Ethno Med, 12(2): 87-95 (2018)
DOI: 10.31901/24566772.2018/12.02.559

Assessment of Bioactive Compounds, Antioxidative Activity and Quantification of Phenols through HPLC in *Solanum* Species

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KEYWORDS Antioxidants. Flavanols. Flavanoids. Phytochemicals. Quantification

ABSTRACT *Solanum torvum, Solanum aethiopicum* and *Solanum macrocarpon* are the popular vegetables of North Eastern India. This study was carried out to evaluate the bioactive properties, antioxidant activity and different phenolic compounds in these *Solanum* species. Sample extract in eighty percent methanol was used for total phenol, flavonols, flavonoid, antioxidant assays and various phenols quantification. All three species were rich in phytochemical content and having the good antioxidant capacity. HPLC fingerprinting for different phenolics compound confirmed that all the three species are having ample amount of phenolics content. The findings of present investigation highlighted the nutraceutical importance of lesser known though very important vegetables and also points towards the need to promote increased consumption and conserve genetic resources of *Solanum species*.

INTRODUCTION

It is a well-established fact that there is an association between the vegetable consumption and the reduced risk of chronic diseases such as cancer, diabetes, cardiovascular disease and arthritis etc. (Joshipura et al. 1999; Dai et al. 2006). Vegetables are a major source of antioxidants, mainly due to high polyphenolics such as tannins, flavonoids and phenolic acids. In India, the genus *Solanum* is represented by fourty-five species including those domesticated for their leaves, fruits or both eaten as a vegetable or used in traditional medicine. *Solanum torvum, Solanum aethiopicum* and *Solanum macrocarpon* bears edible fruits and are cooked as vegetables in North Eastern Hilly (NEH) region of India. The crops have great potential as alternative crops in the organic farming system in the hills of North eastern states of India, owing to high preference by consumers and high remuneration at the local market, despite being neglected in research and development.

Solanum torvum Sw., commonly known as Turkey berry, is widely naturalized in South and South East Asia. It is also found in some parts of Asia. The fruits and leaves are widely used in Cameroonian folk medicine and used for curing pneumonia (Fig.1). Fruits are thin-fleshed and contain a large number of flat, round, brown seeds (Liogier 1995). *Solanum aethiopicum*, one of the leading vegetables in tropical Africa commonly known as the African eggplant or Ethiopian eggplant. *Solanum aethiopicum* has round fruit shape with green strips (Fig.1). Its leaves are eaten as a leafy vegetable and fruits are eaten raw or cooked, whereas berries are used as an ornament in Asia. *Solanum macrocarpon* is a small tropical perennial plant that originated from Africa, cultivated either for fruits, which are 3-10 cm in diameter, flat in shape, non-ribbed, with a smooth surface and white or green coloured at the commercially mature stage, or for its leaves, which are used in the same way as spinach (Fig. 1) (Nyadanu and Lowor 2015).

Though the popularity of the above species in African diet and agriculture is well known, their economic and dietary importance in the Indian context is not studied so far. In many

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Solanum macrocarpon

Solanum aethiopicum

Solanum torvum

Fig. 1. Morphological variation in fruits, flowers and leaves of three species of *Solanum*

African and Asian countries, wild edible plants are used as food and hence, contribute significantly to the nutritional needs of the people. Among the vegetables; eggplant is commonly known as the source of phenols and flavonoids both of which are powerful antioxidants (Kaur et al. 2014). These selected vegetables for this investigation belonging to *Solanum* species are identified as the good source compounds like phenols and flavonoids with high antioxidant activities, as revealed from recent few studies in other part of the world (Ilodibia et al. 2016; Famuwagun et al. 2017; Sathyanarayana et al. 2016; Eletta et al. 2017). These crops have attained a considerable place in the diet of the population of North Eastern states of India. In Sikkim, a Himalayan state of North East India, these crops are found adapted up to an elevation of 5000 ft from MSL and are important components of integrated backyard farm of almost every household. The entire state is now certified as organic, satisfying all the norms and guideline laid down by the regulatory bodies. These crops are more intensified owing to their wide adaptability and tolerance to adverse climatic conditions like winter drought and heavy rainfall. Though the crops are the part of every household, a full understanding of its benefits as bioactive food with antioxidant activity is yet to be established.

Research Objective

The present study was envisaged for analysing the bioactive profile, antioxidant activity and quantification of the phenols will boost the importance of such neglected vegetable crops. So, present investigation aimed to profile bioactive content, antioxidant activity and quantification of phenols of three species of *Solanum*.

METHODOLOGY

Plant Material

The edible part of three species of *Solanum* namely *Solanum torvum, Solanum aethiopicum and Solanum macrocarpon* were collected from all of the four districts of Sikkim from July 2015 to December 2015. In each district, at least five villages and local market were inspected for the collection of samples. At least 2 kg fruit samples of each species were collected from each sampling site. Total 68 samples were collected from all the sampling sites. The collected fruits were placed in a polyethene bag to prevent loss of moisture and transported to the laboratory of the department of Horticulture, Sikkim University, Sikkim, India within 24 hours. Fruits were well washed 2-3 times with running tap water and once with sterile double distilled water and wiped to dry all the water around it as recommended by Badau et al. (2013) and Pillai and Nair (2013). The collected samples of each species were dried in the oven at 25°C and were grinded into powder and stored at room temperature under a dry condition in an airtight plastic container (Nair et al. 2013; Senga et al. 2013). More than 300 g of a sample of each species were blended using the blender after peeling the fruit (except *Solanum torvum* because of its small size and thin skin) and used for analysis as representative samples.

Extract of the Samples

2.0 g of powdered samples of all three species were mixed with methanol (80%) in a ratio of 1:25 and extracted in a Soxhlet apparatus for 5 hours. This process was repeated for 3 times for each sample and the combined extracted samples were concentrated using rotatory evaporator. Extracted samples were further concentrated using Eppendorf Speed Vac/Concentrator Plus at 40^0C and stored at -20^0C. The concentrated samples were used as a sample extract for estimation of phytochemicals, antioxidant activity and quantification of phenols.

Phytochemical Analysis

Total Phenolics

Total phenolics content of the methanol extract were estimated by spectrophotometer using Folin–Ciocalteu reagent (FCR) as described by Lin et al. (2011) where Gallic acid was used as a standard. For one ml of sample extract (80% ethanol), 5.0 ml of Folin–Ciocalteu reagent and 4.0 ml of 7.5 percent Na_2CO_3 solutions were added. The mixture was kept without disturbance for 90 minutes before the absorbance was measured at 760 nm against blank reagent in UV Vis spectrophotometer (Perkin Elmer, Lamb 35). A calibration curve was drawn with standard Gallic acid and results were expressed as Gallic acid equivalent (mg GAE/g DW).

Total Flavonoids Content

Total flavonoids were estimated using Aluminium chloride method as described by Lin et al. (2011). An aliquot (5ml) of extract in 10 ml of volumetric flask having, 0.3 ml of five percent $NaNO_2$ and 0.3 ml of ten percent $AlCl_3 6H_2O$. That mixture was allowed to react for 6 minutes at room temperature. Two ml of 1 mol^{-1} NaOH was added and the solution was diluted to 10 ml with distilled water. The absorbance of the solution versus a blank at 510 nm (Perkin Elmer, Lamb 35) was measured immediately. The results were stated as Rutin equivalent (mg RUE/g DW).

Total Flavonols

Total flavonol contents in the methanol extracts were measured using the method reported by Kumaran and Karunakaran (2006). About 2.0 ml of sample was taken and 2.0 ml of two percent $AlCl_3$ and 3 ml sodium acetate (50 g/L) solutions were added. The absorption was measured at 440 nm (Perkin Elmer, Lamb 35) after 2.5 hours at 20°C. Total flavonols contents were calculated as Rutin (mg RUE/g DW) which was used as a standard.

Antioxidant Activity

Free Radical Scavenging Activity using DPPH Assay

DPPH (2, 2-diphenyl picrylhydrazyl) assay is based on the scavenging ability of antioxidants towards the stable radical DPPH according to Yu et al. (2002) and Aoshima et al. (2004) with slight modification. 2.0 ml of sample extract was added to 5.0 ml of DPPH (0.1 mmol l^{-1}) solution in ninety-five percent methanol and vortexed. After 30 minutes, change in the absorbance of the sample extract was measured at 517 nm with the help of UV Vis spectrophotometer (Perkin Elmer, Lamb 35). The results were expressed as a percentage of inhibition of DPPH which was calculated by the following formula:

Inhibition (%) = $100 \times (A0 - A) / A0$

Where, A0 was the beginning absorbance at 517 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 517 nm. Methanol (95%) was used as a blank.

Hydroxyl Radical Scavenging Activity (HRSA)

The hydroxyl radical scavenging activities of the sample extracts were measured by the method as explained by Yu et al. (2004) with minor modification. This assay is based on Fenton reaction. 2.0 ml of sample extracts were added to 2.0 ml of 0.2 M phosphate buffer, 0.04 ml ferrous sulphate (0.02 M) and 1.0 ml of 1, 10-phenanthroline (0.04 M) in a test tube. 0.1 ml 7mM H_2O_2 was added to test tube to initiate the reaction. The reaction mixture without sample extract was used as a control. The absorbance of colour formed was measured at 560 nm by UV Vis spectrophotometer (Perkin Elmer, Lamb 35) against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following formula:

$$(\%)\ HRSA = (A_{Blank} - A_{sample} / A_{Blank}) \times 100$$

Hydrogen Peroxide Scavenging Activity (HPSA)

Hydrogen peroxide scavenging activity of the sample extracts was determined by Aiyegoro and Okoh (2010) method with slight modification. A 4.0 ml of sample extract was mixed with 2.4 ml of 4 mM H_2O_2 solution prepared in 0.1 M phosphate buffer (pH 7.4) and incubated at room temperature for 10 minutes. The absorbance at 230 nm (Perkin Elmer, Lamb 35 UV Vis spectrophotometer) was measured against a blank, containing the extract without H_2O_2. The result was expressed as a scavenging activity (%) calculated by:

$$(\%)\ HPSA = (A_{Blank} - A_{sample} / A_{Blank}) \times 100$$

Fe^{2+} Chelating Activity (FCA)

The sample extracts were evaluated for their ability to compete with ferrozine for iron (II) in solution by the method of Dinis et al. (1994). 2.0 ml of sample extract was added to the 2.0 ml of ferrous sulphate (0.125 mM). The reaction was initiated by addition of 2.0 ml of 0.3125 mM ferrozine. The mixture was vortexed and kept at room temperature for 10 minutes. The absorbance of the solution was measured at 562 nm (Perkin Elmer, Lamb 35 UV Vis spectrophotometer) against blank solution prepared using ferrous chloride and water. EDTA (0.625-5.0 mg) served as positive control and sample without extract or EDTA served as negative control. The percentage inhibition of ferrozine-Fe (II) complex was calculated using the formula:

(%) Chelating Activity = ($A_{Control}$–A_{sample} / $A_{Control}$) ×100

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

FRAP assay was performed in all three sample extracts according to the procedure of Hazra et al. (2008) with slight modification. 2.0 ml of sample extract was added to the 2.0 ml phosphate buffer (0.2 M, pH 6.6) and 2.0 ml 0.1 percent potassium ferricyanide. The reaction mixture was incubated at 50°C in water bath for 20 minutes. The reaction was stopped by adding 2.0 ml of Trichloroacetic acid (10%). The upper portion of solution (2.0 ml) was mixed with 2.0 ml of distilled water and 2.0 ml of 0.01 percent ferric chloride. The reaction mixture was kept at room temperature for 20 minutes. Absorbance at 700nm (Perkin Elmer, Lamb 35 UV Vis spectrophotometer) was measured at against blank. Results were expressed as mg of Gallic acid equivalent/ g dry weight (mg GAE/g DW) where Gallic acid was used as positive control.

Phosphomolybdenum Assay (PMA)

All the sample extracts were evaluated for antioxidant activity by the green phospho-molybdenum complex as the method described by Prieto et al. (2006). 0.1 ml of sample extract was added to 1.0 ml of reagent solution made up of 0.6 M of sulphuric acid, 28 mM of sodium phosphate and 4 mM of ammonium molybdate in a test tube. The test tubes were incubated for 90 minutes in a water bath at 95°C. The test tubes were cooled at room temperature and the absorbance at 695 nm (Perkin Elmer, Lamb 35 UV Vis spectrophotometer) of the reaction mixture was measured. The result was expressed as mg of ascorbic acid equivalents/g dry weight (mg AAE/ g DW).

Chromatographic Conditions for Quantification of Phenolics

High-performance liquid chromatography was performed with the HPLC system Agilent Series 1100, Agilent Technologies; U.S.A. Reversed-phase chromatographic analyses were carried out in isocratic conditions using RP-C18 column (4.6 mm × 250 mm) packed with 5-μm diameter particles. The mobile phase was acetonitrile-water (10:90, v/v) containing one percent of acetic acid. The flow rate was 0.7 ml/min, injection volume was 40.0 μL, and detection was done at 310 nm. The mobile phase was filtered through a membrane filter (0.45 μm) and then degassed by an ultrasonic sound before use. The crude extract and the solutions of standards (gallic acid, rutin, catechol, ferulic acid and quercetin) were prepared in the same mobile phase of HPLC. All chromatographic operations were performed at room temperature and in triplicate.

RESULTS

Total phenolics, total flavonoid and total flavonols were evaluated in three species of *Solanum* and results are presented in Table 1. Total phenol content was highest in *Solanum aethiopicum* (3.89 mg GAE/g DW) followed by *Solanum torvum* (2.03 mg GAE/g DW) and *Solanum macrocarpon* (1.03 mg GAE/g DW). Total flavonoid was also highest in *Solanum aethiopicum* (27.59 mg RUE/g DW) followed by *Solanum macrocarpon* (12.69 mg RUE/g DW) and *Solanum torvum* (7.43 mg RUE/g DW). Total flavonols were higher in *Solanum aethiopi-*

Table 1: Phytochemical content and antioxidant capacity of three *Solanum* species

Phytochemicals/Antioxidant capacity	*Solanum torvum*	*Solanum aethiopicum*	*Solanum macrocarpon*
Total phenol content (mg GAE/g DW)	2.03±0.04	3.89±0.07	1.03±0.05
Total flavonoid (mg RUE/g DW)	7.43±0.48	27.59±0.92	12.69±0.35
Total flavonols (mg RUE/g DW)	4.86±0.12	9.07±0.09	4.35±0.21
DPPH Assay (%)	77.9±1.4	74.8±0.9	80.4±1.0
Hydroxyl radical scavenging Assay (%)	77.9±0.8	67.5±1.6	75.9±1.3
Hydrogen peroxide scavenging Assay (%)	35.2±0.6	61.5±0.8	45.8±0.4
Ferrous ion chelating assay (%)	24.5±0.2	39.4±0.6	12.3±0.1
FRAP Assay (mg GAE/g DW)	4.26±0.24	20.03±0.17	4.77±0.92
Phosphomolybednum assay mg AAE/g DW)	32.86±1.41	41.13±0.93	40.58±1.63

cum (9.07 mg RUE/g DW) followed by *Solanum torvum* (4.86 mg RUE/g DW) and *Solanum macrocarpon* (4.35 mg RUE/g DW).

The extracts of all the three species showed good capacity [*Solanum macrocarpon* (80.4%) > *Solanum torvum* (77.9%) > *Solanum aethiopicum* (74.8%)] for scavenging free radicals by reducing the stable DPPH radical to the yellow coloured diphenyl picryl hydrazine (Table 1). *Solanum torvum* has the highest hydroxyl radical scavenging activity (77.9%) followed by *Solanum macrocarpon* (75.9%) and *Solanum aethiopicum* (67.5%). Hydrogen peroxide scavenging activity was found highest in *Solanum aethiopicum* (61.5%) followed by *Solanum macrocarpon* (45.8%) and *Solanum torvum* (35.2%). Among them, *Solanum aethiopicum* (39.4%) possesses highest metal chelating activity followed by *Solanum torvum* (24.5%) and *Solanum macrocarpon* (12.3%). Table 1 shows that the reducing power of ferric ion and phospho-molybdenum complex in all the three vegetable extract. *Solanum aethiopicum* shows highest reducing power in both assays followed by *Solanum macrocarpon* and *Solanum torvum*.

Chromatographic fingerprinting of the extracts using reverse phase HPLC is presented in Table 2. Gallic acid, rutin, catechol, ferulic acid and quercetin were identified in the three studied species of *Solanum*. The amount of Gallic acid varied from 46.31 to 1402.14, rutin 33.02 to 94.04, catechol 238.38 to 559.44, ferulic acid 2.90 to 24.70, quercetin 10.06 to 51.17 mg per 100 g dry weight. Among the entire phenolic compound quantified here, Gallic acid was found dominant in all the three species. Gallic acid, rutin and ferulic acid were found highest in *Solanum torvum*, while catechol presence was highest in *Solanum aethiopicum* and quercetin in *Solanum macrocarpon*.

DISCUSSION

Phytochemical Analysis

A phenolic compound in *Solanum* has been recognised as a major bioactive compound which is responsible for their antioxidant effects (Kwon et al. 2008). It is a much-acquainted fact that presence of significant amount of phenolics in daily food gives health-promoting effects due to their antioxidant action. Phenolic compounds of natural origin possess antimicrobial, anti-cancerous and neuroprotective activities and help in improving insulin secretion and reducing unwanted fat in the body (Kaur et al. 2014). A result of this study shows that these species were rich in total phenol content which were in support of the results of earlier experiments (Nisha et al. 2009; Raigón et al. 2010). Phenolic compounds also impart peculiar taste and aroma to the foods through phenolic degradation or mechanisms of Maillard reaction (Jiang and Peterson 2010). This may adequately explain why immature fruits of *Solanum torvum*, *Solanum aethiopicum* and *Solanum macrocarpon* are relished in Northeast India and Africa.

Flavonoids have been found powerful scavengers of singlet oxygen and various other free radicals, related to DNA damage and cancer which makes it beneficial agents for the management of a multitude of diseases states, including cancer, cardiovascular and neurodegeneration (Marchand 2002). Many horticultural resources of wild origin are identified to be reservoirs of high flavonoid content. The results of this study suggest that phenolics, flavonoid and flavonols are important components of *Solanum torvum*, *Solanum aethiopicum* and *Solanum macrocarpon* which could be attributed to playing a significant role in adsorbing and deactivating free radicals, quenching singlet oxygen or decomposing peroxides (Ilodibia et al. 2016; Famuwagun et al. 2017).

Antioxidant Assays

A lot of studies have confirmed that the antioxidants derived from indigenous plant sources are very useful in preventing the damaging effects of oxidative stress (Zahin et al. 2009). A number of assays were designed to measure

Table 2: Gallic acid, rutin, catechol, ferulic acid and quercetin quantification (mg/100g dry weight) in three *Solanum* species

Species	*Gallic acid*	*Rutin*	*Catechol*	*Ferulic acid*	*Quercetin*
***Solanum** torvum*	1402.14	94.04	559.44	24.69	10.82
***Solanum** aethiopicum*	559.55	33.49	927.43	2.90	10.06
***Solanum** macrocarpon*	468.31	33.02	238.38	6.09	51.17

overall antioxidant activity or reducing potential, as an indication of a host´s total capability to endure free radical stress (Gulcin et al. 2010). In order to get a better estimate of antioxidant capacity, multiple antioxidant assays are usually performed rather than rely on the single assay. In this study, antioxidant capacity in three *Solanum* species extracts was evaluated using six in-vitro assays namely, DPPH, HRSA, HPSA, FCA, FRAP and PPMA. These antioxidant assays are commonly used assays among researchers being reliable and economical.

Free radicals are mainly produced as a result of the oxidation process. The high potential for scavenging free radicals could inhibit the spread of oxidation. The conversion of the ion-radical form of DPPH-H, by the antioxidants, could be assessed by monitoring the change of colour from purple to yellow. All the species under study showed better DPPH radical scavenging activity which was in agreement with Loganayaki et al. (2010), Kaur et al. (2014), Eletta et al. (2017) and Famuwagun et al. (2017). The oxidatively induced breaks in DNA strands are produced due to hydroxyl radicals produced through Fenton reaction to yield its open circular or relaxed forms. These radicals are most reactive form among all the dioxygen reduced forms and supposed to cause cell damage *in vivo* (Rollet Labelle et al. 1998). They 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. An extract hydroxyl radical scavenging activity is directly associated with its antioxidant activity (Babu et al. 2001). The ability of extracts to reduce hydroxyl radicals is directly related to prevention of multiplication of lipid peroxidation and they may identify to be a good scavenger of active oxygen species. Hydrogen peroxide (H_2O_2) is a weak oxidizing agent that belongs to a non-radical form of ROS. Hydrogen peroxide can cross cell membranes quickly and form hydroxyl radical which inactivates few enzymes directly (Miller et al. 1993). The result shows that all the three species were good in hydroxyl radical and hydrogen peroxide scavenging activity.

One of the important mechanisms of antioxidant activity is the ability of chelating or deactivating the transition metals that have the capability to catalyse hydroperoxide decomposition. That's why it is important to evaluate the Fe (II) chelating ability of the extracts. The Fe (II) chelating properties of the sample extracts may be accredited to their endogenous chelating agents, mainly phenolics. The Fe^{2+}chelating activity of the extracts was measured by a decrease in absorbance of the ferrozine complex as antioxidants compete with ferrozine in chelating ferrous ion (Elmastas et al. 2006). FRAP assay is the only assay that directly evaluate the reducing ability of antioxidants that react with ferric tripyridyl-triazine (Fe^{3+}–TPTZ) complex and produces a coloured ferrous tripyridyl-triazine (Fe^{2+}–TPTZ) (Benzie and Strain 1996), while phosphomolybdenum complex assay (PPMA), generally assist in the detection of ascorbic acid, phenolics, tocopherols and carotenoids which are used for the evaluation of total antioxidant capacity (Miladi and Damak 2008). The reducing ability of ferric ion by the extracts shown that all the three species have optimal FRAP activity. The difference in the responses of the extract in different antioxidant tests could be described by the fact that the transfer of electrons/hydrogen from antioxidants followed at various redox potential in different assay systems and the transfer also may be subjected to the structure of the antioxidants (Loo et al. 2008).

Quantification of Phenolics

The extracts of natural origin contain several chemical components in varying concentrations, so it is important to use chromatographic methods to analyse these inherently complex mixtures. Various antioxidant activities of these species could be due to the presence of ample amount of various phenolic compounds present in these species. Epidemiological studies have found consumption of foods containing these phenolics have positive association towards a reduced risk of developing several disorders such as cardiovascular diseases, antidiabetic, antimicrobial, inflammatory and neurological activities etc. (Adefegha et al. 2014; Gandhi et al. 2011). Few reports on the phytochemical investigation of these *Solanum* species indicate the presence of these phenolics (Gandhi et al. 2011; Nwanna et al. 2014; Ramamurthy et al. 2012; Plazas et al. 2014; Sathyanarayana et al. 2016). These species may found an important place in pharmaceutical formulations due to their rich content of phenolic compounds.

CONCLUSION

All the three species studied here having a good amount of total phenolics, flavonoid and

flavanols. They also exhibited strong antioxidant activity in various antioxidant assays which could be contributed due to the presence of various phenols. These three species may find a place in people's food basket due to their reducing risk of various disorders and might be a valuable antioxidant natural source with application in healthy medicine and food industry. The findings of the present study encourage eating a variety of vegetables especially indigenous and underutilized because they are the natural sources of antioxidants.

RECOMMENDATIONS

On the basis of findings of this investigation, all the three species namely *Solanum torvum, Solanum aethiopicum and Solanum macrocarpon* which are found commonly in the hilly tracts of Sikkim Himalaya are recommended as a source of antioxidants and phenols and should be included in the daily meal.

ACKNOWLEDGEMENTS

The researchers are very thankful to the department of Horticulture and Chemistry, Sikkim University, Sikkim, India for providing the facilities for the present investigation.

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Paper received for publication on December 2017
Paper accepted for publication on January 2018