Phytochemical Constituents, Total Saponins, Alkaloids, Flavonoids and Vitamin C Contents of Ethanol Extracts of five *Solanum torvum* Fruits

Desmond Ato Koomson¹, Benjamin Danso Kwakye², Williams Kweku Darkwah^{1,3*}, Bismark Odum⁴, Mabel Asante⁵, Gideon Aidoo⁶

Desmond Ato Koomson¹, Benjamin Danso Kwakye², Williams Kweku Darkwah^{1,3*}, Bismark Odum^{3,4}, Mabel Asante⁵, Gideon Aidoo⁶

¹Department of Biochemistry, School of Biological Sciences, University of Cape Coast, Cape Coast, GHANA.

²Department of Mathematics, College of Science, Hohai University, Nanjing, CHINA.

³Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Environmental Engineering Department, College of Environment, Hohai University, Nanjing, CHINA.

⁴Chemical Engineering Department, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, GHANA.

⁵Department of Laboratory Technology, School of Physical Sciences, University of Cape Coast, Cape Coast, GHANA.

⁶Clinical Research Laboratory Department, 37-Military Teaching Hospital, Accra, GHANA.

Correspondence

Williams Kweku Darkwah

Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Environmental Engineering Department, College of Environment, Hohai University, Nanjing, CHINA.

Phone no: +861 52957 82807

E-mail: williams.darkwah@stu.ucc.edu.gh

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ABSTRACT

Introduction: Phytochemicals are frequently used in chemotherapeutic treatment or may be used as chemo preventive agents with chemoprevention. The study report the quantification of phytochemical constituents and vitamin C contents from ethanol extracts of *Solanum torvum* fruits. **Method:** The main objective for this research was to use standard procedures to determine phytochemical and vitamin C content. **Results:** The estimated alkaloids found in mature fruits were 6.32 ± 0.12 mg/g and 16.94 ± 2.3 mg/g in the immature fruits. Total saponins in mature and immature fruits were 8.60 ± 2.6 mg/g and 16.90 ± 9.4 mg/g respectively. Total flavonoids in mature and immature fruits were 21.14 ± 4.4 mg/g and 14.24 ± 1.8 mg/g respectively. Also vitamin C contents were 11.79 ± 2.0 mg/g in mature fruits and 8.70 ± 0.26 mg/g in immature fruits. With the exception of alkaloids whose difference in the mature and immature was significant, other differences obtained were not significant. **Conclusion:** The study showed that the extracts contain diversity of phytochemicals in appreciable amount that can expertly keep the body against oxidative stress triggered by free radicals and therefore be used as a source of potent natural products.

Key words: Solanum torvum fruits, Phytochemicals, Saponins, Flavonoids, Alkaloids, Ethanol extract.

INTRODUCTION

Scientists have suggested that phytochemicals posse different kinds of mechanisms of action. These compounds are known to obstruct microorganisms, and also impede some metabolic routes or control gene expression and signal transduction pathways. Phytochemicals such as alkaloids, phenols, saponins are frequently used in chemotherapeutic treatment or may be used as chemo preventive agents with chemoprevention referring to the use of agents to constrain, reverse, or delay tumorigenesis. With this logic, chemo preventive phytochemicals are pertinent to cancer treatment, since molecular mechanisms may be common to chemoprevention and cancer healing. 3-4

Phytochemicals, for instance, alkaloids, saponins, flavonoids and phenolic compounds also play an important role in the growth and reproduction of most plants, these compounds also act as antifeedants and antipathogens.⁵ These compounds also turn as a natural protective mechanism by hindering mutations in plants.

Solanum torvum fruits are very rich in vitamin C. In spite of being rich in vitamin C, they also provide phytochemicals such as alkaloids, saponins, sugars, phenols and flavonoids folate, potassium and a lot of fiber for pharmaceutical or food production.6 Solanum torvum is a rich source of alkaloids, flavonoids, saponins, glycosides, and tannins.7 According to Pérez-Amador,8 the percentage constituents of various phytochemical compounds within this fruit is highly appreciable. Regardless of broad research on the medicinal properties of most plants, little information is known about the many humid underutilized plants and fruits in most unindustrialized countries specifically Ghana. One of such fruit is Solanum torvum which is a multipurpose constant slender herb. Consequently, in this research, quantification of phytochemical constituents and vitamin C contents by ethanol extracts of Solanum torvum fruits were investigated to evaluate the potential protective or health benefits of this fruits and their linkage to their medicinal properties.

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MATERIALS AND METHODS

Determination of people's knowledge on *Solanum torvum* using questionnaires

A survey was done with the households at Kotokoraba near University of Cape Coast, Cape Coast, Ghana. This was done using questionnaires to help know people's knowledge on *S. torvum*. The data was collected and analyzed using SPSS analysis.

Plant Material

Five fresh fruits *Solanum torvum* were collected from the University of Cape Coast botanical garden, Cape Coast, Ghana with voucher numbers UCCBG000445, UCCBG000448, UCCBG000349, UCCBG000387 and UCCBG000543. The taxonomic character of the fruits was determined on 2nd December, 2014 by a plant taxonomist at the Department of Botany, University of Cape Coast, Ghana. *Solanum torvum* fruits were then washed under with water to get rid of undesirable dirt and other external constituents. The samples were parched under shade until no moisture left. The parched samples then were ground into powder using a blender.

Ethanol extraction

The ethanol extract from *Solanum torvum* fruits was also prepared by drenching 50 g of the grinded sample in 500 mL of sterilized distilled water for 2 h in 90°C water bath at the Department of Biochemistry Research Laboratory, University of Cape Coast, Ghana. The combination was then filtered using Whatman filter paper No 1. The filtrate was concentrated under a reduced pressure using a rotary evaporator at a temperature of 90°C. The resulting extract was weighed and put in storage in impermeable bottles at 35°C for further analysis.

yield (%)=
$$\left(\frac{A_1}{A_0}\right)$$
×100

Where, $\boldsymbol{A_{_0}}$ was the mass of the sample and $\boldsymbol{A_{_1}}$ was the mass of the crude extract.

Phytochemical screening Qualitative analysis

Phytochemical qualitative test of the ethanol extracts of *Solanum torvum* was performed as per standard protocols⁹⁻¹² to reveal the existence of glycosides, tannins, flavonoids, saponins, alkaloids anthraquinones.

Quantitative Analysis Determination of total saponins

The 10 samples were ground and 2 g of each was put into a conical flask and 10 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 20ml of 20% ethanol. The combined extracts were concentrated over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 10ml of diethyl ether was added and shaken strongly. The aqueous layer was recovered while the ether layer was cast-off. The purification process was repeated. 6 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 1ml of 5% aqueous sodium chloride. The residual solution was heated in a water bath. After

evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated.¹³

Determination of total alkaloids

Exactly 1g of the sample was weighed into a 250 ml beaker and 40 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. 14

Determination of total flavonoids

Exactly 1g of the plant sample was extracted repeatedly with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transported into a container and evaporated into dryness over a water bath and weighed to a constant weight. 15

Determination of Vitamin C

About 3.5 g of the cleaned fresh 10 samples was measured. The samples were ground with a clean mortar and pestle which had been rinsed with oxalic acid. Exactly 50 ml of oxalic acid was used in each case to grind and dissolve each sample. The mixtures were filtered using cotton wool and collected in separate labeled beakers. After that, 10 ml of each filtrate was pipetted into a conical flask and 1ml of bromine water was added with constant mixing until the colour of the solution had changed to orange yellow. This was to remove phenolic hydrogen atoms in the ascorbic acid. Thiourea was added to the solutions in drops to expel off excess bromine. The volume of each of these sample solutions was made up to 50 ml by the addition of 4% oxalic acid solution. The same procedure was followed for 10 ml of stock ascorbic acid solution which has been prepared earlier to convert it to dehydroascorbic acid by bromination, followed by expelling excess with 10% thiourea. 0.05 ml, 0.10 ml, 0.15 ml, 0.20 ml, 0.25 ml, 0.30 ml, 0.35 ml, 0.40 ml, 0.45 ml and 0.5 ml of the dehydroascorbic acid were pipette out into a series of test tubes. Similarly 1 ml of each of the brominated samples were pipetted out into a series of tubes and their volumes made up to 3 ml by adding distilled water. To each of the tubes was added 1ml of 2, 4-dinitrophenylhydrazine (2, 4-DNPH) followed by 2 drops of thiourea. A blank (containing 3 ml of distilled water, 1ml of 2, 4-DNPH, and drops of thiourea) was prepared. The contents were mixed thoroughly and then put in the water bath, at 40°C for 3 h. After 3 h of incubation, 6 ml of 80% sulphuric acid was added to each solution and allowed to cool slowly in free air. Absorbance of the blank, the samples, and the standards were measured at 540 nm and tabulated. A standard curve was plotted and from it, the various concentrations of vitamin c in the samples were calculated. This was triplicated. 16

RESULTS AND DISCUSSION

RESULTS

Survey

The survey revealed the knowledge of the local people about the fruits of *S. torvum*. From the survey, the plant is widely known among the local

people (Figure 1). It has been assigned various names such as Beware, Kwau Nsusuwa, Samantroba, Ananantroba, etc.

Qualitative determination of the phytochemical constituents of ethanol extract of *Solanum torvum*

Solanum torvum has been largely considered, precisely its medicinal possessions correlated with stable phytochemicals.¹⁷ Several parts of Solanum torvum have been in use for the separation of an extensive collection of compounds. This plant kind is a precise good source of phytochemical components.⁷ Phytochemical study of the ethanol extract of *S. torvum* revealed the presence of glycosides, tannins, flavonoids, saponins, alkaloids but no anthraquinones (Table 1).

Quantitative determination of total alkaloid, saponin and flavonoid Contents of ethanol extract of *Solanum torvum* fruits

There were different compositions of phytochemicals in the fruit samples of *S. torvum*. There were no significant differences statistically in the levels of flavonoids, and saponins between the mature and young fruits of the *S. torvum* (Table 2; Figure 2-4). There was a statistically significant difference in the levels of alkaloids between the matured and young fruits of the *S. torvum* (Table 2; Figure 2-4).

Quantitative analysis of vitamin C content of ethanol extract of *Solanum torvum* fruits

From the experiment there was no statistical significant difference in the levels of vitamin C contents between the matured and young fruits of the *S. torvum* (Figure 5)

DISCUSSIONS

Most of the people surveyed used the fruits for food, tea or medicine (Figure 1). There has also been a report of the use of the fruits as food in most Asian countries. ¹⁸ The advantages in using the fruits for food were well known to the people surveyed. The fruits have been used by the people from about 2 years to 40 years. This gave the basis to experimentally determine the nutritional or medicinal value of the fruits, especially the phytochemicals and vitamin C constituents.

Sivapriya and Srinivas¹⁹ and Lakshmi²⁰ described phytochemical screening of methanolic and ethanol extracts of sun dried *S. torvum* fruits having the same results as this study had. The presence of these bioactive composites in the extract point out the prospective health remunerations of the plant. The existence of flavonoids and other phytochemical compounds improves the prospect of antioxidant activity, as many studies have informed a robust progressive correlation between these compounds and the antioxidant activity of extracts.^{12,21-23}

Young fruits normally store phytochemicals such as alkaloids to protect them from infections and insects. The alkaloids begin to reduce and their concentrations weaken as the fruits mature and ripen. Environmental influences such as soil type, growing season, geographic location, and mineral status are known to impact intensities of plant secondary metabolites. States are known to impact intensities of plant secondary metabolites. It is to revum fruits that exhibited great altitudes of phytochemicals may be strong to insects and diseases using the phytochemicals as protective chemicals states are times demonstrated that anti-oxidant possessions of flavonoids and their ability to chelate free metallic ions could be operational in reducing the poisonousness of Doxorubicin (DOX) 39. The presence of flavonoids in S. torvum makes the fruit a very potent antioxidant.

The altitudes of vitamin C in vegetables and fruits differ subjected on some factors such as maturity state, position of the fruit on the branch

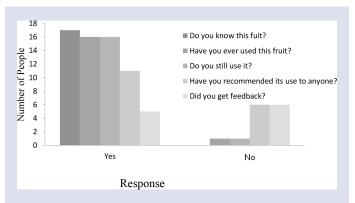


Figure 1: Knowledge of people on Solanum torvum.

Table 1: Phytochemicals present in the fruit of Solanum torvum.

Phytochemicals	Young Fruit	Mature Fruit
Glycosides	Present	Present
Tannins	Present	Present
Flavonoids	Present	Present
Saponins	Present	Present
Anthraquinones	Absent	Absent
Alkaloids	Present	Present

Table 2: Quantitative contents of phytochemicals in *Solanum torvum* (mg/g).

Samples	Flav	Flavonoids		Saponins		Alkaloids	
	Υ	М	Υ	М	Υ	M	
1	14.3	22.2	5.5	9.5	6.7	5.9	
2	18.7	13.1	14.5	2.5	16.0	6.4	
3	7.8	32.1	8.5	9.5	16.0	6.6	
4	15.9	9.2	53.5	17.5	14.9	6.3	
5	14.5	29.1	2.5	4.0	21.1	6.4	

[&]quot;M" Matured fruits

[&]quot;Y" Young fruits

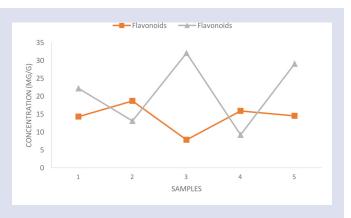


Figure 2: Total flavonoid contents of Solanum torvum (mg/g).

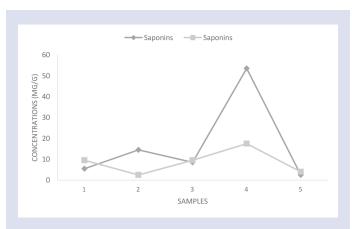


Figure 3: Saponin contents of Solanum torvum (mg/g).

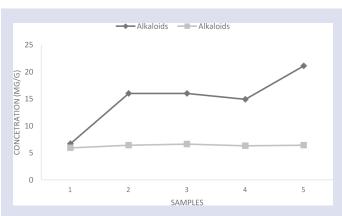


Figure 4: Total Alkaloid contents of Solanum torvum (mg/g).

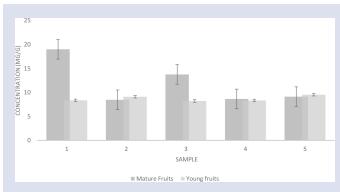


Figure 5: Concentrations of Vitamin C in fruit samples of *Solanum toryum*.

of the stem, temperature, storage, processing, varieties and handling.²⁹ To compare to this study, vitamin C decreases throughout the ripening process. Immature fruit has the highest levels according to previous studies but with this study, matured fruits had the highest content of vitamin C

(Figure 5). Vitamin C is the most important water-soluble antioxidant found in the body. $^{30-32}$ It lowers blood pressure and cholesterol levels. 33 The presence of vitamin C in the fruits of *S. torvum* will help decline the possibility of heart related diseases.

CONCLUSION

The outcomes of the study point out that extracts of *Solanum torvum* fruits comprise of diversity of phytochemical compounds that can expertly protect the body against oxidative stress caused by free radicals and as a result might be used as a source of potent natural food or medicinal compounds. The vitamin C content of *Solanum torvum* could substantiate additional analysis of its other constructive natural properties and regulate its protection.

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CONFLICT OF INTEREST

The Authors declare that they have no conflict of interest.

ABBREVIATIONS

CSC: China Scholarship Council; **M:** Matured fruits; **Y:** Young fruit; **DOX:** Doxorubicin; **2, 4-DNPH: 2, 4-**dinitrophenylhydrazine; **A₀:** Mass of the sample; **A₁:** Mass of the crude extract.

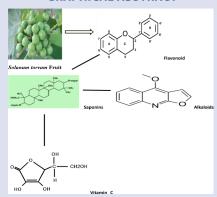
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GRAPHICAL ABSTRACT



SUMMARY

- The study report the quantification of phytochemical constituents and vitamin C contents from ethanol extracts of *Solanum torvum* fruits.
- The main objective for this research was to use standard procedures to determine phytochemical and vitamin C content.
- The estimated alkaloids found in mature fruits were 6.32 ± 0.12 mg/g and 16.94 ± 2.3 mg/g in the immature fruits. Total saponins in mature and immature fruits were 8.60 ± 2.6 mg/g and 16.90 ± 9.4 mg/g respectively. Total flavonoids in mature and immature fruits were 21.14 ± 4.4 mg/g and 14.24 ± 1.8 mg/g respectively. Also vitamin C contents were 11.79 ± 2.0 mg/g in mature fruits and 8.70 ± 0.26 mg/g in immature fruits.
- The study showed that the extracts contain diversity of phytochemicals in appreciable amount that can expertly keep the body against oxidative stress triggered by free radicals and therefore be used as a source of potent natural products

ABOUT AUTHORS



Bismark Odum: Is a Chemical Engineer and Management Consultant. He obtained his B.Sc. qualification in Chemical Engineering from Kwame Nkrumah University of Science and Technology, Kumasi-Ghana in June 2015. He obtained his professional diploma in Professional Consulting from Chartered Management Institute, United Kingdom (UK) in March 2018. He is currently a postgraduate student in Hohai University, Nanjing, China. His current research interest is in flocculation processes of cohesive sediment and its impact on water quality.



Benjamin Danso Kwakye: Is a Ghanaian with Bachelor's degree in Mathematics from Valley View University, Ghana in July 2016 after obtaining his Diploma in Basic Education (Science) in September 2011 at Wesley College of Education (University of Cape Coast), Ghana. He is currently an M.Sc candidate in Mathematics with research interest in Statistics and Statistical Analysis at Hohai University, Nanjing, China.

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