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Research Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF TWO SELECTED 'BIHI' FRUITS USED AS VEGETABLES IN DARJEELING HIMALAYA

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ABSTRACT

The aim of this study was to determine the antioxidant activity as well as total phenol (TPC) and total flavonoid content (TFC) in two fruits, *Solanum anguivi* and *Solanum incanum* grown in Darjeeling Himalaya and used as vegetables in Nepali recipes. The antioxidant activities were examined by five different methods namely DPPH free radical scavenging activity, reducing power, iron chelation, anti-lipid peroxidation, and nitric oxide scavenging activity. The results showed that considerable amount of TPC and TFC was present in these fruit extracts as well as these vegetables contain a vast array of different phytochemicals in their dry form. *Solanum incannum* showed higher antioxidant activity than *Solanum anguivi.* Significant correlations were obtained between free-radical scavenging capacity and TFC, indicating that flavonol group of metabolites were the chief performers of antioxidant activity. Principle Component Analysis (PCA) indicated that polar metabolites along with hydrophilic radical scavengers contributed to the major variability in the antioxidant activity of the plants. Overall, the present results provided basic data for choosing these fruits with high antioxidant capacity for consumption or for the development of antioxidant based medicines as value-added products.

Keywords: *Solanum anguivi, Solanum incanum,* Antioxidants, Vegetables, DPPH, Oxidative stress.

INTRODUCTION

Free radicals which are atomic or molecular chemical species with unpaired electrons are highly unstable and can react with other molecules by giving out or accepting single electron [1]. Oxidation processes are one of the most important routes for producing free radicals in food, drugs and even living systems. These unstable molecules are capable of causing cellular damage, which leads to cell death and tissue injury [2]. Free radicals are linked with the majority of human diseases like ageing, atherosclerosis, cancer, diabetes, liver cirrhosis, cardiovascular disorders, etc.[3, 4]. The most common reactive oxygen species (ROS) are superoxide anion (O_2·-), hydrogen peroxide (H_2O_2), peroxyl radical (ROO·) highly reactive hydroxyl radical (OH·), nitric oxide (NO) and peroxynitrite anion (ONOO·). Antioxidants cease direct ROS attacks and radical mediated oxidative reactions and are important in the prevention of many diseases and health problems[5]. The antioxidants are not only required by our body to combat ROS but also equally important as food additives either in synthetic or naturally occurring forms[6]. Epidemiological and *in vitro* studies on medicinal plants, fruits and vegetables strongly supported the idea that plant constituents with antioxidants are capable of exerting protective effects against oxidative stress in biological systems[7, 8, 9] as well as can be used to prevent complex diseases like Alzeimer's disease and cancer[10]. Many naturally occurring antioxidants from plant sources have been identified as free radical scavengers or active oxygen-scavengers[11, 12]. Recently, natural antioxidants have attracted considerable interest among nutritionists, food manufacturers and consumers, due to their presumed safety and prospective therapeutic values[13].

Solanum anguivi Herb. Lam. ex Dum and *Solanum incanum* L. are two plants locally known as 'Bihi' and 'Bara Bihi' respectively by the people of Darjeeling hills. These plants are shrub like in habit under the family of Solanaceae and are widely cultivated in Darjeeling hills. Fruits of these plants are popular for vegetables of this area. The roots of *S. anguivi* plant are used for curing diarrhoea and skin ailments[14]. The fruits of *S. incanum* are extensively used for the treatment of cutaneous mycotic infections and other pathological conditions[15] and these fruit extracts have high antifungal and DPPH scavenging activity[16]. Due to presence of glycosidal alkaloids the members of Solanaceae family are recognized with antibiotic activity[15] and most of the *Solanum* plants may scavenge reactive oxygen species[17,18,16]. But the detailed information about phytochemical profile of bioactive fractions obtained from the fruits of these two *Solanum* species are still lacking and addressing the functional aspects of health-promoting components like scavenging and detoxification of specific oxygen-based radicals require better understanding and in-depth investigation of antioxidants present in them for channeling their use as functional foods and as ingredients in pharmaceutical and nutracutical industry.

In this study, we investigated the DPPH· radical scavenging, reducing power, metal chelating, nitric oxide scavenging, superoxide scavenging and lipid-peroxidation assay as well as the phytochemical compositions and polyphenol content of the fruits of *S. anguivi* and *S. incanum*. These multiple methods are recommended to determine antioxidant capability of food materials that reflect their potential defensive functions against various oxidative stress induced diseases.

MATERIALS AND METHODS

Plant materials

The fruits of *S. incanum* L. and *Solanum anguivi* Herb. Lam. ex Dum (Figure 1) were collected form from Sorang Basti, Darjeeling, West Bengal, India. Taxonomic position was authenticated by the Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. The materials were deposited in the 'NBU Herbarium' and recorded against the accession no 9542 and 9574 dated 04-03-09.

Animal material

Goat liver, used for anti-lipid peroxidation assay, were collected from slaughter house immediately after slay and the experiment was conducted within one hour after collection.

Chemicals

Methanol (M), 2.2-diphenyl-1-picryl hydrazyl (DPPH), nitroblue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide sodium salt monohydrate (NADH), phenazine methosulphate (PMS), sulfanilamide, glacial acetic acid, napthylethylenediamine dihydrochloride, ferrozine, ferrous chloride, trichloroacetic acid (TCA), thiobarbituric acid (TBA), $FeSO_4.7H_2O$, KOH, KH_2PO_4, ethylene-diamine tetra acetic acid (EDTA), ascorbic acid, vitamin-E, 2-deoxyribose, potassium ferricyanide, ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2), sodium nitroprusside, gallic acid, Folin-Ciocaltue reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), acetone, petroleum ether, Sodium sulphate, and Sodium hydroxide (NaOH) were either purchased from Sigma Chemicals (USA), or of Merck analytical grade.

Fig. 1: Fruit of 'Bara Bihi' [*S. incanum* L.] and 'Bihi' [*S. anguivi* Herb. Lam. ex Dum]

Extraction procedure

Fresh fruits were crushed and under soxhlet extractor crushed fruits were separately extracted with methanol for eight hours. The refluxed samples were separated from the residues by filtering through Whatman No. 1 filter paper. The filtrates were dried under reduced pressure and their total extractive values were calculated on dry weight basis by the formula

$$\% \text{ extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

The samples were then kept in freeze for further use.

DPPH based free radical scavenging activity

The radical scavenging activity of two fruit extracts were measured by DPPH method[20]. The reaction mixture contained 1.8 ml of 0.1mM DPPH and 0.2 ml of each serial dilution (0-800 mg/ml FW) of methanolic fruit extracts. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was served as control.

Radical scavenging activity was expressed as percent inhibition from the given formula:

$$\text{Percent inhibition of DPPH radical} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100.$$

Reducing antioxidant power

The reducing antioxidant power of plant methanolic extracts was determined by the standard method[21]. Different concentrations of 1 ml of fruit extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis. Increased absorbance of the reaction mixture indicates increase in reducing power.

Metal chelating activity

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.*[22], with slight modification. To 0.4 ml of methanol extract, 1.6 ml of methanol was diluted and mixed with 0.04 ml of $FeCl_2$ (2 mM). After 30s, 0.8 ml ferrozine (5 mM) was added. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as

$$\text{Chelating rate (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Determination of Nitric oxide activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction[23]. 320 µL methanol extract, 360 µL (5mM) sodium nitroprusside-PBS solution, 216 µL Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was mixed and incubated at 25°C for one hour. Lastly 2 ml water was added and absorbance was taken at 546nm.

Radical scavenging activity was expressed as percent inhibition from the given formula:

$$\text{Percent inhibition of no. radical} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100.$$

Anti-lipid peroxidation (ALP) assay

The anti-lipid peroxidation activity of the extracts of two *Solanum* fruits was determined by the standard method[24] followed by slight modification with the goat liver homogenate. 2.8 ml of 10% goat liver homogenate, 0.1 ml of 50 mM $FeSO_4$ and 0.1 ml extract was mixed. The reaction mixture was incubated for 30 minutes at 37°C. 1 ml of reaction mixture was added with 2 ml 10% TCA-0.67% TBA in acetic acid (50%) for terminating the reaction. Mixture was boiled for 1 hour at 100°C and centrifuged at 10,000 rpm for 5 minutes. Supernatant was taken for absorbance at 535nm. Vitamin E was used for standard. ALP % was calculated using the following formula:

$$\text{ALP percent} = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{abs. of control}} \times 100$$

Total phenol Estimation

Total phenolic compounds of fruit extracts were determined by Folin-Ciocalteu method[25]. For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2 and 0.3 mg/ml methanolic gallic acid solution was mixed with 5 ml of Folin-Ciocaltue reagent (10 times diluted) and 4 ml sodium carbonate (75 g/L). The absorbance at 765 nm was measured after 1 hr. at 20º C and the calibration curve was drawn. To the similar reagent, 1 ml methanolic fruit extracts (40 mg/ml) was mixed as described above and after 1 hr. the absorbance was measured.

Total flavonoids determination

Aluminum chloride spectrophotometric method was used for flavonoids determination[26]. Each methanolic fruit extracts (0.5 ml of 200mg/ml FW) were separately diluted with 4 ml double distilled water. Diluted extracts of fruits were mixed with 5% (0.3 ml) $NaNO_2$. 10% aluminum chloride was then added with reaction mixture. After 6 minute, 2ml (1.0 M) NaOH and 2.4 ml D.D. water was added and mixed well. Thereafter, absorbance was measured at 510 nm in spectrophotometer. Standard solutions quercetin (0-500 mg L^{-1}) was used as calibration curve.

Phytochemicals evaluation of the crude extracts

The methanolic crude extract (200 mg/ml) of the fruits of the plant was subjected to various chemical tests in order to determine the secondary metabolites present by employing the use of various methods as follows:

Test for Reducing Sugars

To 0.5ml of the extracts, 2ml of a mixture (1:1) of Fehling's solution I (A) and Fehling's solution II (B) were added and the mixture were boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars[27].

Test for Flavonoids

To 1ml of methanolic extracts, a few drops of 10 % ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus[27].

Test for resins

0.5ml of extract was evaporated and dissolved in 2ml of petroleum ether, 2ml of 2% cupper acetate solution was then added and the mixture was shaken vigorously and allowed to separate, a green colour indicated the presence of resin[28].

Test for amino acid

0.5 ml methanolic fruit extracts were treated with few drops of ninhydrin reagent, heated in water bath, a purple colour indicated the presence of amino acids[29].

Test for anthraquinones

1ml methanolic fruit extracts were evaporated and dissolved in 2ml chloroform. 2ml of ammonia was added. Occurrence of red colour suggested the presence of anthraquinones[29].

Test for tannin

0.5 ml methanolic extract of each fruit was added with 0.5 ml 1% lead acetate; a yellow colour precipitation indicated the presence of tannin[29].

Test for triterpenoids

0.5ml of methanolic fruit extracts were evaporated and dissolved in 1ml chloroform. 1ml acetic anhydride was then added and chilled. After cooling, conc.H_2SO_4 was added. If reddish violate colour appeared, the existence of triterpenoids was confirmed[29].

Test for alkaloids

0.5 ml of each fruit extract was added with 0.2ml of 36.5% hydrochloric acid and 0.2 ml Dragendroff's reagent. Production of orange precipitation denoted the presence of alkaloids[29].

Test for glycosides

0.5 ml methanolic extract of fruits were added with 2ml of 50% hydrochloric acid. The mixtures were hydrolyzed for 2 hrs on a water bath. After that 1 ml pyridine, few drops of 1% sodium nitroprusside solution, and 5% sodium hydroxide solution were added. Pink to red colour designated the presence of glycosides[29].

Test for steroid

0.5 ml methanolic fruit extracts were evaporated and dissolved in 2ml chloroform. 2ml of conc. H_2SO_4 was introduced carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of steroid[29].

Test for Saponins

2 ml of double distilled water was added with 1 ml of each methanolic extract. Few drops of olive oil were added and agitated. Formation of soluble emulsion indicated the presence of saponin[30].

Test for cardiac glycoside

0.5 ml of methanolic fruit extracts were evaporated and dissolved in 1 ml glacial acetic acid. One drop of 10% ferric chloride was then added. 1 ml of conc.H_2SO_4 was added by the side of the test tube. Appearance of brown colour ring at the interface indicated of presence of cardiac glycosides[30].

Statistical analysis

The data were pooled in triplicate and subjected to analysis of correlation co-efficient matrix using SPSS (Version 12.00) for drawing the relation between phytochemical properties and antioxidant attributes and MS Excel of Microsoft Office, 2007 was used for comparing the antioxidant attributes of different fruit parts. Smith's Statistical Package (Version 2.5) was used for determining the IC_{50} values of antioxidants and their standard error of estimates (SEE). In order to examine and visualize relationships between different phytochemicals and antioxidant traits, a principal component analysis (PCA) based on the correlation matrix was calculated using Multivariate Statistical Package (MVSP 3.1).

RESULTS AND DISCUSSION

The plant possesses numerous biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine[31]. It was reported that most of the plants of solanaceae contain alkaloids, tannins, steroids, saponins, as well as reducing sugars[32]. Our results (Table 1) of the qualitative phytochemical tests also confirmed that point. Phenolic acids are regarded as one of the functional food components in fruits and are contributed to the health effects of plant-derived products by scavenging free radical species, inhibiting free radical formation, and preventing oxidative damage to DNA[33, 34] due to the presence of hydroxyl groups[35]. The extraction yield, total phenolic content and total flavonoid content of two *Solanum* fruit extracts are presented in Table 2. Percent yield of methanolic extract of *S. incanum* (31.30%) is greater than *S. anguivi* (19.85%). Phytochemicals possess a broad spectrum of biological activities including antioxidant and radical scavenging properties[36, 37, 38], therefore evaluation of antioxidant properties were performed *in vitro* with newly generated free radicals during hydrophilic or hydrophobic reaction.

Figure 2 showed that fruit extracts of two *Solanum* species have got profound antioxidant activity. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance[39]. *S. incanum* fruit extract exhibited a significant dose-dependent inhibition of DPPH activity than *S. anguivi* fruit (Figure 2). These results are also in line of those publications prepared by Priyadarsini *et al*. and Patnibul *et al.* who reported that the fruits of *Solanum* plants have greater capability of free radical scavenging, when compared with other well known vegetables[40, 17].

Simultaneously, according to Jain *et al.*, *Solanum suratense* also showed 84% DPPH radical scavenging activity, which is very significant[41]. The results also (Figure 3) established the effects of reducing potential of *Solanum* fruits' extracts at different concentration (0-200mg/ml FWT). The reducing power of these fruits' extracts increased in a concentration dependent manner. It was well known that the fruit extracts with higher levels of total phenolics also exhibit greater reducing power[42, 26]. In general, the extracts of the tested fruits materials, exhibiting greater total phenol content, also depict good reducing power in the present analysis. Plant phenols constitute the major group of compounds that perform as primary antioxidant[43]. Due to the presence of hydroxyl groups they can react with active oxygen radicals, such as hydroxyl radicals, superoxide radicals and lipid peroxyl radicals and inhibit the lipid peroxidation at an early stage[44, 45]. These plant products also have the potency to neutralize the effect of nitric oxide (NO) formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health[46]. The extracts effectively reduced the generation of nitric oxide from sodium nitroprusside (Figure 4). Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent[23].

Like other free radical scavenging properties *S. incanum* fruit extract exhibited more inhibition of NO, Metal chelating as well as anti-lipid peroxidation activity than *S. anguivi* fruit (Figure 5 and 6). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals[47]. Ferrozine can create complexes with ferrous ions. In the presence of chelating agents, complex (purple colored) formation is interrupted and as a result, the color of the complex is decreased. Thus the chelating effect can be determined by measuring the rate of color reduction. Chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion[48]. This study shows that *Solanum* fruit extracts has a marked capacity for iron binding, suggesting the presence of polyphenols that has potent iron chelating capacity. Naturally occurring polyphenols and flavonoids have been shown to prevent lipid peroxidation, low-density lipoprotein oxidation, and the development of atherosclerosis and heart disease[49]. It was observed (Figure 6) that methanol extract of two *Solanum* fruits have moderate anti-lipid peroxidation effect against goat liver due to the presence of polyphenols and flavonoids content. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex[3] or through – OH radicals by Fenton reaction[47]. In this method the concentration of peroxide decreases as the antioxidant activity increases.

Table 1: Phytochemical profile of two different timbur fruits (semi-quantitative screening)

Plant samples	Alkaloid	Amino acid	Anthrax-quinones	steroids	glycosides	flavonoids	saponins	tannins	Reducing sugar	triterpenes	cardiac glycosides	resin
S. anguivi	++	++++	-	+++	+	+	+	++	++++	++	+	-
S. incanum	++	++++	-	+++	+	+	+	+	++++	+	+	+

(Number of '+' indicates the intensity of phytochemical)

Table 2: Extraction yield, total phenol content and total flavonoid content of *Solanum* fruit extract.

Plant samples	Extraction yield (%)	Total phenol content (mg/g FWT)	Total flavonoid content (mg/g FWT)
S. anguivi	19.85 ± 0.32	1.606 ± 0.19	0.101 ± 0.01
S. incanum	31.30 ± 0.46	2.306 ± 0.37	0.207 ± 0.09

Values are expressed as mean ± SD (n=3)

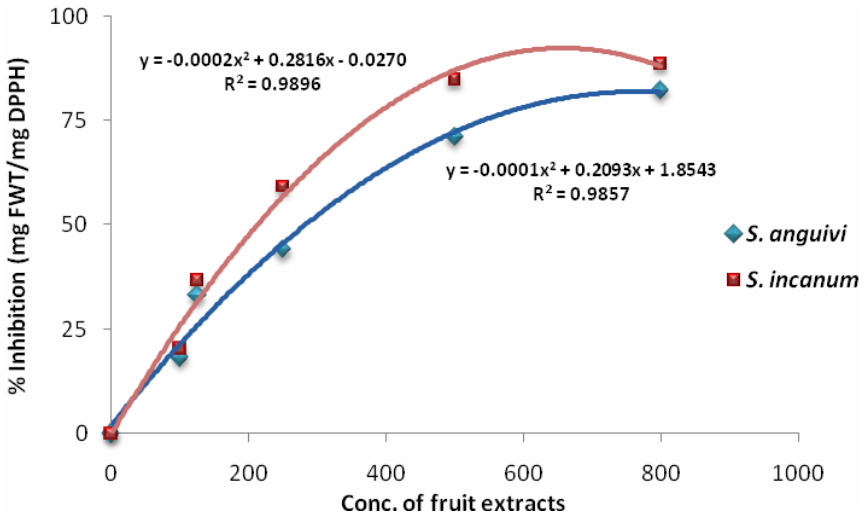

Fig. 2: DPPH radical scavenging (% inhibition) activity of *Solanum* fruits

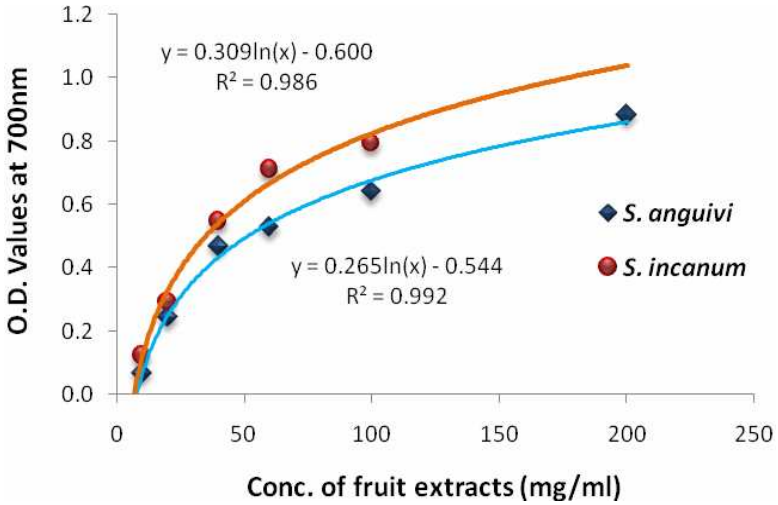

Fig. 3: Reducing Power of *Solanum* fruits

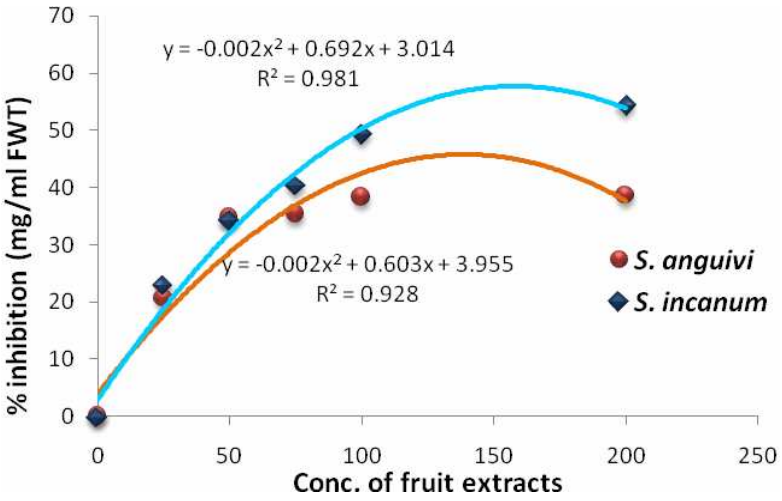

Fig. 4: Nitric oxide scavenging (% inhibition) activity of *Solanum* fruits

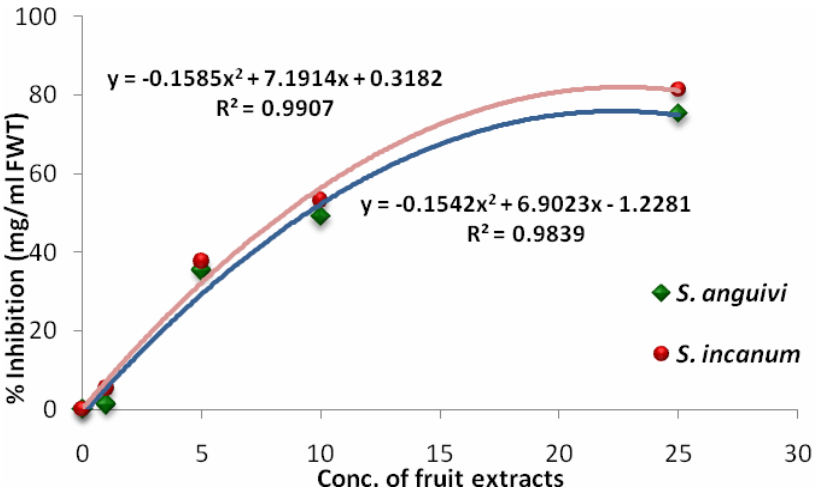

Fig. 5: Metal chelating (% inhibition) activity of *Solanum* fruits

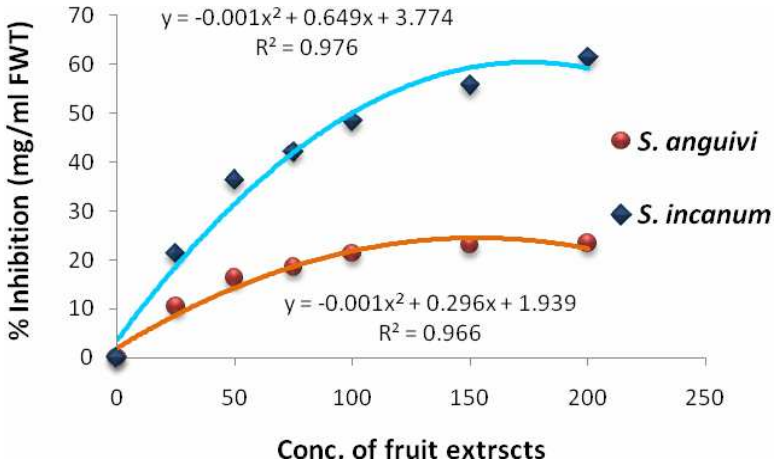

Fig. 6: Anti-lipid peroxidation (% inhibition) activity of *Solanum* fruits

To study the role of phenolic compounds in antioxidant or chelating properties, principle component analysis (PCA) and Pearson's correlation coefficient were performed and analyzed. High correlations were obtained between total phenol content (TPC) and DPPH IC_{50} as well as with reducing potential (Table 3) [p≤0.05]; also TPC is significantly correlated with NO scavenging activity, suggesting that phenolic compounds are the major contributors of antioxidant activity . In this context flavonol class of phenolics may be considered as most potential performers as they showed excellent correlation with DPPH and NO (p≤0.01). Phenolic compounds, which are known as hydrophilic antioxidants, are secondary metabolites that are most abundant in different fruits and vegetables[50]. Gil *et al*. found high correlation (r>0.9; p≤0.05) between DPPH free radical scavenging and ferric reducing potential with total phenol in nectarines, peaches and plums[51]. Also, significant correlation between total phenol and antioxidant activity as determined by ferric reducing antioxidant potential or electron spin resonance spectroscopy were reported in fruit juices[52].

Table 3: Correlations matrix

	DPPH	MC	RP	NO	ALP	Phenol	Flavonol
MC	-0.682						
RP	0.902	-0.301					
NO	0.993*	-0.764	0.845				
ALP	0.508	-0.976	0.088	0.606			
Phenol	-0.972	0.489	-0.979	-0.937	-0.290		
Flavonol	-0.991*	0.775	-0.836	-0.999**	-0.620	0.930	
EV	0.170	-0.837	-0.271	0.285	0.935	0.068	-0.302

* Correlation is significant at the 0.05 level (1-tailed).

** Correlation is significant at the 0.01 level (1-tailed).

PCA was mainly used in the classification of antioxidant activities in fruits of Solanaceae. Methanolic extract of tree tomato [*Cyphomandra betacea* (Cav.) Sendtn.] fruits available in Darjeeling hills were taken as reference sample, as it has been established that this fruit contained remarkable antioxidant activity and several bioactive polyphenols. The loading plots were used to overview and justify importance among different antioxidant assays, metal chelating activity and bioactive phytochemicals. The loading of first and second principle components (PC1 & PC2) accounted for 70.65% and 29.3% of the variance, respectively (Figure 7). PC1was mainly influenced by DPPH, metal chelating, reducing power and nitric oxide scavenging along with phytochemicals like phenols and flavonols, with significant squred cosine values of 0.947, 0.691, 0.608, 987, 0.795 and 0.991 respectively. So these hydrophilic antioxidants and total chelation activity were mainly controlled by polar secondary metabolites like phenolics and flavonoids. Whereas anti-lipid peroxidation (ALP) activity was mostly controlled by some other non-polar metabolites present in extractive, as the ALP and extractive values are mainly loaded on PC2 with squared cosine of 0.521 and 0.847 respectively. Wong *et al*. showed that DPPH radical scavenging, ferric ion reducing power and total phenolic content were heavily loaded on Factor 1 (PC1) in their PCA, when aqueous extracts of edible tropical plants were analyzed[53]. Similar clusters are observed by Wang and Hu, who determined that total phenol content, ferric reducing power and oxygen radical absorbance capacity were positively loaded on PC1, when PCA were performed with antioxidant attributes of mulberry fruits[54]. In a nutshell, two different molecular groups were present in fruits and vegetables, particularly for controlling polar and non-polar class of free radicals in the cellular system.

Etiological factors of several clinical disorders could be prevented or delayed by supplementing the body's natural antioxidant defense. Plant extracts and plant-derived antioxidant compounds potentiate body's antioxidant defense, they are antioxidants of choice because of their lower toxicity and side effects over the synthetic ones as well they are relatively cheaper and are easily accessible. This study provides evidence that *S. anguivi and S. incanum* have high antioxidant properties which may be due to the presence of phenolics, flavonoids, amino acids, steroids and reducing sugars. Therefore, these fruits may have great relevance in the prevention and treatment of diseases in which oxidants or free radicals are implicated.

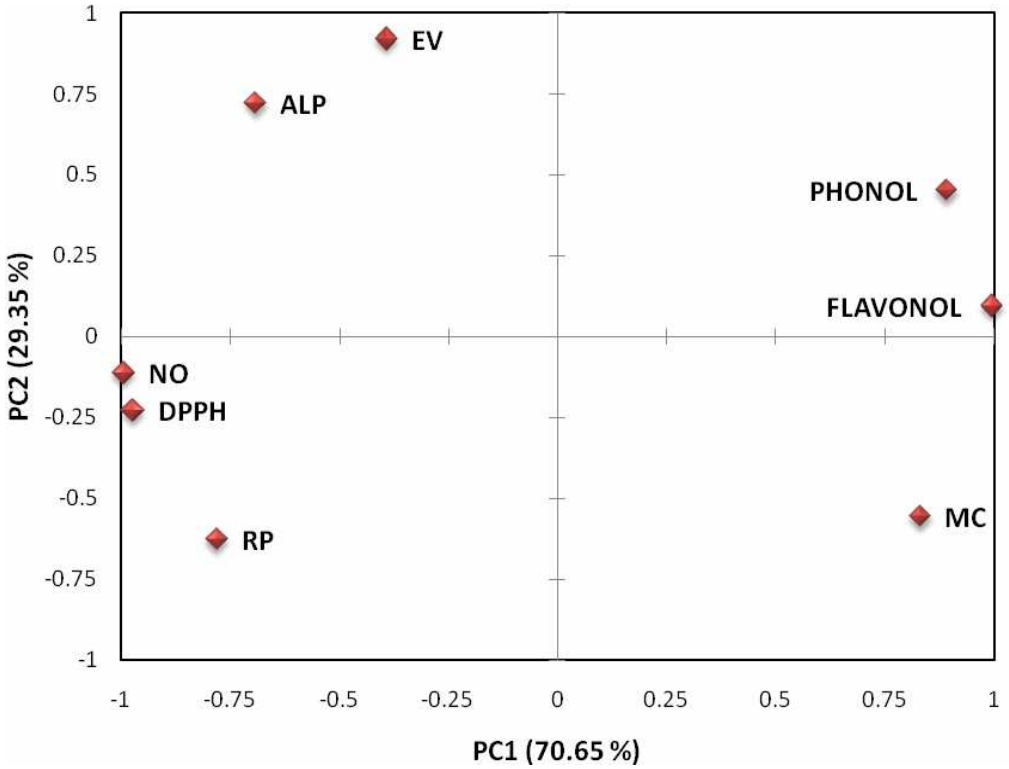

Fig. 7: Principal component analysis of DPPH, MC, NO, ALP, RP, EV, TPC and TFC.

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