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EFFECT OF PROCESSING METHODS ON NUTRITIONAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF GREEN LEAFY VEGETABLES

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Leafy green vegetables are well known for their nutritive value, mineral and fibre content but are less investigated for their phenolic compounds that are responsible for antioxidant and antimicrobial activities. Further, the effect of processing on these quality parameters is also not extensively studied. The present study has been conducted to study the effect of different processing methods (blanching, dry heating and pressure cooking) on nutritional composition and antioxidant activity of green leafy vegetables. The antimicrobial activity in different solvent extracts has also been evaluated. The result obtained revealed that protein (3.94-4.79%), fibre (1.87-2.97%) and ash (3.08-4.23%) content increased significantly on dry heating of leaves. The DPPH radical scavenging ability was highest in Amaranthus leaves followed by makoi, mint and fenugreek leaves. The total phenolic content, flavonoid content, DPPH and FRAP reduced significantly ($p<0.05$) on processing of leaves. The antimicrobial activity of fresh leaf extracts in different solvents was studied against four pathogens viz. E coli, Shigella flexeneri, Salmonella enterica, and Staphylococcus aureus. Ethanolic extracts of Fenugreek leaves showed highest antimicrobial activity against E. coli followed by S. aureus. Among the microbial strains tested, Salmonella enterica and Shigella flexeneri were the most resistant microbes while Staphylococcus aureus and E. coli were the most susceptible strains.

Keywords: Leafy vegetables, Antimicrobial, Antioxidant, Flavonoid, Processing

INTRODUCTION

Vegetables are important as food both from economic and nutritional view points. They occupy an important place among the food crops as they provide adequate amount of vitamins and minerals for humans (Bolaji *et al*, 2008). Their nutritive significance is their richness in minerals which are very essential in the maintenance of human health. Leafy vegetables are low in calories and fat, high in protein per calories and dietary fibres. They contain water-soluble vitamins such as B and C, fat-soluble vitamins including A and D and also carbohydrates and minerals (Whitaker *et al*., 2001; and Afolabi *et al*., 2012). Leaf concentrates made from fractioning fresh green leaves are one of the richest sources of iron. George (2003) stated that although major portion of leafy vegetables is water, they represent a variety of natural pharmacy of minerals, vitamin and phytochemicals. Phytochemicals are metabolites which includes; alkaloids, flavonoids, glycosides, gum, polysaccharides, phenols, tannins, phlobatannins, terpens and terpenoids (Okwu, 2004).

An abundance of research has shown that fresh leafy green vegetables contain important functional food components, such as β-carotene, ascorbic acid, riboflavin, folic acid and minerals (Grusak and DellaPenna, 1999). These

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foods also contain a large amount of polyphenols (e.g., phenolic acids, flavonoids, and aromatic compounds), the most abundant phytochemicals in the human diet. Leafy vegetables are also known for their characteristic color, flavor, and therapeutic value (Gupta *et al.*, 2005; and Faller and Fialho, 2009). Their bioactive substances and phytonutrients have a wide range of biological functions, including antioxidant and antimicrobial activities (Burt, 2004; and Gutierrez *et al*., 2008). Some epidemiological evidences have shown that consumption of fruits and vegetables with high levels of natural antioxidants helps to prevent chronic diseases, such as cardiovascular diseases and cancer (Alia *et al*., 2003). Consequently, leafy green vegetables have received substantial attention from researchers in recent years as a potential source of natural antimicrobial and antioxidant agents. Since antibiotic resistance has long been a significant problem in treatment of bacterial infection, there is always a need for newer and safer options, having easy administration and lesser side effects.

Leafy vegetables are highly perishable food items and require special processing treatments to prevent post harvest losses. Leafy vegetables which are preserved by canning, freezing or dehydration are normally blanched in order to obtain good quality products. These processing methods change the nature and constituents of the vegetables. The objective of the study was to investigate the effect of processing methods on the proximate composition and antioxidant properties of leafy green vegetables. The antimicrobial activity of selected vegetables has also been evaluated.

MATERIAL AND METHODS

Sample Collection and Processing

Four leafy green vegetables viz. Fenugreek leaves (*Trigonella foenum-graecum*), mint leaves (*Mentha sativa*), chaurai leaves (*Amaranthus viridus*) and makoi leaves (*Solanum nigrum*) were procured from local market of Allahabad city. They were washed, dried and stored under refrigeration. The fresh leaves (100g) were blanched using leaves: water ratio of 1:5 (w/v) until they became tender. Pressure cooking of samples (100g) was done in a pressure cooker for 10 min with water ratio of 1:3 (w/v). Water was decanted, boiled and pressure cooked samples were dried at 50 ^{o}C until constant weight reached. Another 100 g of sample was dried at 160 ^{o}C for 10 min in a microwave oven. The fresh, blanched, pressure cooked and dried leaf samples were finely powdered using a Willy Mill of 60 mesh size. All the powdered samples were stored separately in screw capped bottles at a room temperature until further analysis

Proximate Analysis

All the samples were analysed for proximate composition and mineral content (calcium and iron) using AOAC (2005) methods. All the chemicals used were of analytical grade obtained from Merck or Sigma.

Antioxidant Activity

Preparation of Solvent Extract

Raw and processed leaf powders (100 g) were extracted with 500 ml of 70% methanol (w/v) using a shaker, the sample was shaken occasionally for 24 h. The extracts were centrifuged at 5,000 rpm for 20 min and the supernatants obtained were concentrated with a rotary vacuum evaporator (RV-10, IKA) at 45 ^{o}C. The resultant extracts were stored in amber vials at 4 ^{o}C until assayed.

Estimation of Total Phenolic Content (TPC)

Total phenolic content was determined by adopting Folin-Ciocalteu method (Velioglu *et al.,* 1998; and Ying *et al.,* 2013). Basically, 0.2 ml of extract was added to 1.5 ml of Folin-Ciocalteu reagent and mixture was allowed to stand at room temperature for 5 minutes. Then 1.5 ml of sodium carbonate solution (6%) was added into the mixture. Absorbance was measured using spectrophotometer at 725 nm after incubating the sample to stand for 1½ hours at room temperature. Results were expressed as gallic acid equivalent in mg/100 g Dry Weight (DW).

Estimation of Total Tannin Content

Tannin content was determined by the method of Ranganna, 2005. Powdered sample (0.5 g) was boiled with water (75 ml) for 30 minutes and centrifuged at 2000 rpm for 20 minutes and the supernatant was collected. Folin Denis reagent and sodium carbonate were added to the sample extract, solution was diluted to 100ml with water and absorbance is taken at 700 nm after 30 minutes.

Estimation of Total Flavonoid Content

A colorimetric assay (Kim *et al.,* 2003) with some modification was used to quantify total flavonoid content. Briefly, 25 microliter of diluted sample was added to 125 microliter of double distilled H_2O. Subsequently, 7.5 microliter of 5% $NaNO_2$ was added to the mixture and was allowed to stand for 5 minute thereafter 15 microliter of 10% $AlCl_3$ was added. The mixture was incubated at ambient temperature (25 ^{o}C) for additional 5 minutes and 50 microliter of 1 M

NaOH was then added to the mixture. The mixture was immediately diluted by addition of 27.5 microliter of ddH_2O and the absorbance of the mixture was measured at 510 nm against a blank prepared with ddH_2O

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the leaf extracts was estimated by measuring the decrease in absorbance of ethanolic DPPH solution at 517 nm in the presence of the extract (Krings and Berger, 2001; and Koolen *et al.*, 2013). The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. In cases where the absorbance decreased too much before the 30 minutes period the sample was appropriately diluted. The antioxidant activity was expressed as:-

$$DPPH\% = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control absorbance}} \times 100$$

Estimation of Reducing Power

The reducing power of the extracts was determined by using potassium ferricyanide-ferric chloride method (Oyaizu, 1986). Different dilutions of extracts amounting to 1 ml were added to 2.5 ml 0.2 M phosphate buffer (pH = 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 minutes, after which 2.5 ml trichloroacetic acid (10%) was added. 2.5 ml of the mixture was taken and mixed with 2.5 ml water and 0.5 ml of 1% ferric chloride. The absorbance at 700 nm was measured after allowing the solution to stand for 30 minutes.

Antimicrobial Activity

Test Organisms

Four pathogenic bacterial strains were selected for the study to assess antimicrobial activities of selected green leafy vegetables against those strains. The strains were *Escherichia coli* MTCC 1687, *Staphylococcus aureus* MTCC 7443, *Salmonella enterica* MTCC 3219, *Shigella flexeneri* MTCC 1457, obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained on tryptic soy agar (HiMedia) and subcultured regularly. Standard inoculum was prepared by sub-culturing 4-5 freshly grown isolated colonies of each strain in Tryptic Soy Broth (TSB) and incubated at 35-37 ºC for 24 hours. Inocula were standardized with sterile TSB to give final cell load of 10^6-10^7 cfu/ml.

Disc Diffusion Bioassay

The disc diffusion test was performed as described by Jorgensen *et al.* (1999). A 0.5 ml standardized inoculum suspension of each bacterial strain was spread on TSA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Extracts of standard concentrations (10 mg dry weight/ml) were aseptically poured on the discs along with sterile double distilled water or 10% DMSO as negative and streptomycin as positive controls. Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35-37 ºC for 24 hours. The inhibition zone diameters were measured three times and means were represented to nearest mm.

Determination of MICs

Minimum Inhibitory Concentrations (MICs) were determined by broth dilution method in culture tubes (Jorgensen *et al.*, 1999; and Saini and Singh, 2015). Various concentrations (50, 40, 30, 25, 20, 15, 10, 7.5, 5, 2.5, 1.25 mg dry weight/ml) of the extracts were added to broth immediately after inoculating with fresh 0.2 ml culture of the strain, keeping final volume at 5 ml. The cultures were incubated on a rotary shaking incubator at 37 ºC for 48 hours. The lowest concentration of the leaf extracts showing no visible growth was considered as the MIC.

RESULTS AND DISCUSSION

Nutritional Composition

The effect of processing methods on nutritional composition of green leaves is depicted in Table 1. The moisture content was significantly ($p<0.05$) higher in fresh leaves of all the green vegetables ranging from 80.23-88.26%. After processing the pressure cooked samples had higher moisture content (5.39-8.62%) as compared to blanched (5.54-5.98%) and dry heated samples (5.24-5.87%). Oluwalana (2013) has reported that various conventional food processing methods brought a significant (pd”0.05) decrease in the moisture content. Accordingly, reductions in moisture contents resulted in corresponding increase in dry matter contents due to concentration of soluble solids with relatively chemically stable products. The moisture contents of the fresh (raw) and blanched samples were within the range (81.4-90.3%) of some Nigerian green leafy vegetables as reported by Mepba *et al.* (2007), Akubugwo *et al.* (2007) and Olaiya and Adebisi (2010). The higher moisture content observed in the fresh samples is an indication of high susceptibility to microbial spoilage whereas the dried samples which are characterized by lower moisture content will keep longer.

Table 1: Effect of Processing on Nutritional Composition of Green Leaves[1]

Samples	Processing Treatments	Moisture %	Protein %	Fat %	Ash %	Crude Fibre %	Ca (mg/100 g)	Fe (mg/100 g)
Fenugreek leaves	Raw	87.7±1.34^{a}	3.76±0.25^{b}	1.03±0.25^{b}	2.44±0.67^{c}	1.47±0.83^{c}	1.67±0.24^{b}	2.32±0.87^{b}
	Blanched	5.54±1.68^{c}	3.65±0.57^{b}	0.97±0.21^{b}	2.65±0.35^{b}	2.61±0.52^{b}	1.56±0.64^{c}	2.12±0.48^{c}
	Dry heated	5.87±0.88^{c}	3.94±0.42^{a}	1.25±0.41^{a}	2.97±0.65^{a}	4.87±0.26^{a}	1.97±0.45^{a}	2.68±0.84a
	Pressure cooked	7.43±1.75^{b}	3.58±0.63^{b}	1.05±0.53^{b}	2.37±0.74^{c}	2.52±0.37^{b}	1.45±0.37^{c}	2.24±0.56^{b}
Mint leaves	Raw	88.26±1.64^{a}	3.98±0.26^{b}	0.94±0.12^{b}	2.12±0.58^{c}	1.68±0.42^{c}	1.15±0.28^{b}	4.50±0.52^{a}
	Blanched	5.93±1.52^{c}	3.82 ±0.71^{c}	0.88±0.17^{c}	2.9±0.28^{a}	2.94±0.65^{b}	1.05±0.47^{c}	4.11±0.81^{b}
	Dry heated	5.24±0.69^{c}	4.27±0.94^{a}	1.12±0.35^{a}	3.08±0.67^{a}	5.61±0.18^{a}	1.38±0.62^{a}	4.68±0.73^{a}
	Pressure cooked	8.62±1.87^{b}	4.03±0.68^{b}	0.92±0.22^{b}	2.72±0.34^{b}	3.10±0.77^{b}	1.12±0.51^{b}	3.97±0.39^{b}
Amaranth leaves	Raw	80.23±1.45^{a}	4.25±0.55^{b}	0.87±0.14^{a}	2.84±0.75^{d}	2.75±0.38^{d}	1.13±0.68^{b}	11.98±1.04^{a}
	Blanched	5.98±1.32^{c}	4.13±0.49^{b}	0.52±0.19^{b}	3.10±0.49^{c}	3.98±0.31^{b}	0.98±0.24^{c}	9.45±0.23^{b}
	Dry heated	5.56±0.46^{c}	4.79±0.38^{a}	0.80±0.09^{a}	4.23±0.53^{a}	6.64±0.59^{a}	1.27±0.29^{a}	12.67±0.97^{a}
	Pressure cooked	7.42±1.47^{b}	3.95±0.27^{c}	0.68±0.11^{b}	3.57±0.28^{b}	3.35±0.48^{c}	1.02±0.53^{c}	10.13±0.88^{b}
Makoi leaves	Raw	81.66±1.69^{a}	3.68±0.86^{c}	0.76±0.12^{b}	3.11±0.42^{c}	1.93±0.29^{c}	1.87±0.27^{b}	9.87±0.79^{a}
	Blanched	5.72±1.84^{b}	3.89±0.59^{b}	0.71±0.18^{b}	3.23±0.68^{b}	2.18±0.43^{c}	1.66±0.33^{c}	8.56±0.76^{b}
	Dry heated	5.41±0.72^{b}	4.08±0.61^{a}	0.89±0.14^{a}	3.78±0.74^{a}	3.97±0.61^{a}	2.14±0.64^{a}	10.17±0.84^{a}
	Pressure cooked	5.39±1.95^{b}	3.75±0.84^{c}	0.64±0.11^{c}	3.40±0.33^{b}	3.44±0.93^{b}	1.73±0.75^{b}	8.95±0.96^{b}

Note: [1] Each value represents the mean and standard deviation from three lots; Means with different superscripts for each sample are significantly different ($p<0.05$).

The moisture content is in accordance with Yakubu *et al.* (2012) who have also reported a moisture content of 84.4% in bitter leaf samples.

The raw leaves of amaranthus has a protein content of 4.25% whereas the dry heated sample had a higher protein content of 4.79% Similarly in fenugreek leaves also the dry heated sample had a higher protein content (3.94%) than raw leaf sample (3.76%). These results are in accord with those in literature (Parmer *et al.*, 1997; and Atindehou *et al.*, 2004). Lyimo *et al.* (1991) similarly reported protein contents ranging from 3.0-5.0% in bush okro, cocoyam leaves, amaranthus and slippery vine. Appreciable significant ($p<0.05$) increase in crude protein content was noticed in sun-dried and oven-dried vegetable samples (Oboh, 2005). This could be due to increase in concentration of protein as a result of moisture removal. The decrease in protein content during blanching and pressure cooking could be attributed to the fact that some of the proteins were leached off by water during the processing (Oboh, 2005). The reduced protein contents of cooked vegetables could be attributed to the severity of thermal process during cooking also (Sofowara, 1993).

The fat % was low in all the leaf samples ranging from 0.52-1.25% (Table 1). The fresh leaves of Makoi had a significantly lower content of fat (0.76%) as compared to dry heated sample (0.89%). The fat content decreased on blanching and pressure cooking and increased on dry heating. Similarly in mint leaves the dry heated samples a higher fat content of 1.12% as compared to raw (0.94%) blanched (0.88%) and pressure cooked samples (0.92%). Yakubu *et al.* (2012) also reported a decrease in fat content on blanching.

The ash content of amaranthus leaves was 2.84% in raw and 4.23% in dry heated samples. In Makoi leaves the ash content increased from 3.11% (raw) to 3.23% (blanched) and 3.40% (pressure cooked) on processing. Oluwalana (2013) also reported that fresh amaranthus leaf samples had lower ash content than sun dried and oven dried samples. High ash content indicates inherent source of minerals in food. The change in the mineral contents may be due to the breakdown of complex compounds into more simple forms. The total ash contents of fresh and blanched samples were similar to an earlier report by Mepba (2007), Olaiya and Adebisi (2010) and Ashaolu *et al.* (2012).

Crude fibre is useful for maintaining bulk utility and increasing intestinal peristalsis by surface extension of the food in the intestinal tract (Mathenge, 1997). It is necessary for healthy condition, curing of nutritional disorders and for aiding food digestion. The fresh, blanched and pressure cooked samples has a significantly lower crude fiber content compared with dry heated ones. In Amaranthus leaves the raw samples had a crude fibre content of 2.75% whereas dry heated samples had 6.64% (Table 1). In mint leaves also the dry heated samples had crude fibre content of 5.61% which was significantly higher than raw samples (1.68%) ($p< 0.05$). Oluwalana (2013) also reported a crude fibre content of 1.70% in raw leaves and 8.60% in oven dried leaves of *Amaranthus hybridus*. The results are similar to an earlier report by Mepba *et al.* (2007) where the fresh and blanched vegetable samples showed very low crude fibre compared with the sun-dried and oven-dried ones.

The calcium content varied in fenugreek leaves from 1.43% (pressure cooked) to 1.97% (dry heated). The raw leaves had a significantly ($p<0.05$) lower calcium content (1.67%) than dry heated sample (1.97%). Similarly in Makoi leaves the calcium content increased on dry heating of samples and decreased on blanching (1.66%) and pressure cooking (1.73%). Mint leaves also showed slight increase in calcium content on dry heating on samples (1.38%). Iron content was found to be higher in Amaranthus and Makoi leaves as compared to fenugreek and mint leaves. In Amaranthus leaves the dry heated samples (12.67 mg/100 g) had higher iron content than raw (11.98 mg/100 g) samples. Makoi leaves also showed a high iron content of 10.17 mg/100 g in dry heated samples as compared to 8.56mg/100g in blanched sample. Iron content was lower in fenugreek leaves ranging from 2.12-2.68 mg/100 g (Table 1). Yakubu *et al.* (2012) have also reported an iron content of 1.74 mg/100 g in fresh and 12.2 mg/100 g in blanched samples of bitter leaf. These findings are in accordance with a report that green leafy vegetables are good sources of dietary minerals (Akindahunsi and Oboh, 1999). The results are also in agreement with earlier reports by Akindahunsi and Oboh (1999) that abrasion, blanching and soaking may cause a significant decrease in the mineral composition of leafy vegetables.

Antioxidant Activity

A preliminary phytochemical analysis was also carried out to determine the presence of different classes of secondary metabolites. The total phenolic content of fresh fenugreek leaves was 1.41 mg GAE/100 g which reduced significantly on processing. The dry heated samples showed phenolic content of 0.98 mg GAE/100 g. In amaranths leaves the raw samples had a phenolic content of 1.22 mg GAE/100 g whereas in pressure cooked and blanched samples, the content increased to 1.94 mg GAE/100 g and 2.54 mg GAE/100 g, respectively (Table 2). Similarly in fresh makoi leaves the total phenolic content was 1.08 mg GAE/100 g which significantly increased to 1.57 mg GAE/100 g in blanched samples. Siddhuraju (2007) reported that processed samples had lower concentration of phenolic fractions possibly due to the poor extractability by the formation of insoluble tannin- protein and tannin-carbohydrate complexes. The reduction of phenolic content in vegetables may be due to lixiviation (Siddhuraju and Becker, 2003) and the phenols may also bound to other compounds and form insoluble complexes (Fernandez *et al.*, 2003).

Tannin content was also analysed in fresh and processed samples of green leafy vegetables (Table 2). In fenugreek leaves, the raw samples showed tannin content of 4.68 mg tannic acid/100 g which reduced on blanching to 3.75 mg tannic acid/100 g. There was non significant ($p>0.05$) decrease in tannin content on dry heating (4.51 mg tannic acid/100 g) of fenugreek leaves. Tannin content in fresh amaranthus leaves (2.14 mg tannic acid/100 g) showed significant reduction on blanching (1.49 mg tannic acid/100 g) and pressure cooking (1.16 mg tannic acid/100g) of samples. The presence of flavanoids and tannins in all the plants are likely to be responsible for the free radical scavenging effects observed.

Fresh mint leaves showed a flavonoid content of 4.62 mgQ/100 g which reduced to 2.94 mgQ/100 g on dry heating of samples (Table 2). Similarly raw amaranths leaves also showed high flavonoid content of 5.28 mgQ/100 g which decreased to 1.86 mgQ/100 g on dry heating and 3.07 mgQ/100 g on pressure cooking of samples. Phenolic compounds including tannins, flavonoids, saponins and alkaloids have been implicated in pharmacological activities such as antioxidants, antimicrobial and anti-inflammatory activities (Wichtl, 1994). DPPH content was higher in amaranths leaves as compared to fenugreek and mint leaves. In amaranthus, the fresh leaves depicted a free radical scavenging ability of 60.98% which increased to 85.23% on dry heating and 83.78% on blanching of samples (Table 2). The raw makoi leaves showed a radical scavenging activity of 58.99% which also increased to 77.72% on dry heating of samples. In fenugreek leaves, the raw (23.2%) and dry heated samples (21.04%) showed no significant difference in free radical scavenging activity (Table 2).

Table 2: Effect of Processing Methods on Antioxidant Activity of Green Leaves[1]

Samples	Processing Treatments	Total Phenolic Content (mg GAE/100 g)	Total Tannin Content (mg Tannic Acid/100 g)	Flavonoid Content (mgQ/100 g)	DPPH (%)	FRAP (mg Ascorbic Acid/100 g)
Fenugreek leaves	Raw	1.41±0.79^{a}	4.68±0.72^{a}	3.56±0.62^{a}	23.2±0.26^{a}	4.76±0.08^{a}
	Blanched	1.06±0.13^{b}	3.75±0.67^{c}	2.94±1.45^{b}	20.7±0.36^{b}	3.37±0.08^{c}
	Dry heated	0.98±0.29^{b}	4.51±0.32^{a}	2.25±0.89^{b}	21.04±0.04^{a}	3.02±0.19^{c}
	Pressure cooked	1.27±0.16^{a}	4.25±0.49^{b}	2.64±0.7^{b}	20.1±0.17^{b}	3.72±0.05^{b}
Mint leaves	Raw	2.87±0.17^{a}	5.05±0.13^{a}	4.62±1.12^{a}	52.28±0.57^{a}	5.07±0.18^{a}
	Blanched	1.22±0.20^{c}	4.38±0.13^{c}	3.98±0.98^{a}	47.16±0.21^{b}	3.26±0.11^{b}
	Dry heated	1.13±0.19^{c}	4.67±0.13^{b}	2.94±1.06^{b}	47.85±0.44^{b}	2.76±0.12^{c}
	Pressure cooked	1.73±0.21^{b}	4.08±0.16^{d}	3.27±1.13^{b}	43.73±0.64^{c}	3.35±0.38^{b}
Amaranth leaves	Raw	1.22±0.89^{c}	2.14±0.18^{a}	5.28±1.97^{a}	60.98±1.27^{c}	4.41±0.15^{a}
	Blanched	2.54±0.57^{a}	1.49±0.16^{b}	3.44±0.65^{b}	83.78±1.52^{a}	3.28±0.37^{c}
	Dry heated	1.36±0.86^{c}	1.53±0.28^{b}	1.86±0.93^{c}	72.53±1.45^{b}	4.23±0.12^{b}
	Pressure cooked	1.94±0.29^{b}	1.16±0 .35^{c}	3.07±0.90^{b}	85.23±1.36^{a}	3.42±0.53^{c}
Makoi leaves	Raw	1.08±0.58^{c}	2.14±0.18^{a}	3.43±1.52^{b}	58.99±1.27^{b}	9.06±0.18^{a}
	Blanched	1.57±0.51^{a}	1.10±0.41^{c}	5.81±1.79^{a}	63.17±2.40^{b}	7.04±0.41^{b}
	Dry heated	1.18±0.90^{b}	1.74±0.78^{b}	4.22±1.08^{b}	77.72±1.36^{a}	7.08±0.36^{b}
	Pressure cooked	1.20±0.74^{b}	1.12±0.21^{c}	5.04±1.14^{a}	62.74±2.53^{b}	6.04±0.78^{c}

Note: FRAP: Ferric reducing (mg Ascorbic Acid/100 gm); [1] Each value represents the mean and standard deviation from three lots; Means with different superscripts for each sample are significantly different ($p<0.05$).

Greater inhibition of free radicals in leafy vegetables may be due to the presence of high amount of alkaloids, flavonoids, phenolics and tannins which are known antioxidants. The presence of these compounds in the extracts of these vegetables may also be the main cause of their high radical-scavenging activity and popularly reported high health beneficial properties (Oboh *et al*., 2006; and Uusiku *et al*., 2010). According to Tsai and She (2006) a change in phenolic compounds after heating might be contributed to a decrease in DPPH-scavenging ability.

The reducing power was highest in makoi leaves (9.06 mg Ascorbic Acid/100 g). The reducing power was higher in fresh leaf samples and decreased on processing. Fresh fenugreek leaves had a reducing power of 4.76 mg AA/100 g which reduced to 3.37, 3.02, 3.72 mg AA/100 g on blanching, dry heating and pressure cooking, respectively. Similarly, the fresh mint leaves had a reducing power of 5.07 mg AA/100 g which decreased significantly ($p<0.05$) on blanching to 3.26 mg AA/100 g and on pressure cooking to 2.76 mg AA/100 g. The decrease in reducing power of pressure cooked samples correlates with the low level of phenolic contents since, during cooking, a part of phenolics diffuse from the seed coat to cooking water (Rocha-Guzman *et al.*, 2007).

Antimicrobial Activities

The antimicrobial activity of fresh leaf extracts in different solvents was studied against four pathogens viz. *E coli, Shigella flexeneri, Salmonella enterica*, and *Staphylococcus aureus*. The antimicrobial activity was higher in fenugreek and mint leaves as compared to amaranthus and makoi leaves. The ethanolic extracts with inhibition zone of 33-37 mm showed higher antimicrobial activities than other extracts (Table 3). Ethanolic extracts of fenugreek leaves showed highest antimicrobial activity against *E. coli* followed by *S. aureus*. The hexane and petroleum ether extract of fenugreek showed no antimicrobial activity against any pathogen (Table 3). Aqueous extract of Amaranthus leaves showed higher antimicrobial activity with ethanolic and petroleum ether extracts. Mint leaf extracts of amaranthus showed broad spectrum antimicrobial activity with zone of inhibition ranging from 14-39 mm. Extracts with more than 14mm zone of inhibition were considered as having good antimicrobial activity (Aligiannis *et al*., 2001).

Table 3: Antimicrobial Activities, Indicated by Diameter of Inhibition Zone (DIZ, mm, for 10 mg Dry Weight/Disc, Mean ± SD)

Leaves	Sample Extracts	*Escherichia coli*	*Shigella*	*Salmonella enterica*	*Staphylococcus aureus*
Fenugreek	Aqueous	18±1	20±1	20±2	19±1
	Acetone	26±2	26±1	25±1	26±2
	Hexane	0	0	0	0
	Ethanol	37±2	33±1	34±1	35±1
	Petroleum ether	0	0	0	0
Mint	Aqueous	28±1	23±1	28±1	24±2
	Acetone	21±1	20±2	26±1	20±1
	Hexane	18±2	14±1	16±2	14±1
	Ethanol	37±2	36±1	35±1	39±2
	Petroleum ether	15±1	15±1	20±1	19±1
Amaranth	Aqueous	14±1	10±1	11±1	8±1
	Acetone	14±1	9±1	10±1	12±1
	Hexane	12±1	11±1	11±1	12±1
	Ethanol	12±1	9±1	15±1	8±1
	Petroleum ether	10±1	8±1	12±1	5±1
Makoi	Aqueous	18±1	15±1	10±1	12±1
	Acetone	0	5±1	0	8±1
	Hexane	10±1	12±1	11±1	11±1
	Ethanol	9±1	10±1	12±1	12±1
	Petroleum ether	12±1	10±1	9±1	10±1
Streptomycin		15±3	16±2	11±2	12±1

Table 4: Antimicrobial Activities, Minimum Inhibitory Concentration of Green Leafy Vegetables (MIC for mg Dry Weight/ml)

Leaves	Sample Extracts	*Escherichia coli*	*Shigella*	*Salmonella enterica*	*Staphylococcus aureus*
Fenugreek	Aqueous	15	40	30	5
	Acetone	30	30	20	15
	Hexane	*	*	*	*
	Ethanol	10	30	20	7.5
	Petroleum ether	*	*	*	*
Mint	Aqueous	20	30	40	10
	Acetone	20	10	30	15
	Hexane	30	40	40	15
	Ethanol	15	15	30	10
	Petroleum ether	30	40	30	25

Table 4 (Cont.)

Amaranth	Aqueous	20	20	20	15
	Acetone	25	20	40	20
	Hexane	*	40	40	25
	Ethanol	10	40	20	10
	Petroleum ether	40	40	40	40
Makoi	Aqueous	20	20	40	20
	Acetone	40	40	40	30
	Hexane	20	20	40	40
	Ethanol	15	10	30	10
	Petroleum ether	40	40	20	20
Streptomycin		7.5	5	5	10

Note: "*" shows no MIC value.

The MIC assay of different extracts showed that fenugreek had the highest antimicrobial action against the strains tested, followed by mint, makoi and amaranthus leaves. Whether the aqueous or ethanolic extract would work well on microbes, it all depends on active constituents (de Boer *et al*., 2005). Among the microbial strains tested, *Salmonella enterica* and *Shigella flexeneri* were the most resistant microbes while *Staphylococcus aureus* and *E. coli* were the most susceptible strains. Differential antimicrobial activity of herbs against different bacteria might be due to presence of different active phyto-compounds. Among different antimicrobial compounds; phenolic compounds, terpenoids, and alkaloids are very important for antimicrobial or antioxidant effects (Hoult and Paya, 1996; and Rios and Recio, 2005). Streptomycin, used as standard showed a high inhibitory effect on all the strains tested. The study also revealed that ethanolic extracts showed higher inhibitory activity than other extracts. Previous studies (Akinnibosun *et al*., 2008) confirm that alcohol is a better solvent for the extraction of antimicrobials from *P. pellucida* and *P. umbellatum* for the inhibition of growth of some pathogenic bacteria. Similarly studies (Olajumoke *et al*., 2012), with aqueous extracts of *P. pellucida* on *Staphylococcus aureus* strain showed that water is not a good solvent for the extraction of solutes which have inhibitory activity.

CONCLUSION

Vegetables are highly perishable; they lose their eating quality very rapidly after harvesting. These processing methods can help to prolong the shelf life of the vegetable as well as enhance the nutritive properties of the vegetable. The presence of phytochemicals such as tannins, phenolics and flavonoids in the selected leafy vegetables may have crucial roles in the observed antioxidant and antibacterial potential of the leaves. Therefore, further research is required towards the isolation and identification of the active ingredients in these vegetables. The inhibitory factor responsible for the antimicrobial activity can further be identified and used as an alternative to currently used drugs against the pathogenic microbes under study.

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