In vitro Anti-oxidant Assay, HPLC Profiling of Polyphenolic Compounds, AAS and FTIR Spectrum of Malaysian Origin *Solanum torvum* Fruit

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ABSTRACT

Objective: *Solanum torvum swartz, a* Solanaceae family plant is used by traditional systems of medicine. The aim of this study is to determine the phytochemical content and antioxidant potential of the fruits of *Solanum torvum* of Malaysian origin. **Method:** A preliminary phytochemical screening was carried out for the analysis of important phytochemicals present in *Solanum torvum* Fruit. Anti-oxidant potential of *S. torvum* Fruit was evaluated by various scavenging models including DPPH, FRAP and HPLC method used for Identification of polyphenolic compounds. Elemental analysis and Functional group analysis were done by Atomic absorption spectrophotometer (AAS) and Fourier Transform Infrared Spectrophotometer (FTIR) *methods* respectively. **Results:** Phytochemical screening was done for the aqueous, ethanolic and methanolic extracts of *S. torvum*. Quantitative assessment of total phenols and flavonoid content, DPPH and FRAP assay was done in the ethanolic extracts of *S. torvum*. Qualitative analysis of each extract showed the presence of reducing sugars, saponins, alkaloids, phenols and flavonoids except anthraquinones. Quantitative determination of total phenols and flavonoids in STE showed 16.4 mg GAE/g extract of *S. torvum* and 2.8 mg QE/g extract of *S. torvum* respectively. In DPPH radical scavenging assay, the IC_{50} value of the extract was found to be 1.62 mg/ml and in FRAP assay, FRAP value of ethanolic fruit extract was found to be 470 mg $FeSO_4$ E/gr of *S. torvum* fruit extract. Extract analyzed by High performance liquid chromatography revealed presence of polyphenolic compounds such as gallic acid, rutin, quercetin and ascorbic acid. Elemental determination by Atomic absorption spectrophotometer (AAS) showed the presence of essential elements. Fourier Transform Infrared Spectrophotometer (FTIR) report shows the presence stretching vibrations of OH groups in phenyl, CH_2 asymmetric stretch of methyl groups, C-O stretching vibrations ring of phenyls, CH bending vibration. Based on the above obtained data, it can be concluded that fruits are a rich source of antioxidants. **Conclusion:** *S. torvum* a local Malaysian medicinal plant fruit ethanolic extract possesses good amount of phenols and flavonoids responsible for the antioxidant property.

Key words: Polyphenolic compounds, Antioxidant property, HPLC, AAS, FTIR.

INTRODUCTION

The genus solanum family comprises of 1500 species. Botanical classification of *Solanum torvum*. Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Solanales, Family: Solanaceae, Genus: Solanum, Species: *Solanum torvum* sw. It is a small shrub widely distributed in Malaysia. In Malay it is also known as terong belanda, terong perak, terong pokok, terong puyoh, terong rapok. It is known as Turkey berry,

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Egg plant or Devils figure, West Indian Turkey Berry in English, Sanskrit: Brihati, Marathi: Marang, Hindi: Bhurat, Bhankatiya, Folk Ran: Baingan, goth-begun, Kaatuchhunta in Malayalam and Sundaikkai in Tamil.[1]

Its edible fruits are considered as an essential ingredient in Malay and Thai cuisine. People of Tamilnadu use this fruit of *S. torvum* in their daily diet. It is a plant which is found all over the Indian subcontinent and West Indies, Bermuda, Indonesia, Malaya, China, Philippines and tropical America.[2-5]

The pericap of the fruit contains high amount of phenolic and flavonoid compounds that are the secondary metabolites of the plant. So the fruits and leaves of *S. torvum* are widely used in Cameroonian folk medicine. A decoction of fruits useful for cough ailments and in cases of liver and spleen enlargement.[6,7]

It is used as a sedative and diuretic. The leaves and ripened fruits are used for as a haemopoietic and haemostatic agents.[8] It is used worldwide in the traditional systems of medicine as poison anti-dote, for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension.[9]

Biological properties of fruits include properties like Antimicrobial,[10-13] Anti-viral,[14] Immuno-secretory,[15] Anti-oxidant,[16] Analgesic and Anti-ulcerogenicactivities,[17] Cardioprotective,[18] Nephroprotective,[19] Anti-diabetic,[20] Angiotensin and Serotonin receptor blocking activities.[21] The natural antioxidant properties of the plant depend on the total phenols and flavonoids content. Geographic location of plant, age of the plant, season, associated micro flora, nutritional status, and environmental stress determines the production of natural antioxidants of particular plant species.[9]

An antioxidant is a molecule capable of preventing the oxidation of free radicals. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radicals and free radical intermediates; inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents.[10]

Scientists and Nutritionists encouraged the use of antioxidants as food supplements in order to prevent diseases due to oxidative stress and to maintain good health. Hence, the present study is focused to evaluate the phytochemical contents and antioxidant activity of the fruits of *S. torvum* of Malaysian origin.

MATERIALS AND METHODS

Collection of Plant Material

S. torvum fruits were collected during the month of March and April from the Bedong, Sungai Petani, Kedah, Malaysia. The collected fruits were authenticated by Botanist Dr. Xavier. The voucher specimens were deposited in herbarium, Department of faculty of medicine, AIMST University, Malaysia for future reference. The work was carried out in the Department of Biotechnology and Faculty of Medicine Multi disciplinary lab.

The fruits were washed with distilled water and dried under shade, mechanically pounded to get coarse powder and passed through number 40 sieve meshes. The sample powders were processed in such a way that they are useful for phytochemical studies.

Chemicals

Quercetin, Aluminium chloride, Diphenyl picryl hydrazine (DPPH), Trichloroacetic acid (TCA) and FeCl3. DPPH was obtained from Hi media laboratories Pvt. Ltd. Mumbai. Aluminium chloride, TCA, FeCl3 were obtained from Merck, Mumbai, India; Quercetin was obtained from Sisco research laboratories Pvt. Ltd. (SRL) Mumbai, India.

Preparation of extract

Shade dried fruits of *S. torvum* were finely powdered with a blender and used for the preparation of aqueous, methanolic and ethanolic extracts. The aqueous extract was prepared by cold maceration process for a period of 72 h with occasional stirring. Then the mixture was filtered and the filtrate was collected and the solvent was removed under reduced pressure. Ethanolic and methanolic extracts were prepared by maceration process and concentrated using rotary evaporator. The extracts were dried under fume hood.

Phytochemical screening

The aqueous, ethanolic and methanolic extracts of *S. torvum* fruits were screened for the presence of phytochemical constituents, reducing sugars, saponins, alkaloids, anthraquinones, phenols and flavonoids using standard method.[10-13]

Determination of total phenols

Principle

Folin-Ciocalteu reagent is formed from a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, after oxidation of the phenols, is reduced to a mixture of blue oxides of

tungsten(W_8O_{23}) and molybdenum(Mo_8O_{23}). The blue color produced is proportional to the total quantity of phenolic compounds originally present.

Method

Total phenolic content in the *S. torvum* fruit extract was determined using the Folin-Ciocalteu method as described by Singleton *et al.* (1999) with slight modifications. Briefly, different concentrations of ethanolic fruit extract of *S. torvum* ranging from 125 to 500 µg/ml was prepared and 1.5 ml Folin-Ciocalteau reagent was added to the extracts and incubated at room temperature for 5 min followed by the addition of 4 ml of 20% Sodium carbonate (Na_2CO_3) solution. The contents were made up to 10 ml with distilled water and incubated at room temperature for 30 min and absorbance was recorded at 738 nm. Gallic acid was used as the standard.[21] The content of phenol in ethanolic leaf extract of *S. torvum* fruits was expressed in terms of gallic acid equivalent (Figure 1).

Determination of total flavonoids

Principle

Acid stable complexes are formed with the C-4 keto group or either C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A-or B-ring of flavonoids .

Method

The flavonoid content was determined by aluminium chloride colorimetric method. The method of Cetkovic *et al.*, (2008) was used with slight modifications. Varying concentrations of *S. torvum* fruits ethanolic extract ranging from 250-2000 µg/ml was prepared and was added with 3 ml ethanol, 0.2 ml aluminum chloride, 0.2 ml potassium acetate solution and 5.6 ml distilled water and the contents were mixed well. The absorbance was measured at 415 nm.[22,23] Quercetin was used to plot the standard calibration curve (Figure 2). Extract was analysed in triplicate and the results were expressed as milligrams of quercetin equivalent per gram dried weight (mg QE/g DW).

DPPH free radical scavenging assay

Principle

This method is based on the reduction of DPPH in ethanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non radical form DPPH-H, the colour changes from purple to yellow after reduction.

Method

In vitro DPPH radical scavenging activity of *S. torvum,* fruits extract was carried out by slightly modified method of Blois (1958).The antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.[24] The DPPH (Diphenyl–picryl-hydrazyl) is a stable free radical and is widely used to assess the radical scavenging activity of anti-oxidant compounds. The reaction mixture (3.0 ml) consists of 1.0 ml of DPPH in ethanol (0.3 mM), 3.0 ml of the extract. Subsequently, the mixture was immediately vortexed, incubated in the dark for 30 min and then the absorbance is measured at 517 nm.

This results in a colour change from purple to yellow. Radical scavenging activity increases with the increase in percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging activity of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses its colour depending on the number of electrons taken up.

In this assay, quercetin solution was used as the positive control to calibrate the standard curve.

The percentage of inhibition was calculated using the following formula:

$$Inhibition\ (\%) = (A_0 - A_1 / A_0) \times 100$$

Where;

A_0 is the absorbance of control and A1 is the absorbance of test.

Each experiment was carried out in triplicate and results are expressed as mean % antiradical activity (Figure 3).

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration of extracts that inhibits the formation of DPPH radicals by 50% Figure 4.

Ferric reducing antioxidant power (FRAP) assay

Principle

This method is based on the potential antioxidant which will reduce the ferric ion (Fe3+) to ferrous ion (Fe2+) which results in a blue complex formation (Fe2+/ tripyridyltriazine (TPTZ)).[25]

Method

Ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain (1999) with slight modification.[25] 100 µl extracted samples were mixed with 4.5 ml FRAP reagent in test tubes and thoroughly mixed by vortexing. The blank was prepared with FRAP and ethanol and the samples

at different concentrations prepared with FRAP, fruit extract and blank were incubated in water bath for 30 min at 37°C. The absorbance of the samples was determined against blank at 593 nm. Series of stock solution at 5, 2.5, 0.125, 0.625, 0.312, 0.156 mg/ml were prepared (R^2= 0.987) using aqueous solution of $FeSO_4$ $7H_2O$ as standard. The values obtained were expressed as mg of ferrous equivalent Fe (II) per gram of extract. Absorbance value for sample will be substituted in the y= mx+c and its reducing power were determined (Figure 5).

High performance liquid chromatography (HPLC) system

Chemicals

Standards of Gallic acid (GA) (purity 98.0%), rutin hydrate (RH) (purity 98.1%), quercetin (QU) (purity 98.5%), ascorbic acid (AS) (purity 99.0%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC), ammonium acetate (HPLC), methanol (HPLC) were purchased from Merck (Darmstadt, Germany), Water (HPLC grade) and all other chemicals used were of analytical grade.

Preparation of standard solutions

10 mg of each reference standard was accurately weighed and transferred into a 10 ml volumetric flask and dissolved in methanol and made up to the volume with the same solvent to produce a 1 mg/ml of each. The stock solution was stored in refrigerator at -20 ± 2°C until analysis.

Preparation of Samples

10 mg of each sample were accurately weighed and transferred into a 10 ml volumetric flask and dissolved in methanol and made up to the volume with the same solvent to produce a 1 mg/ml of each then filtered with whatman filter paper. The filtered solutions were used for the analysis.

HPLC Apparatus and chromatographic conditions

The following method is described by Laghari *et al.* (2011) with slight modifications.[26] The chromatographic separation was achieved on a Shimadzu high-pressure liquid chromatographic system equipped with a binary LC-20AD solvent delivery system, SPD-20A Photodiode Array (PDA) detector and SIL-20ACHT injector with 100 µL loop volume. The LC solution version 1.25 data acquisition system was used for data collecting and processing (Shimadzu Corporation, Japan). Thermo C_{18} (250×4.6 mm i.d., 5.0 µ) column was used for analysis (Thermo scientific, USA).

The column and auto sampler tray temperature were kept constant at 40°C and 4°C respectively. The mobile phase consisted of a mixture of acetonitrile: 5 mM ammonium acetate (40:60) (PH 4.5) and was delivered at a flow-rate of 1.0 ml/min with detection at 278 nm. The mobile phase was filtered through a 0.45 µm, membrane filter. The sample injection volume was 25 µl. Standard Peak areas for quercetin, rutin, gallic acid and ascorbic acid detector response peak response vs run time were recorded. Peak areas for all components were automatically integrated using the LC solution version 1.25 data acquisition system (Shimadzu Corporation, Japan). All chromatography operations were carried out at ambient temperature and in triplicate.

Elemental Determination by Atomic Absorption Spectrophotometer (AAS)

Principle

Atomic absorption is a process involving the absorption by free atoms of an element of light at a wavelength specific to that element, or put more simply, it is a means by which the concentration of metals can be measured.

Chemicals

Magnesium (Mg), Manganese (Mn), Copper (Cu), Lead (Pb), Zinc (Zn), Calcium (Ca), Iron (Fe), Nickel (Ni), Sodium (Na), Sulphuric acid (H_2SO_4), Perchloric acid ($HCIO_4$), Nitric acid ($HNO_{3)}$, deionized water.

Equipments

Volumetric flasks, crucible, filter paper.

Method

Five grams (5 g) of *S. torvum* fruits dried sample was weighed into a crucible. The crucible was placed in a hot furnace and ashed at 600°C for 3 h. The furnace was cooled to about 120°C. The crucible was then removed and placed in a desiccators for 1 h to cool before weighing. The process was repeated until a constant weight was obtained. The ashed sample (0.5 g) were weighed and transferred into the digestion tube. 5 ml each of distilled water, concentrated Nitric acid (HNO_3) and Hydrochloric acid ($HCIO_4$) were added and the content mixed. The tubes were placed into the digestion block inside a fume cupboard and the temperature control of the digester was set at 150°C and digested for 90 min. The temperature was then increased to 230°C and digested for another 30 min. The digester temperature was reduced back to 150°C followed by the addition of 1 ml of hydrochloric acid to the tubes within a few min. The concentrated digest was not allowed to cool to room temperature to prevent for-

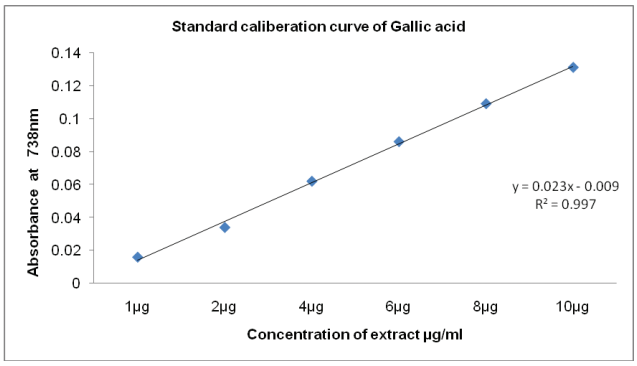

Figure 1: Calibration curve of standard Gallic acid for determination of total phenolic content

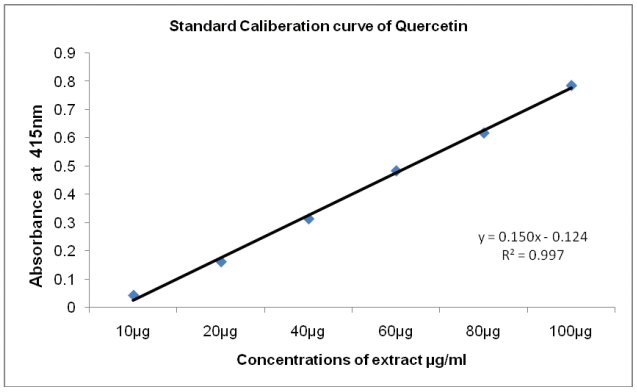

Figure 2: Calibration curve of standard Quercetin for determination of total flavonoid content

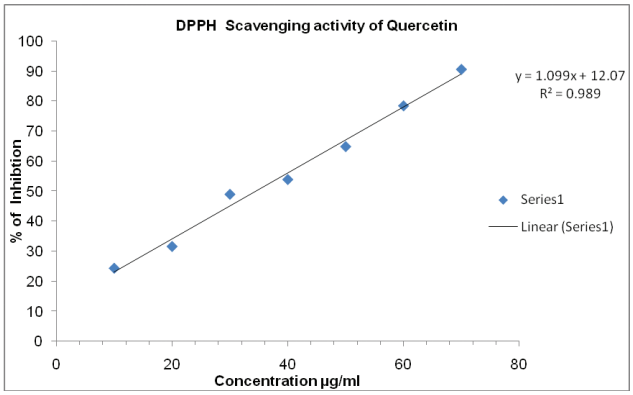

Figure 3: Results of DPPH radical scavenging assay of Quercetin

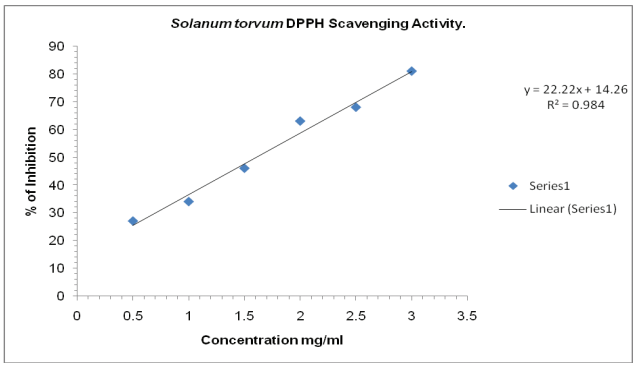

Figure 4: Results of DPPH radical scavenging assay of *S. torvum* fruit

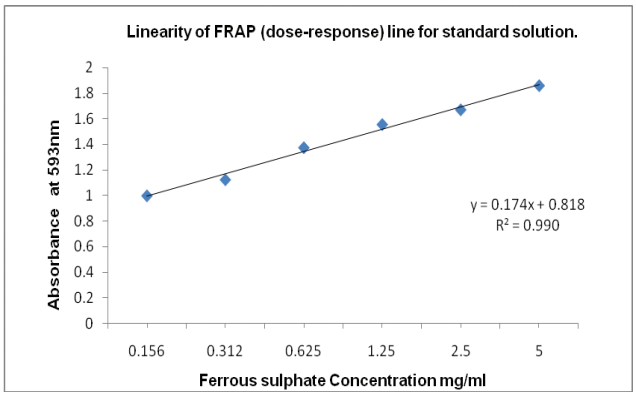

Figure 5: Results of FRAP radical scavenging assay of Ferrous sulphate

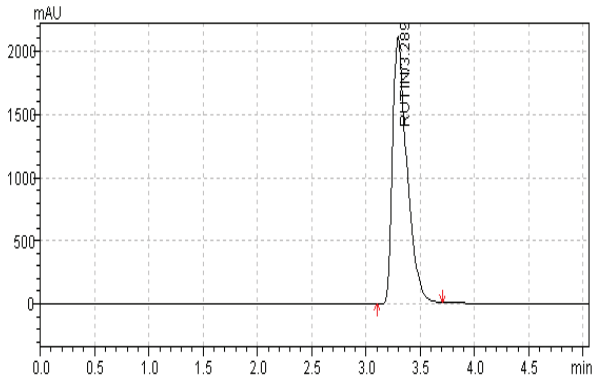

Figure 6: HPLC chromatogram of Rutin at concentration of 25 µg/ml

mation of insoluble precipitate (potassium per chlorate). More water was added to the tube to make up to mark and the content was mixed and filtered. The resulting solution was used for the elemental analysis using atomic absorption spectrophotometer (AAS) (A. Analyst 400 Model) at an appropriate wavelength, temperature and lamp-current for the different elements.[27] The following elements were determined, Calcium (Ca), Copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb), Zinc (Zn), Nickel (Ni), Magnesium (Mg), and Sodium (Na).

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful method for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic

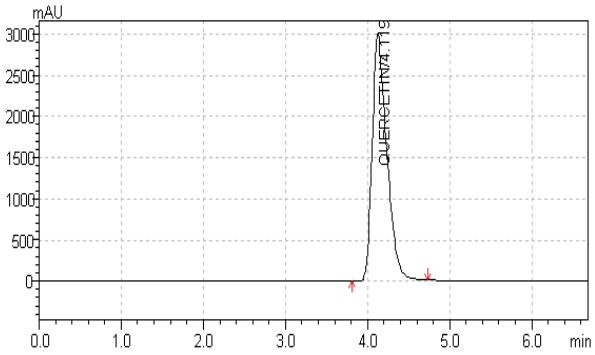

Figure 7: HPLC chromatogram of Quercetin at concentration of 25 µg/ml

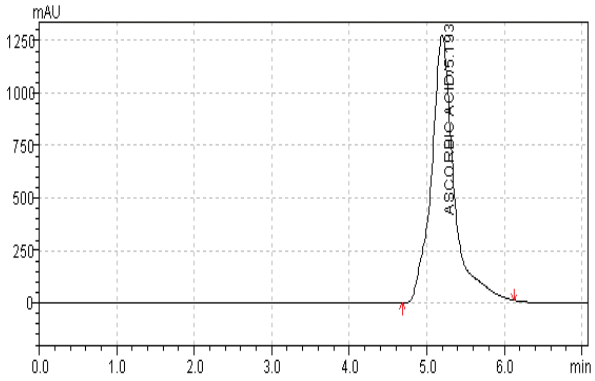

Figure 8: HPLC chromatogram of Ascorbic acid at concentration of 25 µg/ml

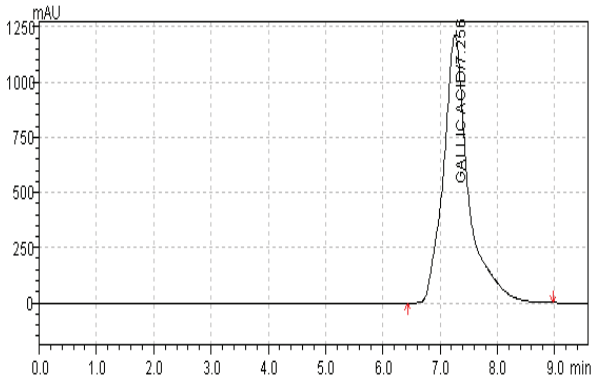

Figure 9: HPLC chromatogram of Gallic acid at concentration of 25 µg/ml

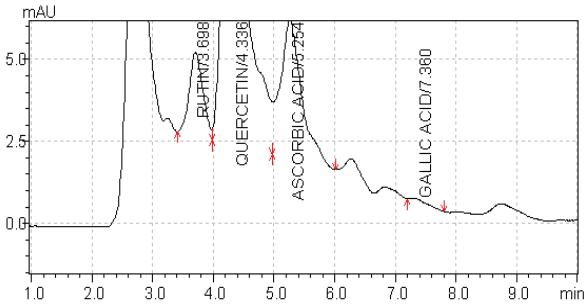

Figure 10: HPLC chromatogram of *S. torvum* extracts. Peaks: Rutin hydrate, Quercetin, Ascorbic acid and Gallic acid

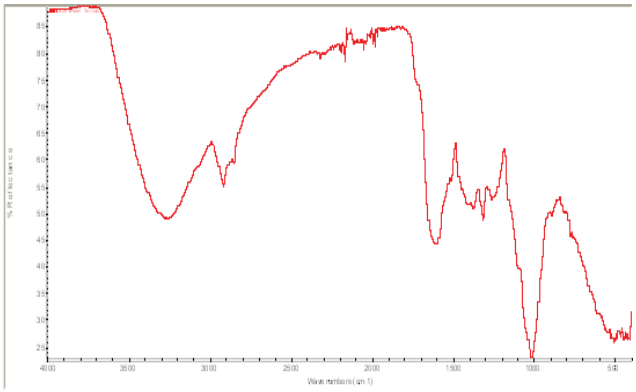

Figure 11: Results of FTIR analysis

of the chemical bond as can be seen in the annotated spectrum. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum. Dried powder of *S. torvum* fruit was used for analysis. 10 mg of the dried powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs.[28] The powdered fruit sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Statistical analysis

The experiments were performed in triplicate and the data were statistically analysed as mean ± SE. All graphs were plotted using MS Excel ®software 2010.

RESULTS AND DISCUSSION

Qualitative and Quantitative Phytochemical screening

Solanaceae family includes many medicinal plants. *S. torvum* fruits preliminary qualitative phytochemical screening of the extract was carried out to assess the bioactive components. Qualitative phytochemical analysis showed the presence of reducing sugars, alkaloids, saponins, tannins, phenols, flavonoids (Table 1).

Quantitative analysis showed the presence of total phenolic content i.e. 16.4 mg GAE/gr of ethanolic extract of *S. torvum* fruits. The total flavonoid content of ethanolic extract of *S. torvum* fruits is 2.8 mg E/gr extract of *S. torvum* fruits (Table 2). The medicinal

properties of the plants depend on phenolic and flavonoid content. Being a Solanaceae family, *S. torvum* fruits also showed significant levels of phenols and flavonoids which may play a critical role as a source of phytomedicine.

Phenolic compounds are most widely distributed secondary metabolites in the plant kingdom. The quantity of phenolic compounds present in plants are influenced by genotype, storage method and environmental conditions.[29] Plant phenolic constituents are the major group that accounts for antioxidant properties of a plant. Phenolics are able to scavenge reactive oxygen species (ROS) due to their electron donating properties.

Antioxidant activity

The ethanolic extract of *S. torvum* fruits showed prominent IC_{50} value of 1.62 mg/ml as compared to Quercetin 34.51 µg/ml. (Table 3) which is a well known antioxidant. The ferric reducing antioxidant potential (FRAP) assay ethanolic fruit extract showed value of 470 mg $FeSO_4$ E/gr of *S. torvum* fruits extract (Table 3).

HPLC Studies

Identification of individual polyphenolic compounds in the *S. torvum* fruits were analysed by HPLC system. In this study, we used four different polyphenolic standards, C_{18} column with 250 mm length, The wavelength between 210 and 380 nm was used for the detection of polyphenolic compound.[30,31] The wavelength of 259 nm was more sensitive for determining the analyte. In this study, the flow-rate of the mobile phase was also screened; 1 ml/min was a suitable flow rate to get an optimum retention time for *S. torvum* chromatogram.

Therefore, for detection of standards 254 nm for ascorbic acid, gallic acid, and 366 nm wavelength, rutin, quercetin were selected in this study. It can be observed that a good separation can be achieved within 40 min using the above condition described. Symmetrical, sharp and well-resolved peaks were observed for the four polyphenolic standards. The elution order and the retention times for RU, QU, AS, and GA were 3.28, 4.11, 5.19 and 7.25 min respectively. (Figure 6, 7, 8, 9) and in the *S. torvum* elution order and retention time for, RU, QU, AS and GA were 3.69, 4.33, 5.25 and 7.36. (Figure 10) From the above observed data, it can be interpreted that there is presence of rutinhydrate, quercetin, ascorbic acid and gallic acid. The present HPLC study could be indicative to a potential application to identify and quantify the polyphenolic compounds in any medicinal plant extract since phenolic compounds are of great interest. The antioxidant activity showed by the *S. torvum* ethanolic extract are mainly due presence of (polyphenolic compounds) quercetin, rutin, gallic acid and ascorbic acid. (Figure 10). On the basis of this study, fruit extract showed significant antioxidant activity compared to standard compounds *in vitro*.

Atomic Absorption Spectrophotometer (AAS) studies

The elemental analysis result shows the presence of Calcium (Ca), Copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb), Zinc (Zn), Nickel (Ni), Magnesium (Mg), and Sodium (Na) in the fruit sample at 5 ppm concentration (Table 4). The concentrations of the essential elements appear to be lower which is within safety limit according to W.H.O (1996).[32]

The lower concentration of iron (Fe), zinc (Zn), and copper (Cu) is an indication of little or no toxicity of the plants as heavy metals are known to cause cancer, liver and kidney problems. The elements (Mg, Ca, Cu, Mn) are used extensively in chemotherapy and are essential in human and animal health. Magnesium and calcium are known to help in bone and teeth development.[33,34]

Fourier Transform Infrared Spectrophotometer (FTIR) studies

S. torvum spectrum showed characteristic absorption bands at 3350.0 cm^{-1} for a hydroxyl (-OH) group, 2915 cm^{-1} (for C-H asymmetric stretching), 2850 cm^{-1} (for C-H symmetric stretching), 1520.0 cm^{-1} (for C-H bending), 1350.0 cm^{-1} (for OH bending) , 900.0 cm^{-1} (for CH asymmetric bending) (Table 5,6). *S. torvum* gave bands from 3320-3562 cm^{-1} for the stretching vibrations of OH groups in phenyl, 2915 cm^{-1} for CH_2 asymmetric stretch of methyl groups mainly of lipids ; all in the functional group region.[35] In the fingerprint region, sharp peaks at 900 cm^{-1}, 1350 cm^{-1} and 1520 cm^{-1} and a broad band at 1630 cm^{-1} were shown (Figure 11).

This inference the presence stretching vibrations of-OH groups in phenyl, -CH_2 asymmetric stretch of methyl groups, -C-O stretching vibrations ring of phenyls, -CH bending vibration(Table 5,6). Based on the obtained and cited data it can be concluded that fruits are a rich source of anti-oxidants.

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are the most frequent pro-oxidants which originate from normal metabolism or induced by UV radiation and different pollutants. Harmful effects are prevented by intake of antioxidant substances.[36-38] The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect.[39-41] Therefore, the Research on biological activity and chemical composition of medicinal plants are required to find out the natural antioxidants and importance in drug discovery.

Table 1: Phytochemical screening of the various extracts of the *S. torvum* fruits

Phytochemicals	Extracts		
	Water	Methanol	Ethanol
Reducing sugars	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Tannins	–	–	–
Anthraquinones	–	–	–
Phenols	+	+	+
Flavonoids	+	+	+

Note: (+) = Present and (-) = Absent.

Table 2: Total phenols and flavonoid content in *S. torvum* fruits

Phytochemicals	Concentration
Total phenols	16.4 mg GAE/gr of *S. torvum*
Flavonoids	2.8 mg QE/gr of *S. torvum*

Table 3: *S. torvum* fruits ethanolic extract IC_{50} and FRAP values

Name of the plant and test	Value
S. torvum DPPH activity IC_{50}	1.62 mg/ml
Quercetin DPPH activity IC_{50}	34.51 µg/ml
S. torvum FRAP assay	470 mg $FeSO_4$ E/gr of *S. torvum* extract

Table 4: Elemental analysis of the *S. torvum* fruit

S.NO.	Metals	mg/ml for 5 ppm
1.	Calcium (Ca)	0.763 ± 0.092
2.	Copper (Cu)	0.568 ± 0.210
3.	Iron (Fe)	0.288 ± 0.312
4	Manganese (Mn)	0.197 ± 0.218
5.	Lead (Pb)	0.237 ± 0.310
6.	Zinc (Zn)	0.0472 ± 0.193
7.	Nickel (Ni)	0.039 ± 0.021
8.	Magnesium (Mg)	0.539 ± 0.093
9.	Sodium (Na)	0.322 ± 0.021

Table 5: Peaks and Band range of *S. torvum* fruit

Peak (cm^{-1})	Functional Groups
3350.0	-OH
2915.0	-CH (alkane)
2850.0	=CH (aldehyde)
1630.0	Bending mode of H_2O
1520.0	CH_2 bending
1350.0	OH bending
1020.0	C-O-C Pyranose ring
900.0	CH a symmetric bending

Table 6: Functional group and Fingerprint region of *S. torvum* fruit

Sample	Fingerprint region-wave number RANGE(800-1600 cm-1)	Functional group-wave number RANGE (2400-3600 cm^{-1})	Region Inference
Solanum torvum	816, 1200-1500	3320-3562,2850, 2915,	Stretching vibrations of -OH groups in phenyl, -CH_2 asymmetric stretch of methyl groups, -C-O stretching vibrations ring of phenyls, -CH bending vibration

CONCLUSION

Presence of bioactive polyphenolic compounds are responsible for the medicinal properties of plants. Plants belonging to the family solanaaceae have been used as a source of phytomedicine due to the presence of considerable amount of phenols and flavonoids. Due to presence of phenols and flavonoids, the extract showed significant amount of antioxidant activity. The study suggests that the fruits of the plant might be a potential source of natural antioxidants. Thus *S. torvum* fruits can be a good candidate for novel phytomedicine that can be used to treat several diseases. The future study shall be directed towards the identification of bio-active compounds and quality standards for developing a potential drug.

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CONFLICT OF INTEREST

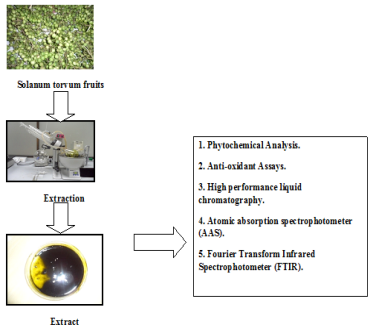
We declare that we have no conflict of interest.

REFERENCES

1. Forest S, Kim S, Lloyd L. *Solanum torvum*. United States Geological Survey-Biological Resources Division Haleakala Field Station. Maui, Hawaii. 2003;1-4.

2. Ghani A. Medicinal plants of Bangladesh: chemical constituents and uses. Asiatic society of Bangladesh: Dhaka, Bangladesh. 1998;2:267.
3. Muhammad A, Sheeba F. Pharmacognostical studies and evaluation of total phenolic and flavonoid contents of traditionally utilized fruits of *solanum torvum* swartz. Indian natural products and resources. 2011;2(2):218-24.
4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal and Industrial Research. Council of Scientific and Industrial Research: New Delhi. 1956;2(1):218.
5. Adjanohoun J, Aboubakar N, Dramane K. Traditional medicine and pharmacopeia-contribution to ethnobotanical and floristic studies in Cameroon. In: CNPMS. Porto-Novo Benin. 1996;50-2.
6. Jaiswal BS. *Solanum torvum*: A review of its traditional uses, Phytochemistry and Pharmacology. International Journal of Pharma and Bio Sciences. 2012;3(4):104-11.
7. Siemonsma J, Piluek K. Plant Resources of South-East Asia 8 (PROSEA). Bogor Indonesia. 1994;(6):412.
8. Kala CP. Ethno medicinal botany of the Apatani in the Eastern Himalayan region of India. J Ethno and Ethno Med. 2007;1(18):104,510-7.
9. Ndebia EJ, Kamga R, Nchunga anye N. Analgesic and anti-inflammatory properties of aqueous extract from leaves of *Solanum torvum*. AJTCAM. 2007;4(2):240-4.
10. Ajaiyeoba EO. A comparative phytochemical and antimicrobial study of Solanum macrocarpum and *Solanum torvum* leaves. Fitoterapia. 1999;70(2):184-6.
11. Chah KF, Muko KN, Oboegbulem SI. Antimicrobial activity of methanolic extract of *Solanum torvum* fruit. Fitoterapia. 2000;71(2):187-9.
12. Arthan D, Svasti J, Kittakoop P. Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry. 2002;59(4):459-63.
13. Israf DA, Lajis NH, Somchit MN, Sulaiman MR. Enhancement of ovalbumin specific IgA responses via oral boosting with antigen co-administered with an aqueous *Solanum torvum* extract. Life Science. 2004:75(4):397-406.
14. Siva priya M, Srinivas L. Isolation and purification of a novel antioxidant protein from the water extract of Sundakai (*Solanum torvum*) seeds. Food Chemistry. 2007;104(2):510-7.
15. Nguelefack TB, Mekhfi H, Dimo T, Afkir S. Cardiovascular and anti-platelet aggregation activities of extracts from *Solanum torvum* (Solanaceae) fruits in rat. JCIM. 2008;5(7):180-92.
16. Mohan M, Jaiswal BS, Kasture S. Effect of *Solanum torvum* on blood pressure and metabolic alterations in fructose hypertensive rats. J Ethnopharmacol. 2009;126(1):86-9.
17. Mohan M, Kamble S, Kasture S. Protective effect of *Solanum torvum* on doxorubicin induced nephrotoxicity in rats. Food and Chem Toxico. 2010;48(1):436-40.
18. Gandhi GR, Ignacimuthu S, Paul raj MG, Sasikumar P. Anti-hyperglycemic activity and antidiabetic effect of methyl caffeate isolated from *Solanum torvum* Swartz. Fruit in streptozotocin induced diabetic rats. Eur J Pharmacol. 2011;670(23):623-31.
19. Jaiswal BS, Mohan M. Effect of *solanum torvum* on the contractile response of isolated tissues preparation in fructose fed rat. IntJ Pharm Bio Sci. 2012;3(3):161-9.
20. Sivapriya M. Isolation and purification of a novel antioxidant protein from the water extract of sundakai *(Solanum torvum)* seeds. Food Chem. 2007;104(2):510-7.
21. Singleton V, Orthofer R, Lamuela R. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 1999;299:152-78. doi: 10.1016/S0076-6879(99)99017-1.
22. Pal D, Sannigrahi S, Mazumder U. Analgesic and anticonvulsant effects of saponin isolated from the leaves of *Clerodendrumin ortunatum* Linn in mice. Indian Journal of Experimental Biology. 2009;47(9):743-7.
23. Cetkovic G, Canadanovic J. Assessment of Polyphenolic content and *in vitro* antiradical characteristics of *apple pomace*. Food Chemistry. 2008;109(2):340-7.
24. Blois MS. Antioxidant determinations by the use of actable free radical. Nature. 1958;181:1199-50. doi: 10.1038/1811199a0
25. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth Enzymol. 1999;299:15–27. doi:10.1016/S0076-6879 (99)99005-5
26. Lag HariAH, Memon S, Nelofar A. Determination of free phenolic acids and antioxidant activity of methanolic extracts obtained from fruits and leaves of *Chenopodium album*. FoodChem. 2011;126(4):1850-5.
27. Olajide O, Afolayan M, Adewusi AJ, Adeyanju O. Antimicrobial activity and Elemental analysis of *Cassia siberiana* leaves Using Atomic Absorption Spectrometer. Scholars Research Library. J Nat Prod. Plant Resour. 2012;2(1):9-18.
28. AshokKumar R, Ramaswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. Int J Curr Microbiol App Sci. 2014;3(1):395-6.
29. Jutarat Tawekij P, Jindarat P. Determination of flavonoid content and antioxidant activity from *ferns* by ultrasonic extraction. TIChE International Conference; Hatyai, Songkhla, Thailand. 2011;1-4.
30. Shui G, Leong PL. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high performance liquid chromatography. J Chromatograma. 2002;997(1):89-96.
31. Tormo M, Izco JM. Alternative reversed phase High-Performance Liquid Chromatography method to analyze organic acids in dairy products. J Chromatogr. 2004;1033(2):305-10.
32. World Health Organization. WHO Guidelines for elemental concentration, trace elements in health and human Nutrition. 1996;50-68.
33. Ogugbuaja VO, Akinniyij A, Abdurrahman FI. Elemental Contents of Medicinal plants. Department of chemistry faculty of science, University of Maiduguri, Nigeria. A Monograph. 1997.
34. Khan IZ. The role of Chemistry in health disease and ageing. A seminar presentation in the Department of chemistry, University of Maiduguri, Nigeria. 1996.
35. Lu X, Rasco BA. Determination of antioxidant content and antioxidant activity in foods using infrared spectroscopy and chemometrics: a review. Food science Nutri. 2012;52(10):853-75.
36. Daniel M, Maciej B. Estimation of total polyphenols contents in Ocmum basilicum, Origanum vulgare and Thymus vulgaris commericial samples. Kerba Polonica. 2009;55(1):35-41.
37. Ghosh T, Maity KT, SenGupta P, Dash KD. Antidiabetic and *in vivo* antioxidant activity of ethanolic extract of Bacopamonnieri Linn aerial parts: a possible mechanism of action. Iranian J Pharm Res. 2008;7(1):61-8.
38. Ognjanovic BI, Markovic SD, Pavlovic SZ. Effect of chronic cadmium exposure on antioxidant defense system in sometissues of rats: protective effect of selenium. Physiol Res. 2008;57(3):403-11.
39. Chia-Chi C, Ming-huaY, Hwei-mei W. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food and Drug Analysis. 2002;10(3):178-82.
40. Chen C, Pearson MA, Gray IJ. Effects of synthetic antioxidants on the mutagenicity of iq-like compounds. Food Hem. 1992;43(3):177-83.
41. Kahl R, Kappus H. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin. Forsch. 1993;196(4):329-38.

PICTORIAL ABSTRACT



ABBREVIATIONS USED

DPPH: Diphenyl picryl hydrazine; **FRAP :** Ferric reducing antioxidant power; **AAS:** Elemental Determination by Atomic Absorption Spectrophotometer; **FTIR:** Fourier Transform Infrared Spectrophotometer; **HPLC:** High performance liquid chromatography.

SUMMARY

- A preliminary Qualitative analysis *Solanum torvum swartz* extract showed the presence of reducing sugars, saponins, alkaloids, phenols and flavonoids except anthraquinones. Quantitative determination of total phenols and flavonoids in STE showed 16.4 mg GAE/g extract of *S. torvum* and 2.8 mg QE/g extract of *S. torvum* respectively.
- In DPPH radical scavenging assay, the IC_{50} value of the extract was found to be 1.62 mg/ml and in FRAP assay, FRAP value of ethanolic fruit extract was found to be 470 mg $FeSO_4$ E/gr of S. torvum fruit extract.
- Extract analyzed by High performance liquid chromatography revealed presence of polyphenolic compounds such as gallic acid, rutin, quercetin and ascorbic acid.
- Elemental determination by Atomic absorption spectrophotometer (AAS) showed the presence of essential elements.
- Fourier Transform Infrared Spectrophotometer (FTIR) report shows the presence stretching vibrations of OH groups in phenyl, CH2 asymmetric stretch of methyl groups, C-O stretching vibrations ring of phenyls, CH bending vibration.

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