Journal of Food and Drug Analysis

Volume 20 | Issue 4 Article 21

# Differential effects of processing methods on total phenolic content, antioxidant and antimicrobial activities of three species of Solanum

*Journal of Food and Drug Analysis, Vol. 20, No. 4, 2012, Pages 844-854* doi:10.6227/jfda.2012200414

# Differential Effects of Processing Methods on Total Phenolic Content, Antioxidant and Antimicrobial Activities of Three Species of *Solanum*

**SRINIVASAN NITHIYANANTHAM, SUBRAMANIAN VARADHARAJAN AND PERUMAL SIDDHURAJU***

*Bioresource Technology Lab, Department of Environmental Sciences, Bharathiar University, Coimbatore, India*

(Received: October 11, 2011; Accepted: June 11, 2012)

## ABSTRACT

The present study evaluated the antioxidant and antimicrobial activities of raw and processed fruit extracts from *Solanum torvum*, *S. xanthocarpum* and *S. violaceum* and assessed the relationship with their total phenolic content. Total phenolic and tannin contents of raw and processed fruit sample extracts ranged from 5.0-7.6 g/100g and 4.5-7.0 g/100g, respectively. As compared to raw samples, processed samples had significant decrease in total phenolic and tannin content, FRAP, linoleic acid emulsion system, antihaemolytic and superoxide anion radical scavenging activity. However, processing the samples caused significant ($p$<0.05) increases in DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $^{\bullet}$OH radical scavenging capacities, metal chelating ability and phosphomolybdenum assay. After processing, the total antioxidant activity increased or remained unchanged depending on the type of samples. All the fruit samples showed optimal antimicrobial activity against Gram negative bacteria. Interestingly, the extracts from raw and processed *S. torvum* showed no inhibitory effect on the growth of *E. coli*.

Key words: Solanaceous vegetable, antioxidant activity, phenolics, tannins, free radical scavenging capacity and antimicrobial activity

## INTRODUCTION

Antioxidants can inhibit or delay the oxidation of oxidizable substrates and this appears to be very important in the prevention of oxidative stress which is suggested as the leading cause of many oxidation related diseases[1]. Recently, mainly due to undesirable side effects such as toxicity and carcinogenicity of synthetic additives, interest has considerably increased for searching for naturally occurring antioxidant and antimicrobial compounds suitable for use in food and/or medicine[2,3]. In this regard, research was conducted on many plant species in order to find new natural bioactive compounds. Vegetables are a major source of antioxidants. It is therefore desirable to assess the antioxidant activity of different foods and to compare different storage or processing methods. Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids. A number of reports studied the antioxidant properties of either isolated plant constituents such as flavonoids[4-6], flavonols[7], essential oils[8], tannins[9] or plant extracts obtained from vegetables, fruits, spices, and wine[10,11]. The effects of processing on food antioxidant properties[12-14] were also extensively studied. The family *Solanaceae* comprises about 80 genera and 3000 species, from which 1500 belong to the genus *Solanum*. This genus is widespread over the world although it is concentrated mainly in the tropics and subtropics. The genus has toxic alkaloids which are distributed in all parts of the plant[15]. Several *Solanum* species contain free and glycosilated alkaloids, important substrates for the synthesis of steroidal hormones[16,17], thus making these species very important economically. *Solanaceae* species play an important role by favouring the colonization of open areas and consequently in forest regeneration processes. Many of these species are used as tonics, antirheumatic, remedies for cold, fever dizziness and are consumed as vegetables for high nutritive value or as mild anticonvulsants. Research has shown that some *Solanum* species have antiviral, anticancer, anticonvulsant and anti-infective agents[18]. The objectives of this study were (i) to determine the total phenolic content, antioxidant and antimicrobial activities of three species of *Solanum*, (ii) the effects of cooking on total phenolic content and antioxidant activity and (iii) to evaluate free radical scavenging, peroxidation inhibition and metal chelating activities of these species of *Solanum*.

* Author for correspondence. Tel: 91-422-2428394;
Fax: 91-422-2422387; E-mail: siddhurajubrt@yahoo.com

*Journal of Food and Drug Analysis, Vol. 20, No. 4, 2012*

## MATERIALS AND METHODS

### I. *Chemicals*

Ferric chloride, 2, 2′-diphenyl-1picrylhydrazyl (DPPH), potassium persulfate, 2,2 azinobis (3-ethylbenzo-thiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy -2,5,7,8-tetramethylchroman 2-carboxylic acid (trolox), linoleic acid, ferrous chloride, ammounium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra acetic acid (EDTA) disodium salt and were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

### II. *Fruit Samples and Processing*

The fresh fruit samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum* were collected from the market in Coimbatore district, Tamilnadu. Fifty grams of raw fruits of each variety were boiled (processed) for 10 min, with water ratio of 1 : 5 (w/v). Then water was decanted and then boiled fruit samples were dried at 40°C until constant weight was reached. The raw samples were cut into small pieces, air-dried in the shade. The raw and processed fruit samples were finely powdered using a blender. All the powdered samples were stored separately in a screw capped bottle at room temperature until further analysis.

### III. *Solvent Extraction*

The raw and boiled ground fruit samples (15 g) were extracted by stirring with 105 mL 80% methanol at 25°C for 48 h and filtered through a Whatman No. 4 filter paper. The residues were re-extracted with an additional 75 mL 80% methanol, as described above, for 3 h. The combined extracts were dried at 40°C in an incubator until constant weight. The dried extract was used directly for total phenolics and tannins estimation and also for the assessment of antioxidant capacity using various *in vitro* assays.

### IV. *Extract Recovery Percentage*

From the extract, a known volume was taken, dried in an oven at incubator temperature of 40°C until sample getting a constant weight and the recovery percent was calculated as follows:

$$\text{Recovery\%} = \frac{(\text{Extract+container (g)}) - (\text{Empty container (g)})}{\text{Sample weight (g)}} \times 100$$

### V. *Estimation of Total Phenolics and Tannins*

The total phenolic content was determined according to Folin-Ciocalteau method (FCM)[19]. FCM actually measures a sample's reducing capacity and can be considered as antioxidant (electron transfer) capacity assay. For the assay, aliquots (100 µL) of extracts were taken in test tubes and made up to 1 mL with distilled water. Then 0.5 mL of Folin-Ciocalteau phenol reagent (1 : 1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. After vortexing, the test tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE). Tannins in the extracts were estimated after treatment with polyvinyl poly pyrrolidone (PVPP). One hundred milligrams of PVPP was weighed in a 100 mm×12 mm test tube and to this 1.0 mL of distilled water and then 1.0 mL of tannin containing phenolic extract were added. The content was vortexed and kept at 4°C for 4 h. Then the sample was centrifuged at 3000 ×g for 10 min at room temperature and the supernatant was collected. This supernatant has only simple phenolics other than tannins since the tannins would have been precipitated along with the PVPP. The phenol content of the supernatant was measured, as mentioned above and expressed as the content of non-tannin phenolics on a dry matter basis[20]. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

### VI. *Ferric Reducing/Antioxidant Power (FRAP) Assay*

The antioxidant capacity of phenolic extracts of raw and processed *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruit samples was estimated according to the procedure described by Benzie and Strain[21] as modified by Pulido *et al.*[22]. FRAP reagent (900 µL), prepared freshly and incubated at 37°C, was mixed with 90 µL distilled water and 30 µL test sample, or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The FRAP reagent contained 2.5 mL 20 mM TPTZ (2,4,6-tripyridyl-1,3,5-triazine) solution in 40 mM HCl plus 2.5 mL 20 mM $FeCl_3.6H_2O$ and 25 mL of 0.3 M acetate buffer, pH 3.6[21]. At the end of incubation the absorbance readings were taken immediately at 593 nm on a Spectrophotometer. Methanolic solutions of known Fe (II) concentration ranging from 100 to 2000 µmol/L ($FeSO_4.7H_2O$) were used to construct the calibration curve. The parameter Equivalent Concentration ($EC_1$) was defined as the concentration of antioxidant which has a ferric- TPTZ reducing ability equivalent to that of 1 mM $FeSO_4.7H_2O$. $EC_1$ was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mM Fe (II) solution determined using the corresponding regression equation.

### VII. *Phosphomolybdenum Assay*

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex according to the method of Prieto *et al.*[23]. An aliquot of 100 µL of sample solution was combined with 1 mL of reagent solution (0.6 M

sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 mL vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the mixture was measured at 695 nm. The results reported are mean values expressed as μmol of ascorbic acid equivalents/g extract (ascorbic acid equivalent antioxidant activity).

VIII. *Stable Free Radical Scavenging Activity by DPPH$^{\bullet}$ Method*

The antioxidant activity of the *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruit extracts, ascorbic acid and BHA was measured in terms of hydrogen donating or radical scavenging ability, by DPPH$^{\bullet}$ method[24] modified by Sanchez-Moreno *et al*.[25]. A methanol solution (0.1 mL) of the sample extracts at various concentrations was added to 3.9 mL (0.025 g/L) of DPPH$^{\bullet}$ solution. The solution was incubated at room temperature for 60 min and then the decrease in absorbance at 515 nm was determined. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the samples and was calculated using the following formula:

(%) radical scavenging activity=((Control OD–Sample OD)/Control OD)×100

IX. *Total Antioxidant Activity Assay by Radical Cation (ABTS$^{\bullet+}$)*

ABTS$^{\bullet+}$ was dissolved in water to a 7 mM concentration, ABTS radical cation (ABTS$^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1 : 89 v/v) and equilibrated to 30°C to give an absorbance at 734 nm of 0.700±0.02 in a 1 cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 μL aliquot of each dilution into the assay, they produced between 20-80% inhibition of the blank absorbance. After the addition of 1 mL diluted ABTS$^{\bullet+}$ solution to 10 μL of samples or Trolox standards (final concentration 0-15 μM) in ethanol, absorbance was taken at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run for each assay. Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration[26]. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox with equivalent antioxidant activity expressed as μmol/g sample extracts.

X. *Antioxidant Activity in Linoleic Acid Emulsion System*

The antioxidant activity of the extracts was determined by the thiocyanate method of Mitsuda *et al.*[27] as described by Yen and Hsieh[28]. Each sample (1 mg/mL) in 0.5 mL absolute ethanol was mixed with a linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0) in phosphate buffer (2 mL, 0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing and homogenizing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL phosphate buffer. The reaction mixture was incubated at 37°C. Aliquots of 0.1 mL were taken at several intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (4.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl). After the mixture has rested for 3 min, the peroxide value was determined by monitoring the absorbance at 500 nm. A control was performed with linoleic acid without the samples. The degree of oxidation was measured for every 24 h until a day after the absorbance of the control reached its maximum. The lipid peroxidation inhibition (LPI) % was calculated as:

$$LPI\ (\%) = (1–(A–A_1))\times 100$$

Where A represents absorbance of sample at 48$^{th}$ h, while $A_1$ represents absorbance of control at 48$^{th}$ h.

XI. *Superoxide Anion ($O_2^{\bullet-}$) Radical Scavenging Activity*

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich[29], described by Zhishen *et al*.[30]. All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were performed using 20 W fluorescent lamps. The concentration of extracts in the reaction mixture was 50-200 μg/mL. The total volume of the reactant mixture was 5 mL and the concentrations of the riboflavin, methionine and nitro blue tetrazolium (NBT) was $3\times10^{-6}$, $1\times10^{-2}$ and $1\times10^{-4}$ M, respectively. The reactant was illuminated at 25°C for 25 min. The photochemically reduced riboflavins generated $O_2^{\cdot-}$ which reduced NBT to form blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance (A) was measured at 560 nm. The sample extracts were added to the reaction mixture, in which $O_2^{-\bullet}$ was scavenged, thereby inhibiting the NBT reduction. Absorbance (A) was measured and the decrease in $O_2^{-\bullet}$ was represented by $A_1$. The degree of the scavenging was calculated by the following equation:

$$Scavenging\ (\%) = ((A–A_1)/A)\times 100$$

XII. *Antihaemolytic Activity*

Bovine erythrocytes were separated by centrifugation and washed with phosphate buffer (pH 7.4) until the supernatant appeared colourless. The erythrocytes were then diluted with saline or phosphate buffer to give 4% suspension. Five hurdred microgram of sample/mL saline buffer were added to 2 mL of the suspension of erythrocytes and the volume was made up to 3-5 mL with saline buffer. This mixture was pre-incubated for 5 min and then 0.5 mL $H_2O_2$ solutions of appropriate concentration in saline buffer were added. The

concentration of $H_2O_2$ in the reaction mixture was adjusted so as to bring about 90% haemolysis of blood cells after 240 min. After incubation, the reaction mixture was centrifuged at 1,000 ×g for 10 min and the extent of haemolysis was determined by measurement of the absorbance (at 540 nm) corresponding to haemogloblin liberation[31].

(%) Antihaemolytic activity=((Control OD–Sample OD)/Control OD) ×100

XIII. *Metal Chelating Activity*

The extracts (100 µL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract[32].

XIV. *Hydroxyl Radical Scavenging Activity*

The scavenging activity of the extracts of raw and processed fruit samples on hydroxyl radical were measured according to the method of Klein *et al*.[33]. Various amount (100, 200 and 300 µg) of extracts were added to 1.0 mL iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL EDTA solution (0.018%) and 1.0 mL dimethyl sulfoxide (DMSO; 0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in water bath. After incubation, the reaction was terminated by addition of 1.0 mL ice-cold TCA (17.5% w/v). Three milliliter Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following formula:

(%) HRSA = (1–(difference in absorbance of sample / difference in absorbance of blank)) ×100

XV. *Statistical Analysis*

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple-range test ($p<0.05$) using software SPSS 13. Values expressed are means of triplicate determinations±standard deviation. Pearson's correlation test was conducted to determine the linear correlations among variables.

XVI. *Microorganisms and Culture Conditions*

The microorganisms used for this antimicrobial assay were procured from MTCC, IMTECH, Chandigarh, India. This includes *Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 424), *Salmonella typhi* (MTCC 3215) and *Escherichia coli* (MTCC 40). They were further subcultured and maintained on nutrient agar medium at 4°C.

XVII. *Antibacterial Activity*

The antibacterial activity of extracts was analyzed by agar disc diffusion method of Bauer *et al*.[34] and Skytta *et al*.[35] with slight modifications. One milliliter overnight bacterial culture was inoculated into 100 mL of nutrient broth and incubated at 37°C for 3 h to get the exponential phase culture. They were diluted to the optical densities at 600 nm ranging from 0.150-0.250. One hundred µL fresh bacterial culture (approximately $10^6$ CFU/mL) were inoculated into sterile Muller Hinton agar plates using a sterile spreader. Sterile discs (6 mm) were loaded with 20 µL (2000 µg/mL) of extracts dissolved in 5% DMSO and left to dry for 1 to 2 h under sterile conditions. The dried discs were placed on the inoculated plates with the positive controls of standard antibiotics such as gentamycin, ciprofloxacin, amikacin, tetracycline and streptomycin (10 µg/disc) and negative control as sterile disc treated with DMSO. The plates were then incubated at 37°C for 24-36 h. Diameter of inhibition zones around each disc were measured and recorded at the end of the incubation time.

## RESULTS AND DISCUSSION

I. *Antioxidant Activity Studies*

There is great interest in the use of antioxidants that intercept ROS to ameliorate oxidative stress-induced diseases. Numerous plant materials, such as vegetables, fruits, spices and herbs, are established sources of natural antioxidants. In the present investigation, an attempt was made to study the antioxidant activity of the raw and processed fruit samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum*. There are many different antioxidant components in plants, and it is relatively difficult to measure each antioxidant component separately. Therefore, several different methods have been developed to evaluate the antioxidant activity of biological samples[36-38].

II. *Extract Yield Percentage, Total Phenolic and Tannin Content of Fruit Extracts*

The extract yield percentage, total phenolics and tannins of extracts obtained from raw and processed fruit samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum* were shown in Table 1. Among the samples maximum yield was obtained from the extracts of *S. violaceum* (26.7%) processed sample. The extractable total phenolic and tannin content of raw samples was found to be higher than that of processed samples. This study indicated that cooking caused loss of phenolics in all the fruit samples, which is

**Table 1.** Solvent extract recovery percentage, Total phenolics and Tannin contents of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits

| Samples | Extract yield (%) | Total phenolics (g/100g extract) | Tannins (g/100g extract) |
|---|---|---|---|
| *S. torvum* | | | |
| Raw | 13.9 | $5.8^{bc}$±0.4 | $5.3^{bc}$±0.4 |
| Processed | 10.2 | $5.0^{c}$±0.6 | $4.5^{c}$±0.6 |
| *S. xanthocarpum* | | | |
| Raw | 5.7 | $7.6^{a}$±0.3 | $7.0^{a}$±0.4 |
| Processed | 9.3 | $6.1^{abc}$±0.3 | $5.6^{abc}$±0.4 |
| *S. violaceum* | | | |
| Raw | 2.9 | $6.6^{ab}$±1.4 | $6.2^{ab}$±1.4 |
| Processed | 26.7 | $5.0^{c}$±1.0 | $4.7^{bc}$±0.9 |

Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($p$<0.05) different.

consistent with previous findings. Sahlin *et al.*[39] found that cooking by boiling, baking and frying resulted in a significant reduction ($p$<0.01) in the total phenolic, ascorbic acid and lycopene contents in tomatoes. In the case of boiling or pressure cooking lixiviation phenomenon occurs that leads to a 64% loss of total carotenoids and a 49% loss of total phenolics[40]. The phenols enter the cooking water and complex phenol proteins are found, reducing by 90% or more according to Barroga *et al.*[41] and Rocha-Guzman *et al.*[42]. Total phenolics are usually stored in vegetables in pectin or cellulose networks and can be released during thermal processing. Individual phenolics may sometimes increase because heat can break supramolecular structures, releasing the phenolic sugar glycosidic bonds, which react better with the folin-ciocalteau reagent[40].

III. *Ferric Reducing/Antioxidant Power (FRAP) Assay*

Antioxidants can be explained as reductants. The inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Since FRAP measures the reduction of Fe (III)-TPTZ to Fe (II)-TPTZ, there are some pitfalls to this method that need to be discussed. Apart from measuring the potential of biological antioxidants, it also measures chemical reductants that would reduce the ferric complex to the ferrous form and not all of these reductants are antioxidants. Some antioxidants such as glutathione (GSH) are unable to reduce Fe (III), therefore, this test does not account for the SH-group of antioxidants[43]. Table 2 shows the reducing power of raw and processed fruit samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum.* The ferric reducing ability of the extracts revealed that all of them posses optimal FRAP activity (7.0-39.0 mmol Fe(II)/µg extract). The highest activity was observed for SVR (39.0 mmol Fe(II)/µg extract) followed by SVP (29.5 mmol Fe(II)/µg extract). It is widely believed that many food antioxidant components can be significantly lost as a consequence of industrial sterilization, pasteurization and dehydration as well as domestic cooking[44]. However, processing does not always result in the destruction of the antioxidant components. In some cases, processing factors could induce the formation of compounds[14,45].

IV. *Phosphomolybdenum Assay*

The phosphomolybdate method has been routinely used to evaluate the antioxidant capacity of extracts[23]. In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum V complex, which shows a maximum absorbance at 695 nm. In the present study, the highest activity was noted for SVP (64.7 µmol ascorbic acid/g extract) followed by STR (56.4 µmol ascorbic acid/g extract). The measurements and the ascorbic acid equivalence of reducing power are reported in Table 2. The differential response of the extracts in various antioxidant tests may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occurred at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants[46].

V. *Free Radical Scavenging Activities on DPPH$^{\bullet}$ and ABTS$^{\bullet+}$*

The DPPH and ABTS are decolorization assays which measure the relative antioxidant potentials of natural extracts to scavenge free radicals (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, respectively) generated in the assay system[47]. Both assays are the most commonly used antioxidants methods due to excellent reproducibility under certain assay conditions. However, they show significant differences in their response to antioxidants. DPPH is a free radical and stable at room temperature, which is violet in ethanol. Reduction of DPPH by antioxidants results in a loss of absorbance. The DPPH values of the antioxidant extracts are presented in Table 3. In summary, STP (1.9 g extract/g DPPH) had the highest radical scavenging ability followed by the SVR (2.0 g extract/g DPPH). A lower value of $IC_{50}$ indicates a higher antioxidant activity. However, it is worth noting that this increase in antioxidant activity with cooking, agrees with earlier report on the effect of cooking on the antioxidant properties of tropical green leafy vegetables[48].

ABTS is one of the radicals generally used for testing the preliminary radical scavenging activity of a compound or plant extract. The ABTS$^{\bullet+}$, generated from oxidation of ABTS by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavengers of lipid peroxyl radicals)[49]. Among the samples, SXP (236.1 µmol/g extract) exhibited the greatest scavenging ability followed by SVP (234.4 µmol/g extract) and STR (226.1 µmol/g extract). Halvorsen *et al.*[50] reported large increases in antioxidant

activities for several vegetables such as carrots, spinach, mushrooms, asparagus, broccoli, cabbage, red cabbage, green and red peppers, potatoes and tomatoes after steaming and boiling. Furthermore, thermal processing could lead to the formation of novel compounds with antioxidant activity or the release of bound phenolic compounds[14,48,51,52].

VI. *Antioxidant Activity in Linoleic Acid Emulsion System*

The ability of antioxidants to scavenge peroxyl radicals through hydrogen donation during polyunsaturated fatty acids (PUFA) oxidation was assessed by the ferric thiocyanate method[53,54]. Thiocyanate reacts with ferric ion, formed through reduction of the ferrous ion by peroxides, to produce a red-colored complex, the absorbance of which is monitored every 24 h until the reaction is complete[55]. Figure 1 shows that the peroxidation inhibition activity of raw and processed methanol extracts of *S. torvum*, *S. xanthocarpum* and *S. violaceum.* The peroxidation inhibition in the presence of different extracts SXR (99%), SVR (99%), STR (98%), STP (98%), SXP (98%) and SVP (98%). All the samples of raw and processed fruit extracts showed higher inhibition of peroxidation when compared to the positive controls such as BHA (90%), BHT (91%), RUT (89%), QUE (93%) and TRO (91%). The findings of the present study are similar to the Muricia *et al.*[56] which reported that raw vegetables were able to scavenge lipoperoxyl radicals, being very good antioxidant with a percentage of inhibition higher than 75% for beet root, spanish, swiss chard, broad bean and artichoke.

VII. *Superoxide Anion ($O_2^{\bullet-}$) Radical Scavenging Activity*

Superoxide is a reactive oxygen species, which can cause damage to the cells and DNA leading to various diseases. It was, therefore, proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical[57]. Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as *via* non enzymatic reactions such as autooxidation by catecholamines. Figure 2 shows the percentage inhibition of superoxide radical generation by *S. torvum*, *S. xanthocarpum* and *S. violaceum* in comparison with BHA, BHT, Rutin and Quercetin. Among the samples, STR (85%) exhibited a strong radical scavenging activity when compared with positive controls. The present finding is contradictory to that by Sini and Devi[58] which reported that 97% scavenging effect was exhibited in *S. trilobatum,* and in *S. melongena* was found about 45% in raw and 70% in the boiled samples[59]. The major source of free radical production *in vivo* is through superoxides, which are produced by the leakage of a free electron during its transport in mitochondria. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxy radicals.

VIII. *Antihaemolytic Activity*

The oxidation of PUFA in biological membranes can lead to the formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and even destruction of membrane lipids. Many of these biochemical activities can lead to the production of breakdown products that are highly toxic to most cell types[60]. The antihaemolytic activity of raw and processed samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum* are presented in Figure 3. Among the various samples, SVR (63%) and SVP (63%) exhibited high antihaemolytic activity.

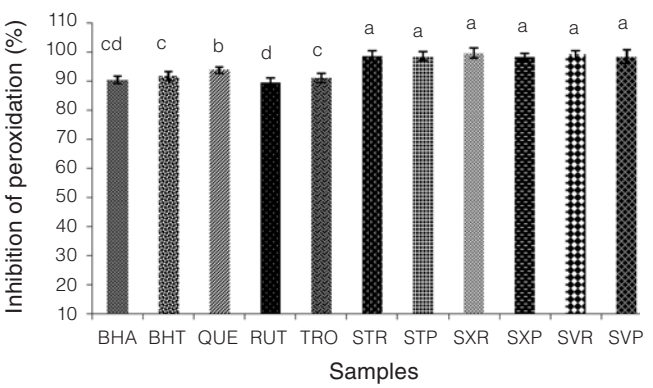


**Figure 1.** Peroxidation inhibiting activities of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits. Peroxidation inhibiting activities of raw and processed samples of *Solanum* spp. BHA-Butylated hydroxy anisole; BHT- Butylated hydroxy toluene; QUE- Quercetin; RUT- Rutin; TRO- Trolox; STR- *Solanum torvum* raw; STP- *Solanum torvum* processed; SXR- *Solanum xanthocarpum* raw; SXP- *Solanum xanthocarpum* processed; SVR- *Solanum violaceum* raw; SVP- *Solanum violaceum* processed. Values are means of triplicate determinations ± standard deviation. Bars having different letters are significantly different ($p < 0.05$).

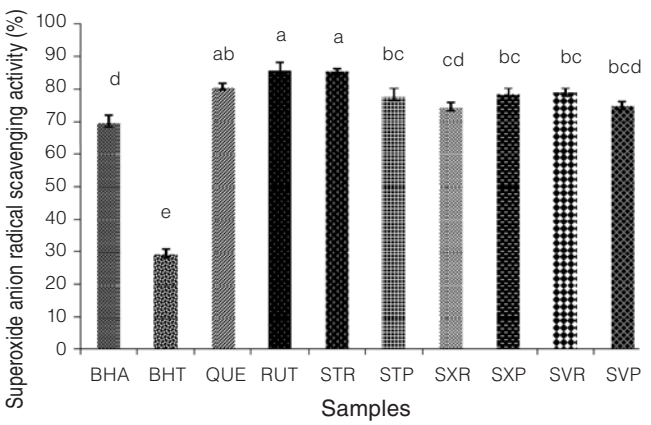


**Figure 2.** Superoxide anion radical scavenging activities of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits. Superoxide anion radical scavenging activities of raw and processed samples of *Solanum* spp. BHA- Butylated hydroxy anisole; BHT- Butylated hydroxy toluene; QUE- Quercetin; RUT- Rutin; STR- *Solanum torvum* raw; STP- *Solanum torvum* processed; SXR- *Solanum xanthocarpum* raw; SXP- *Solanum xanthocarpum* processed; SVR- *Solanum violaceum* raw; SVP- *Solanum violaceum* processed. Values are means of triplicate determinations ± standard deviation. Bars having different letters are significantly different ($p < 0.05$).

Nonetheless, when compared to the positive controls like BHA (53%) and RUT (50%), the raw and processed samples of *S. violaceum* had higher antihaemolytic activity. Lipid oxidation of bovine blood erythrocyte membrane mediated by $H_2O_2$ induces membrane damage and subsequently haemolysis[61]. Hence, the red blood cell (RBC) haemolysis is a more sensitive system for evaluating the antioxidant properties of phytoceuticals.

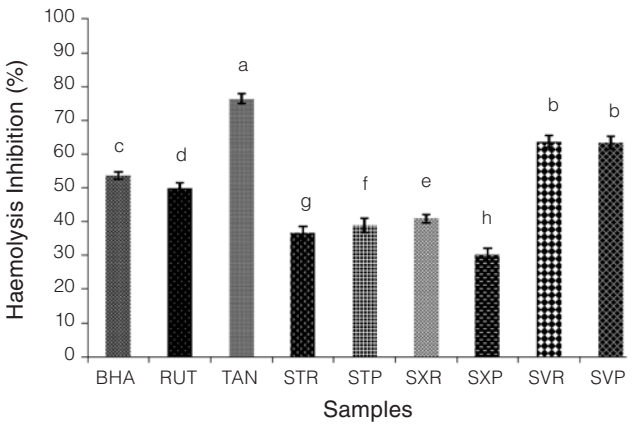


**Figure 3.** Antihaemolytic activities of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits. Antihaemolytic activities of raw and processed samples of *Solanum* spp. BHA- Butylated hydroxy anisole; RUT- Rutin; TAN-Tannic acid; STR- *Solanum torvum* raw; STP- *Solanum torvum* processed; SXR- *Solanum xanthocarpum* raw; SXP- *Solanum xanthocarpum* processed; SVR- *Solanum violaceum* raw; SVP- *Solanum violaceum* processed. Values are means of triplicate determinations ± standard deviation. Bars having different letters are significantly different ($p < 0.05$).

IX. *Metal Chelating Activity*

The ability of antioxidants to form insoluble metal complexes with ferrous ion or to generate steric hindrance that prevent interaction between metal and lipid is evaluated using the iron chelating capacity assay[62]. Activity is measured by monitoring the decrease in absorbance of the red $Fe^{2+}$/ferrozine complex as antioxidants competing with ferrozine in chelating ferrous ion[55]. Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play important roles as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions *via* Fenton chemistry[63]. These processes can be delayed by iron chelation and deactivation. Therefore, the ability of the extracts to chelate iron (II) ions was evaluated and expressed as % chelation capacity. The metal chelating property of raw and processed fruit samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum* are presented in Table 2. Among the samples, the highest activity was noted for STP (29.2 mg EDTA/g extract) followed by SXR (29.0 mg EDTA/g extract). No correlation was found between iron-chelating capacity and phenolic content. This may indicate the presence of other antioxidants responsible for metal chelation. Non-phenolic metal chelators include phosphoric acid, citric acid, ascorbic acid, carnosine, some amino acids, peptides and proteins such as transferrin and ovotransferrin[53].

X. *Hydroxyl Radical Scavenging Activity*

Hydroxyl radicals generated *via* Fenton reaction are

**Table 2.** Metal chelating activity, Phosphomolybedum and FRAP assay of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits

| Samples | Metal chelating (mg EDTA/g extract) | Phosphomolybdenum (μmol ascorbic acid/g extract) | FRAP (μg extract/mmol Fe(II))[A] |
|---|---|---|---|
| *S. torvum* | | | |
| Raw | $11.9^{ef}$±2.5 | $56.4^{c}$±4.8 | $7.2^{e}$±0.0 |
| Processed | $29.2^{c}$±1.4 | $14.6^{d}$±0.2 | $28.9^{c}$±0.0 |
| *S. xanthocarpum* | | | |
| Raw | $29.0^{c}$±1.0 | $12.4^{de}$±1.1 | $7.0^{e}$±0.0 |
| Processed | $24.1^{cd}$±2.1 | $14.9^{d}$±0.5 | $28.5^{d}$±0.0 |
| *S. violaceum* | | | |
| Raw | $6.5^{f}$±2.4 | $9.1^{e}$±0.4 | $39.0^{a}$±0.1 |
| Processed | $6.1^{f}$±2.1 | $64.7^{b}$±1.9 | $29.5^{b}$±0.1 |
| BHA | $18.4^{de}$±10.4 | $24408.1^{a}$±48.9 | $2.3^{f}$±0.0 |
| Rutin | $3.8^{f}$±5.4 | $9749.1^{a}$±24.4 | $1.9^{g}$±0.0 |
| Tannic Acid | $227.1^{a}$±11.9 | $22122.5^{a}$±97.7 | $2.5^{f}$±0.0 |
| Trolox | $106.9^{b}$±5.9 | $6574.7^{a}$±24.2 | - |
| Citric Acid | $32.8^{c}$±2.9 | - | - |
| Ascorbic acid | - | - | $1.4^{h}$±0.0 |

Values are means of triplicate determinations ± standard deviation.
Mean values followed by different superscript in the same column are significantly ($p < 0.05$) different.
[A:] Ferric reducing/antioxidant power assay (concentration of substance having ferric-TPTZ reducing ability equivalent to that of 1 mM Fe (II)).

known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms. Hydroxyl ($OH^-$) radicals are extremely reactive and may be generated in the human body under physiological conditions, where they can react with non-selective compounds such as proteins, DNA, unsaturated fatty acids and almost all biological membrane. Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are thought to initiate cell damage *in vivo*[64]. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity[65]. Among the samples, STP (52%) and SXR (46%) showed higher scavenging activity when compared to the positive controls such as BHT, RUT and TRO (Figure 4). These results are contradictory to Muricia *et al.*[56] which reported that raw vegetables were very good hydroxyl scavenger with 80% inhibition potential such as beet root (87.9%), spinach (83.5%), swiss chard (80.5%), broad bean (80%) and the radish (54%). In biological systems metal binding can occur on DNA leading to partial site-specific hydroxyl radical formation. Polyphenols are potential protecting agents against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions. The ability of extracts/fractions to quench hydroxyl radicals seems to be directly related to the prevention of propagation of lipid peroxidation and they seem to be good scavengers of active oxygen species, thus reducing the rate of reaction.

Variability is common in the evaluation of antioxidant capacities among some fruits and vegetables, measured by different investigators even when the same assay was employed. The variance could be due to changes in phytochemical compositions, which are effected by cultivars,

**Table 3.** $DPPH^•$ radical and $ABTS^{•+}$ cation radical scavenging activities of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits

| Samples | $DPPH^•$ $IC_{50}$ (g extract/g $DPPH^•$)[A] | TAA (μmol/g extract)[B] |
|---|---|---|
| *S. torvum* | | |
| Raw | $7.6^{a}$±0.2 | $226.1^{c}$±20.2 |
| Processed | $1.9^{ab}$±0.2 | $80.3^{c}$±0.6 |
| *S. xanthocarpum* | | |
| Raw | $2.1^{ab}$±0.2 | $144.4^{c}$±12.1 |
| Processed | $2.5^{ab}$±0.0 | $236.1^{c}$±3.9 |
| *S. violaceum* | | |
| Raw | $2.0^{ab}$±0.3 | $111.9^{c}$±43.6 |
| Processed | $2.7^{ab}$±0.2 | $234.4^{c}$±6.4 |
| Ascorbic acid | $0.1^{b}$±0.0 | $971.4^{b}$±65.6 |
| Rutin | $0.1^{b}$±0.0 | $1301.0^{b}$±73.0 |
| Tannic Acid | - | $21087.7^{a}$±1037.2 |
| BHA | $0.1^{b}$±0.0 | $1555.6^{b}$±51.0 |
| BHT | $0.2^{b}$±0.0 | - |

Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly ($p < 0.05$) different.
[A]: g of extract required to decrease one g of the initial DPPH concentration by 50%
[B]: Total antioxidant activity (μmol equivalent Trolox performed by using ABTS radical cation)

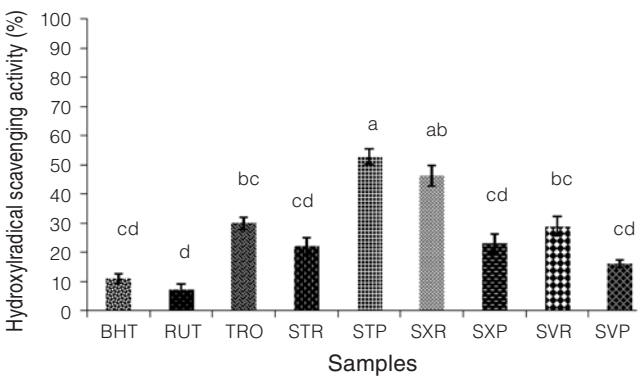


**Figure 4.** Hydroxyl radical scavenging activities of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits. Hydroxyl radical scavenging activities of raw and processed samples of *Solanum* spp. BHT- Butylated hydroxytoluene; RUT- Rutin; TRO - Trolox; STR- *Solanum torvum* raw; STP- *Solanum torvum* processed; SXR- *Solanum xanthocarpum* raw; SXP- *Solanum xanthocarpum* processed; SVR- *Solanum violaceum* raw; SVP- *Solanum violaceum* processed. Values are means of triplicate determinations ± standard deviation. Bars having different letters are significantly different ($p < 0.05$).

**Table 4.** Antimicrobial activities of raw and processed methanol extract of *S. torvum*, *S. xanthocarpum* and *S. violaceum* fruits

| Microorganisms | INHIBITION ZONES (mm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | STR | STP | SXR | SXP | SVR | SVP | Gen | Cip | Tet | Ami | Str |
| *S. aureus* | 8 | 7 | - | - | - | - | 18 | 30 | - | - | - |
| *K. pneumoniae* | 6 | 6 | 8 | 8 | 10 | 9 | - | - | 15 | 25 | - |
| *P. aeruginosa* | 13 | 11 | 12 | 9 | 7 | 6 | 20 | 25 | - | - | - |
| *S. typhi* | - | - | - | - | - | - | - | - | 22 | 20 | - |
| *E. coli* | - | - | 11 | 9 | 12 | 10 | 14 | - | - | - | 12 |

-: no inhibition.
STR- *Solanum torvum* raw; STP- *Solanum torvum* processed; SXR- *Solanum xanthocarpum* raw; SXP- *Solanum xanthocarpum* processed; SVR- *Solanum violaceum* raw; SVP- *Solanum violaceum* processed; Gen- Gentamycin; Cip- Ciprofloxacin; Tet- Tetracycline; Ami- Amikacin and Str- Streptomycin.

growing region, harvest season, maturity stage, storage conditions, as well as the part of the foods tested (for example, apples with or without the peel)[66,67]. The variance could also be attributed to different extracting solvents used. In this study, 80% methanol was used, which may result in variable results from those reported by others.

XI. *Antimicrobial Activity*

The maximum inhibition zones for microbial strains sensitive to the raw and processed samples of *S. torvum*, *S. xanthocarpum* and *S. violaceum* were in the range of 6-13 mm (Table 4). All the samples showed good antimicrobial activity against Gram negative bacteria. Interestingly, the extracts from *S. torvum* showed no inhibitory effect on the growth of *E. coli*. As far as the bacterial species are concerned, our results were in agreement with Chah *et al.*[68] and Dayang *et al.*[69] in which the methanol extract from *S. torvum* exhibited similar profile of growth inhibiting activity against the bacterial species. This can be attributed to the extra protective outer membrane, Gram negative bacteria are usually considerably more resistant to antibacterial agents than their Gram positive counterparts[70]. In this regard, the *S. torvum* extracts showed its moderate antibacterial activity in disc diffusion test on Gram positive bacteria (*S. aureus*). The extracts from *S. xanthocarpum* and *S. violaceum* showed no inhibitory effect on the growth of *S. aureus*. The antibacterial activity of standard antibiotics such as gentamycin, ciprofloxacin, amikacin, tetracycline and streptomycin were also tested, and they gave moderate to good antimicrobial activity for all the microorganisms tested. The observed antibacterial activities of *Solanum* species are well known and probably caused by the alkaloids[71,72]. According to Norrby[73], there has been a transition from a dominance of Gram negative to Gram positive aetiology of hospital-acquired infections. This is in line with Perumal Samy *et al.*[74] which reported that most of the Indian medicinal plants exhibited high degree of antibacterial activity towards Gram negative bacteria.

## CONCLUSIONS

In summary, the results implies that the raw and processed fruit sample extracts exhibited good antioxidant activity and it shows that *Solanum* species were rich in antioxidant components and moreover, the activities are not greatly affected by the processing conditions. The lack of correlation between phenolic content and antioxidant activity (reducing power ($R^2$=-37.4), ABTS ($R^2$=-15.8)) suggested that the antioxidant activity is a result of a different compounds with synergic and antagonistic effects. Consumption of these fruits can supply substantial antioxidants which may provide health promoting and disease preventing effects. Indeed, there is need for availability of new plant derived bioactive molecules, thus these fruit samples may be a great natural source for the development of new drugs.

## REFERENCES

1. Halliwell, B., Gutteridge, J. M. C. and Cross, C. E. 1992. Free radicals, antioxidants, and human diseases: where are we now? J. Lab. Clin. Med. 119: 598-620.
2. Losso, J. N., Shahidi, F. and Bagchi, D. 2007. Anti-angiogenic functional and medicinal foods. Boca Raton FL: Taylor and Francis.
3. Scalbert, A., Manach, C. and Morand, C. *et al*. 2005. Dietary polyphenols and the prevention of diseases. Crit. Rev. Food Sci. Nutr. 45: 287-306.
4. Hertog, M. G. L., Hollman, P. C. H. and Venema, D. P. 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food Chem. 40: 1591-1598.
5. Hertog, M. G. L., Hollman, P. C. H. and Katan, M. B. 1992a. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J. Agric. Food Chem. 40: 2379-2383.
6. Lee, Y., Howard, L. R. and Villalon, B. 1995. Flavonoids and antioxidant activity of fresh pepper. J. Food Sci. 60: 473-476.
7. Bilyk, A., Cooper, P. L. and Sapers, G. M. 1984. Varietal differences in distribution of quercetin and kaempferol in onion (*Allim cepa* L.) tissue. J. Agric. Food Chem. 32: 274-276.
8. Farag, R. S., Badel, A. Z. M. A. and Hewedi, F. M. *et al*. 1989. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. J. Am. Oil Chem. Soc. 66: 792-799.
9. Laughton, M. J., Evans, P. J. and Moroney, M. A. *et al*. 1991. Inhibition of mammalian 5-lipoxygenase and cyclo-oxigenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. Biochem. Pharmacol. 42: 1673-1681.
10. Onyeneho, S. N. and Hettiarachchy, N. S. 1991. Effect of nany bean hull extract on the oxidative stability of soy and sunflower oils. J. Agric. Food Chem. 39: 1701-1704.
11. Kinsella, J. E., Frankel, E. and German, B. *et al*. 1993. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol. 4: 85-89.
12. Gazzani, G. 1994. Anti- and prooxidant activity of some dietary vegetables. Riv. Sci. Aliment 23: 413-420.
13. Al-Saikhan, M. S., Howard, L. R. and Miller, J. C. 1995. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). J. Food Sci. 60: 341-347.
14. Nicoli, M. C., Anese, M. and Parpinel, M. T. *et al*. 1997. Loss and/or formation of antioxidants during food processing and storage. Cancer Lett. 114: 71-74.
15. Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, 1262.
16. Lewis, D. C. and Liljegren, D. R. 1970. Glycoalkaloids from *Archaesolanum* species. Photochemistry 9: 2193-2195.
17. Maitf, P. C., Mookherjea, S. and Matew, R. *et al*. 1979.

Studies on Indian *Solanum I.* Alkaloid content and detection of Solasodine. Econ. Bot. 33: 75-77.
18. Gbile, Z. O. and Adesina, S. K. 1988. Nigerian *Solanum* species of economic importance. Ann. Mo. Bot. Gard. 75: 862-865.
19. Siddhuraju, P. and Becker, K. 2003. Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var. utilis) extract and certain non-protein amino/imino acids through in vitro models. J. Sci. Food Agric. 83: 1517-1524.
20. Siddhuraju, P. and Manian, S. 2007. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chem. 105: 950-958.
21. Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power”: The FRAP assay. Anal. Biochem. 239: 70-76.
22. Pulido, R., Bravo, L. and Saura-Calixto, F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 48: 3396-3402.
23. Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269: 337-341.
24. Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28: 25-30.
25. Sa´nchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F. 1998. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. 76: 270-276.
26. Re, R., Pellegrini, N. and Proteggente, A. *et al*. 1999. Antioxidant activity applying an improved ABTS• radical cation decolorization assay. Free Radical Biol. Med. 26: 1231-1237.
27. Mitsuda, H., Yasumoto, K. and Iwami, K. 1966. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Nihon Eiyo Shokuryo Gakkaishi 19: 210-214.
28. Yen, G. C. and Hsieh, C. L. 1998. Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models in vitro. J. Agric. Food Chem. 46: 3952-3957.
29. Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.
30. Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chem. 64: 555-559.
31. Naim, M., Gestetner, B. and Bondi, A. *et al*. 1976. Antioxidative and antihemolytic activities of soybean isoflavones. J. Agric. Food Chem. 24: 1174-1177.
32. Dinis, T. C. P., Madeira, V. M. C. and Almeida, L. M. 1994. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.
33. Klein, S. M., Cohen, G. and Cederbaum, A. I. 1981. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. Biochemistry 20: 6006-6012.
34. Bauer, A. W., Kirby, W. M. and Sherris, J. C. *et al*. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.
35. Skytta, E. and Mattila-Sandholm, T. 1991. A quantitative method for assessing bacteriocins and other food antimicrobials by automated turbidometry. J. Microbiol. Method 14: 77-88.
36. Chang, W. H., Luu, H. X. and Cheng, A. C. 1983. A TLC-fluorescent method of detecting and evaluating individual antioxidative components. J. Food Sci. 48: 658-659.
37. Lingnert, H., Vallentin, K. and Eriksson, C. E. 1979. Measurement of antioxidative effect in model system. J. Food Process. Preserv. 3: 87-103.
38. Lopez, M., Martinez, F. and Del Valle, C. *et al*. 2003. Study of phenolic compounds as natural antioxidants by a fluorescence method. Talanta. 60: 609-616.
39. Sahlin, E., Savage, G. P. and Lister, C. E. 2004. Investigation of the antioxidant properties of tomatoes after processing. J. Food Compos. Anal. 17: 635-647.
40. Bunea, A., Andjeldkovic, M. and Socaciu, C. *et al*. 2008. Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). Food Chem. 108: 649-656.
41. Barroga, F. C., Laurena, A. C. and Mendoza, E. M. T. 1985. Polyphenols in Mung bean (*Vigna radiate,* L.): determination and removal. J. Agric. Food Chem. 33: 1006-1009.
42. Rocha-Guzman, N. E., Gonzalez-Laredo, R. F. and Ibarra-Perez, F. J. *et al*. 2007. Effect of pressure-cooking on the antioxidant of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. Food chem. 100: 31-35.
43. Prior, R. L. and Cao, G. 1999. In vivo total antioxidant capacity: comparison of different analytical methods. Free Radical Biol. Med. 27: 1173-1181.
44. Nicoli, M. C., Anese, M. and Parpinel, M. 1999. Influence of processing on the antioxidant properties of fruit and vegetables. Trends Food Sci. Technol. 10: 94-100.
45. Manzocco, L., Anese, M. and Parpinel, M. 1998. Antioxidant properties of tea extracts as affected by processing. LWT Food Sci. Technol. 31: 694-698.
46. Loo, A. Y., Jain, K. and Darah, I. 2008. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem. 107: 1151-1160.
47. Apak, R., Guclu, K. and Demirata, B. *et al*. 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 12: 1496-1547.
48. Adefegha, S. A. and Oboh, G. 2011. Cooking enhances the antioxidant properties of some tropical green leafy

vegetables. Afr. J. Biotechnol. 10: 632-639.
49. Leong, L. P. and Shui, G. 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. 76: 69-75.
50. Halvorsen, B. L., Carlsen, M. H. and Phillips, K. M. *et al*. 2006. Content of redox-active compounds (i.e, antioxidants) in foods consumed in the United States. Am. J. Clin. Nutr. 84: 95-135.
51. Dewanto, V., Wu, X. and Liu, R. H. 2002. Processed sweet corn has higher antioxidant activity. J. Agric. Food Chem. 50: 4959-4964.
52. Dewanto, V., Wu, X. and Adom, K. K. *et al*. 2002a. Thermal processing enhances the nutritive value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50: 3010-3014.
53. Lee, J., Koo, N. and Min, D. B. 2004. Reactive oxygen species, aging and antioxidative nutraceuticals. Compre. Rev. Food Sci. Food Safety 3: 21-33.
54. Huang, D., Ou, B. and Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53: 1841-1856.
55. Elmastas, M., Gulcin, I. and Isildak, O. *et al*. 2006. Radical scavenging activity and antioxidant capacity of bay leaf extracts. J. Iran. Chem. Soc. 3: 258-266.
56. Muricia, M. A., Jimenez, A. M. and Martinez-Tome, M. 2009. Vegetables antioxidant losses during industrial processing and refrigerated storage. Food Res. Int. 42: 1046-1052.
57. Vani, T., Rajani, M. and Sarkar, S. *et al*. 1997. Antioxidant properties of the ayurvedic formulation triphala and its constituents. Int. J. Pharmacogn. 35: 313-317.
58. Sini, H. and Devi, K. S. 2004. Antioxidant activities of the chloroform extract of *S. trilobatum*. Pharm. Biol. 42: 462-466.
59. Lo Scalzo, R., Fibiani, M. and Mennella, G. *et al*. 2010. Thermal treatment of egg plant (*Solanum melongena* L.) increase the antioxidant activity content and the inhibitory effect on human neutrophil burst. J. Agric. Food Chem. 58: 3371-3379.
60. Siddhuraju, P., Maheshu, V. and Loganayaki, N. *et al*. 2008. Antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from processed indigenous legumes, *Macrotyloma uniflorum* (Lam.) Verdc. and *Dolichos lablab* L. Food 2: 159-167.
61. Sowndhararajan, K., Siddhuraju, P. and Manian, S. 2011. Antioxidant and free radical scavenging capacity of the underutilized legume, *Vigna vexillata* (L.) A. rich. J. Food Compos. Anal. 24: 160-165.
62. Hsu, C. L., Chen, W. and Weng, Y. M. *et al*. 2003. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 83: 85-92.
63. Halliwell, B. 1997. Antioxidants: the basics – what they are and how to evaluate them. Adv. Pharmacol. 38: 3-20.
64. Rollet Labelle, E., Grange, M. J. and Elbim, C. *et al*. 1998. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. Free Radical Biol. Med. 24: 563-572.
65. Babu, B. H., Shylesh, B. S. and Padikkala, J. 2001. Antioxidant and hepatoprotective effect of *Alanthus icicifocus*. Fitoterapia 72: 272-277.
66. Drogoudi, P. D., Michailidis, Z. and Pantelidis, G. 2008. Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. Sci. Hortic. 115: 149-153.
67. Van der Sluis, A. A., Dekker, M., De Jager, A. and Jongen, W. M. 2001. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. J. Agric. Food Chem. 49: 3606-3613.
68. Chah, K. F., Muko, K. N. and Oboegbulem, S. I. 2000. Antimicrobial activity of methanolic extract of *Solanum torvum* fruit. Fitoterapia 71: 187-189.
69. Dayang, F. B., Razinah, S. and Paden, M. 2005. Antimicrobial activities of ethanol and ethyl acetate extracts from the fruits of *Solanum torvum*. Malays. Appl. Bioi. 34: 31-36.
70. Saier, M. H. Jr. 2009. Cell membrane, prokaryoitc. In "Encyclopedia of microbiology". 3rd ed. pp.341-356. Schaechter, M. Academic Press. U.S.A.
71. Mohamed, S., Saka, S. and El-sharkawy, S. H. *et al*. 1996. Antimycotic screening of 58 Malaysian plants against plant pathogens. Pestic. Sci. 47: 259-264.
72. Kambizi, L. and Afolayan, A. J. 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J. Ethnopharmacol. 77: 5-9.
73. Norrby, S. R. 1995. Emerging antibiotic resistance in Gram positive bacteria: return to the pre-antibiotic era? Hong Kong Med. J. 1: 129-135.
74. Perumal Samy, R., Ignacimuthu, S. and Sen, A. 1998. Screening of 34 Indian medicinal plants for antibacterial properties. J. Ethnopharmacol. 62: 173-182.