harmful radical have made it possible to search for the potent superoxide scavenging natural agent which can be supplemented partially by *Cucumis dipsaceus* as per the moderate activity. Moreover, by reducing the risk of superoxide radicals the risk of other harmful radicals like hydrogen peroxide and hydroxyl radicals can also be reduced.

**Phosphomolybdenum assay**

The results in Table 4 depicted that in fruit, highest activity is in chloroform extract (547.2 mg AA/g extract) when compared to other extracts where it was found low in hot water (67.8 mg AA/g extract) extract. The phosphomolybdenum assay is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds and the formation of formation of green phosphate/Mo (V) complex (Sowndhararajan et al., 2010). Hence the estimation of Mo reduction activity by *Cucumis dipsaceus* became an essential report in determining its antioxidant potential.

**Conclusion**

Antioxidant and nutritional properties of this plant has been evaluated for the first time. The carbohydrate, proteins, minerals and amino acids has shown that it could contribute its role as a potent fruit growing in wild with an appreciable nutritional content. However the observed anti-nutritional factor can be reduced by heat treatment or cooking. Hence the fruit could only be recommended best for human consumption after proper cooking. The results obtained have also shown good radical scavenging activity which can be taken as evidence to cure several free radical associated diseases. Nutritional contents may also highlight the importance of this wild fruit.

**Acknowledgement**

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