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Quantitative Determination of Secondary Metabolites of Fractions Obtained from *Solanum aethiopicum* (L.) Fruit

Abdullahi Rabiu Abubakar [1*], Ibrahim Haruna Sani [2], Sani Malami [3], Abdullahi Hamza Yaro [4]

[1,3,4] Department of Pharmacology and Therapeutics,
Faculty of Pharmaceutical Sciences,
Bayero University, Kano.
Post code: 700233.
P.M.B: 3452, Kano, Nigeria.

[2]Unit of Pharmacology,
College of Health Sciences,
Yusuf Maitama Sule University,
PMB 3099, Kano, Nigeria.

Email: raabdullahi.pha@buk.edu.ng

Abstract

Solanum aethiopicum (L.), family *Solanaceae* is popularly known as garden egg or Ethiopian egg plant. In Nigeria it is called *gauta* by Hausa tribe, *igbagba* by Yoruba tribe and *afufa* by Igbo tribe. The aim of this research is to quantify secondary metabolites present in the crude methanol extract and fractions of *Solanum aethiopicum* fruits. The powdered fruit was extracted using 6L of 70% methanol. The crude extract was dissolved in water and fractionated using n-hexane, chloroform, ethylacetate, and n-buthanol. Phytochemical screening was conducted to determine the chemical composition of crude methanol extract of *Solanum aethiopicum* and its fractions. Quantitative analysis of total alkaloids, flavoniods, saponins, tannins, steroids and cardiac glycosides was also carried out. The percentage yield of methanol extract of *Solanum aethiopicum* was 31.2%. The phytochemical screening conducted revealed presence of saponins, tannins, flavonoids, alkaloids, steroids and cardiac glycosides. The crude extract fractionated produced 0.2% of n-hexane, 0.1% of chloroform, 2.2% of ethylacetate, 36.8% of n-buthanol and 60.7% of residual aqueous fractions. *Solanum aethiopicum* fruits crude extract and its fractions contain large amount of alkaloids, flavonoids and steroids which justifies its use traditionally in the treatment of various chronic diseases.

Keywords: Fractionation, Phytochemical, Quantitative, Metabolites, *Solanum-aethiopicum*

**Author for Correspondence*

INTRODUCTION

Medicinal plants are extracted, purified and taken as traditional medicine or use for research purposes. Preparation of medicinal plants for experimental purposes involves proper and timely collection of the plant, identification by an expert, proper drying and grinding. This is followed by extraction, fractionation and isolation of the bioactive compound where possible. Furthermore, it involves evaluation of quantity and quality of secondary metabolites (Sasidharan *et al.,* 2011; Doughari, 2012; Pandey and Tripathi, 2014; Azwanida, 2015; Ingle et *al.,* 2017; Abubakar *et al.,* 2020). The primary goal of fractionation is to separate bioactive molecules such as alkaloids, flavonoids, terpenes, saponins, steroids, glycosides etc from inert material using an appropriate solvent and standard extraction procedure (Abubakar *et al.,* 2020). Plant materials with high content of phenolic compounds and flavonoids possess antioxidant properties; hence their use in treatment of age related diseases such as Alzeihmers disease, Parkinsonism, anxiety and depression (Sasidharan *et al.,* 2011; Azwanida, 2015).

Solanum aethiopicum (L.), family *Solanaceae* is popularly known as garden egg, Ethiopian egg plant, or bitter tomato. In Nigeria it is called *gauta* by Hausa, *igbagba* by Yoruba and *afufa* by Igbo tribes (Burkill, 2000; Osei *et al.,* 2010; Chinedu *et al.,* 2011; Anosike *et al.,* 2012; Eze and Kanu, 2014; Eletta *et al.,* 2017). The plant was initially known as *Solanum anguivi* or *Solanum gilo* which shows potentials to treats various chronic ailments (Anosike *et al.* 2012; Eze and Kanu, 2014). In Nigeria we have about 25 families of *Solanum* but the most famous among them medicinally is *Solanum aethiopicum*. The eggplant is eaten raw and shared to appreciate visitors during weddings and to celebrate new born babies. In addition, the fruit is used for cooking varieties of foods and traditional vegetable sauces (Burkill, 2000; Osei *et al.,* 2010; Chinedu *et al.,* 2011; Eletta *et al.,* 2017). *Solanum aethiopicum* has various medicinal properties and it is useful in the treatment of insomnia, diabetes, constipation, skin infection, allergy, pain, and dyspepsia (Burkill, 2000; Osei *et al.,* 2010; Chinedu *et al.,* 2011; Eletta *et al.,* 2017). Previous experiments conducted on *Solanum aethiopicum* reported anti-inflammatory (Anosike *et al.,* 2012). The fruit also decreased body weight and blood glucose (Okafor *et al.,* 2016; Emiloju and Chinedu, 2016). It reduced cholesterol level (Chinedu *et al.,* 2013). In addition, it has antifungal activity (Watanabe *et al.,* 2001). The plant possesses laxative activity (Saba *et al.,* 2003). Antiulcer property was also reported (Chioma *et al.,* 2011). Lastly, *Solanum aethiopicum* showed significant antioxidant activity (Eletta *et al.,* 2017). This study focussed on fractionation and quantification of secondary metabolites present in *Solanum aethiopicum* fractions.

METHODOLOGY

Plant Materials

The whole plant material was collected from Fallau Town, Dawakin Kudu Local Government, Kano State. The *Solanum aethiopicum* plant is available in their farmland because it is cultivated by irrigation. The identification and authentication was done by the Department of Plant Biology, Bayero University, Kano. Voucher number was collected as BUKHAN 0501 and kept for future references.

Extraction

The fruits were first washed, shade dried, and grinded into a coarse powder using motar and pestle. The powdered fruit (2 kg) was macerated using 6L of 70% methanol v/v with occasional shaking for 7 days and filtered using Whatman No10 filter paper. The filtrate was evaporated to dryness *in vacou* at 40^{o}C to yield residue (Deng *et al.,* 2007).

Fractionation
The dried crude methanol extract 392g was treated with n-hexane, chloroform, ethyl acetate, n-buthanol and water to obtain their various fractions using separating funnel to produce n-hexane fraction (HF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and n-buthanol (BF) and residual aqueous fraction (RAF) (Deng *et al*., 2007).

Phytochemical Screening
The chemical composition of crude methanol extract and fractions was determined using phytochemical screening (Trease and Evans, 2002).

Test for Saponins
Frothing Test: In this test, 3g of a powdered *Solanum aethiopicum* crude extract was mix with 5 ml of distilled water in a test tube and shaken vigorously. The frothing produced was mix with few drops of olive oil and mixed briskly. The formation of foam indicated the presence of saponins.

Test for Tannins
Ferric Chloride Test: To 1ml solution of *Solanum aethiopicum* crude extract, HF, CHF, EAF, BF, and RAF in a test tube, 1% gelatin solution containing ferric chloride was added and shaken. Formation of bluish-black colour showed the presence of phenols.

Test for Flavonoids
Shinoda's Test: 1ml of *Solanum aethiopicum* extract, HF, CHF, EAF, BF, and RAF solution were transferred into a test tube, then few drops of concentrated HCl acid added, followed by 0.5mg of magnesium ribbon and shaken. Emergence of pink coloration indicates the presence of flavonoids.

Test for Alkaloids
Dragendoff's Test: In this test, 1ml of *Solanum aethiopicum* extract, HF, CHF, EAF, BF, and RAF were added to 1ml of potassium bismuth iodide solution (Dragendoff's reagent) and shaken. An orange red precipitate formed indicated the presence of alkaloids.
Wagner's Test: 1ml of plant extract, HF, CHF, EAF, BF, and RAF were added to 1ml of potassium iodide (Wagner's reagent) and shaken. Appearance of reddish brown precipitate signified the existence of alkaloids.
Mayer's Test: 1ml of plant extract, HF, CHF, EAF, BF, and RAF were added to 1ml of potassium mercuric iodide (Mayer's reagent) and shaken. Emergence of whitish precipitate confirmed the presence of alkaloids.

Test for Anthraquinones
Bontrager's Test: 10ml of benzene was added to 6g of the *Solanum aethiopicum* crude extract, HF, CHF, EAF, BF, and RAF powdered sample in a conical flask and soaked for 10 minutes, then filtered. A 10ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds. Appearance of pink colour in the ammonia phase showed the occurrence of anthraquinones.

Test for Steroids
Libermann Bruchard's Test: 5ml aqueous plant crude extract, HF, CHF, EAF, BF, and RAF were transferred into a test-tube. Then 2ml of chloroform and concentrated H_2SO_4 were added, appearance of red colour at the lower chloroform layer signified the presence of steroids.

Cardiac Glycosides

Keller Killiani's Test: 10ml of S*olanum aethiopicum* extract, HF, CHF, EAF, BF, and RAF solution were transferred into a test-tube. Then 4ml of glacial acetic acid was added, followed by 1drop of 2% $FeCl_3$ solution and shaken. Then 1ml of concentrated H_2SO_4 was added to the mixture. A brown ring formed between the two layers indicated the existence of cardiac glycosides.

Quantitative Analysis

Total Alkaloids

The sample of *Solanum aethiopicum* crude extract, HF, CHF, EAF, BF, and RAF 5g were taken into a 250ml beaker. Then 200 ml of 10% acetic acid in ethanol was transferred, covered and allowed to stand for 4 hrs. The mixture was filtered and then concentrated over water bath to ¼ of the initial volume. Later, few drops of concentrated HN_4OH were added to the extract until complete precipitate was formed. The whole mixture was allowed to settle and the precipitate was collected and washed with dilute HN_4OH and then filtered. The residue produced was dried and weighed as alkaloid (Harbone, 1973).

Total Flavoniods

In this test, powdered sample of *Solanum aethiopicum* crude extract, HF, CHF, EAF, BF, and RAF 2.5g were mixed with 50ml of 80% aqueous methanol in 250ml beaker, and allowed to stand for 24 hours at room temperature. The supernatant layer was discarded, and the residue was re-extracted three times with 50ml of ethanol. The solution produced were filtered using Whatman filter paper number 42 (125mm). The filtrates were later evaporated to dryness over a water bath. The content was cooled in a desiccator and weighed until constant weight was obtained (Boham and Koupai-Abyazan, 1974). The percentage yield for flavonoid was calculated as follows:

% Yield = Weight of flavonoid / Weight of sample ×100.

Total Saponins

Solanum aethiopicum crude extract, HF, CHF, EAF, BF, and RAF 20 g each were placed in conical flask and 100ml of 20% aqueous ethanol was added. The mixture was heated on water bath at 55^o C for 4 hours with continuous stirring. It was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at about 90^o C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether added and vigorously shaken. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was purified and 60 ml of n-butanol was added. The n-butanol extract washed twice with 10 ml of 5 % aqueous NaCl. The remaining solution was evaporated and dried in the oven to a constant weight (Obadoni and Ochuko, 2002). The saponin content was calculated using the formula below:

% Yield = Weight of saponin / Weight of sample ×100.

Total Tannins

In this test, the sample of *Solanum aethiopicum* crude extract, HF, CHF, EAF, BF, and RAF 500mg were weighed into a 50 ml plastic bottle. About 50 ml of distilled water was added and shaken for 1 hour. This was filtered into a 50 ml volumetric flask and made up to the mark. Subsequently, 5 ml of the filtrate was pipetted into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance of the mixture was measured at 120 nm within 10 min (Van-Buren and Robinson, 1969). The percentage yield of tannin was calculated using the formula below:

Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard.
% Yield = Weight of saponin / Weight of sample ×100.

Total Steroids
The solution of *Solanum aethiopicum* crude extract, HF, CHF, EAF, BF, and RAF 1ml was transferred into 10 ml volumetric flasks. In addition, H_2SO_4 acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated over water-bath at 70 ± 2° C for 30 minutes with occasional shaking. The volume was made up to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Mahdu *et al.,* 2016). The total steroid was calculated using the formula below:
Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard.
% Yield = Weight of saponin / Weight of sample ×100.

Total Cardiac Glycosides
In this test, 10% of *Solanum aethipicum* extract, HF, CHF, EAF, BF, and RAF were mixed with 10 mL freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). The mixture was allowed to stand for 1 hour. This is followed by dilution with 20 mL distilled water and the absorbance was measured at 495 nm using UV spectrophotometer (Solich *et al.,* 1992). The percentage yield of cardiac glycosides was calculated as follows:
Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard.
% Yield = Weight of saponin / Weight of sample ×100.

RESULTS

Percentage Yield
The 2kg powdered *Solanum aethiopicum* fruit macerated produced 624.2g of dried crude methanol extract giving 31.2% yield.

Fractions Obtained
Three hundred and ninety-two grams (392g) of the crude extract was fractionated and the results are presented in Table 1:

Table 1: Percentage yield

S/N	Solvent	Yield (g)	% Yield
1	n-hexane	0.6	0.2
2	Chloroform	0.4	0.1
3	Ethylacetate	8.4	2.2
4	n-buthanol	140.1	36.8
5	Residual Aqueous Fraction	231.3	60.7

Phytochemical Constituents
The phytochemical constituents present in *Solanum aethiopicum* crude extract and fractions are saponins, tannins, flavonoids, alkaloids, steroids and cardiac glycosides. The result is shown in Table 2:

Table 2: Phytochemical screening of crude, HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum*.

S/N	CONSTITUENT	TEST	CRUDE	HF	CHF	EAF	BF	RAF
1	Saponins	Frothing Test	+++	++	++	-	+	++
2	Tannins	Ferric Chloride Test	++	+	+	+	+	+
3	Flavonoids	Shinoda's Test	++	++	++	++	++	++
4	Alkaloids	Dragendoff's Test	+	+	+	+	+	+
		Wagner's Test	+	+	+	+	+	+
		Mayer's Test	+	+	+	+	+	+
5	Anthraquinones	Bontrager's Test	-	-	-	-	-	-
6	Steroids/Triterpenes	Lieberman Buchard's Test	++	+	+	+	+	++
7	Cardiac Glycosides	Keller Killiani's Test	+	+	+	+	+	-

HF= n-Hexane Fraction, CHF= Chloroform Fraction, EAF= Ethylaceatate Fraction, BF= n-Buthanol Fraction, RAF= Residual Aqueous Fraction.

Quantity of Secondary Metabolites

The results indicated that highest quantity of saponins 37mg/g, alkaloids 56mg/g, and cardiac glycosides 63mg/g was found in crude extract. Similarly, largest weight of flavonoids 198mg/g and steroids 19.6mg/g were in RAF. Also, the highest quantity of tannins 37.4mg/g was found in BF. Total quantity of all the secondary metabolites was expressed as mg per g of dried extracts.

The result of quantitative analysis is shown in Table 3:

Table 3: Quantity of secondary metabolites in crude extract and fractions

S/N	CONSTITUENT	QUANTITY (mg/g)					
		CRUDE	HF	CHF	EAF	BF	RAF
1	Saponins	37.0	23.7	18.6	-	8.0	26.0
2	Tannins	31.2	19.6	21.5	24.2	37.4	30.0
3	Flavonoids	162	134	128	156	167	198
4	Alkaloids	56	41	46	34	38	44
5	Steroids	9.2	5.3	3.4	4.8	5.2	19.6
6	Cardiac Glycosides	63	20.2	18.5	25.1	26.5	-

HF= n-Hexane Fraction, CHF= Chloroform Fraction, EAF= Ethylaceatate Fraction, BF= n-Buthanol Fraction, RAF= Residual Aqueous Fraction.

DISCUSSION

The powered *Solanum aethiopicum* fruit produced reasonable yield when extracted with aqueous methanol solution which indicated that it is an appropriate solvent of extraction. The outcome is similar to what was reported earlier (Anosike *et al.,* 2012; Okafor *et al.,* 2016). Preliminary phytochemical screening of *Solanum aethiopicum* crude extract and fractions reveals the presence of saponins, tannins, flavonoids, alkaloids, steroids and cardiac glycosides. Similar outcome was obtained by other researchers (Anosike *et al.,* 2012; Ossamulu *et al.,* 2014; Eze and Kanu, 2014; Eletta *et al.,* 2017; Tunwagun *et al.,* 2020).

During fractionation, the crude extract of *Solanum aethiopicum* produced 60.7% residual aqueous fraction, followed by 36.8% n-buthanol fraction, 2.2 % ethylacetate fraction, 0.2% of n-hexane fraction and finally 0.1% of chloform fraction.

Furthermore, highest quantity of saponins, alkaloids, and cardiac glycosides were found in crude extract. This is comparable to the result reported by another study (Eze and Kanu, 2014). But it is in contrast with outcome produced by other researchers (Ossamulu *et al.,* 2014; Okafor *et al.,* 2016). In addition, largest quantity of non-polar compounds such as flavonoids and steroids were found in polar residual aqueous fraction. This is because the method of extraction was not successive according to increasing order of polarity of solvents. But rather the initial step began with aqueous methanol in order to produce crude extract. The aqueous methanol was able to extract both polar and non-polar secondary metabolites (Makin *et al.,* 2010). Subsequent, fractionation with non-polar solvent was unable to remove all non-polar constituents from residual aqueous fraction because of poor miscibility between non-polar solvents and water (Makin *et al,* 2010). The study established the types and quantities of secondary metabolites presence in fractions of *Solanum aethiopicum* fruit.

CONCLUSION

Solanum aethiopicum crude methanol extract and its fractions contains large quantities of alkaloids, flavonoids and steroids which justifies its use in long term treatment of cardiovascular and mental disorders by the traditional medicine practitioners. It can be suggested that bioassay of both crude methanol extract and its fractions carried out in animal models of various chronic diseases.

REFERENCES

Abubakar, A.R. and Haque, M., 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy And Bioallied Sciences*, *12*(1), p.1-10.

Anosike, C.A., Obidoa, O. and Ezeanyika, L.U., 2012. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). *DARU Journal of Pharmaceutical Sciences*, *20*(1), p.76.

Azwanida, N.N., 2015. A review on the extraction methods use in medicinal plants, principle, *Aromat Plants*, *4*(196), pp.2167-0412.

Boham, B.A. and Kocipai-Abyazan, R., 1974. Flavonoids and condensed tannins from leaves Vaccinium vaticulatum and V. calycinium. *Pacific sci*, *48*(4), pp.458-463.

Burkill, H.M., 2000. The useful plants of west tropical Africa, Vol 5, Families S–Z, Addenda. *Royal Botanic Gardens, Kew, Richmond, United Kingdom*. pp 686.

Chinedu, S.N., Eboji, O.K. and Rotimi, S.O., 2013. Effects of Solanum aethiopicum fruit on plasma lipid profile in rats. *Advances in Bioresearch*, *4*(4), pp.79-84.

Chinedu, S.N., Olasumbo, A.C., Eboji, O.K., Emiloju, O.C., Arinola, O.K. and Dania, D.I., 2011. Proximate and phytochemical analyses of Solanum aethiopicum L. and Solanum macrocarpon L. fruits. *Research Journal of Chemical Sciences*, *1*(3), pp.63-71.

Chioma, A., Obiora, A. and Chukwuemeka, U., 2011. Does the African garden egg offer protection against experimentally induced ulcers?. *Asian Pacific Journal of Tropical Medicine*, *4*(2), pp.163-166.

Deng, C., Liu, N., Gao, M. and Zhang, X., 2007. Recent developments in sample preparation techniques for chromatography analysis of traditional Chinese medicines. *Journal of Chromatography A*, *1153*(1-2), pp.90-96.

Doughari, J.H., 2012. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In *Phytochemicals-A global perspective of their role in nutrition and health*. (Ed.). ISBN: 978-953-51-0296-0, InTechOpen. www.intechopen.com

Eletta, O.A.A., Orimolade, B.O., Oluwaniyi, O.O. and Dosumu, O.O., 2017. Evaluation of proximate and antioxidant activities of Ethiopian eggplant (Solanum aethiopicum L) and Gboma Eggplant (Solanum macrocarpon L). *Journal of Applied Sciences and Environmental Management*, *21*(5), pp.967-972.

Emiloju, O.C. and Chinedu, S.N., 2016. Effect of Solanum aethiopicum and Solanum macrocarpon fruits on weight gain, blood glucose and liver glycogen of wistar rats. *World*, *4*(1), pp.1-4.

Eze, S.O. and Kanu, C.Q., 2014. Phytochemical and nutritive composition analysis of Solanum aethopicum L. *Journal of Pharmaceutical and Scientific Innovation (JPSI)*, *3*(4), pp.358-362.

Harborne, J. 1973. Phytochemical methods. *Chapman and Hall, Ltd*. London. p.49-88.

Ingle, K.P., Deshmukh, A.G., Padole, D.A., Dudhare, M.S., Moharil, M.P. and Khelurkar, V.C., 2017. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry*, *6*(1), pp.32-36.

Madhu, M., Sailaja, V., Satyadev, T.N.V.S.S. and Satyanarayana, M.V., 2016. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of Pharmacognosy and Phytochemistry*, *5*(2), pp.25-29.

Makin, H.L., Honour, J.W., Shackleton, C.H. and Griffiths, W.J., 2010. General methods for the extraction, purification, and measurement of steroids by chromatography and mass spectrometry. In *Steroid analysis* (pp. 163-282). Springer, Dordrecht.

Obadoni, B.O. and Ochuko, P.O., 2002. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of pure and applied sciences*, *8*(2), pp.203-208.

Okafor, H.K., Odugbemi, A.I., Okezie, C.B. and Achebe, M.K., 2016. Antidiabetic and Hypolipidaemic Effects of Garden Egg (Solanum aethiopicum) Leaf Extract in Beta-cells of Streptozotocin Induced Diabetic Male Wistar Rats. *Annual Research & Review in Biology*, pp.1-11.

Osei, M.K., Banful, B., Osei, C.K. and Oluoch, M.O., 2010. Characterization of African eggplant for morphological characteristics. *Journal of Agricultural Science and Technology*. 4(3): 33-37.

Ossamulu, I.F., Akanya, H.O., Jigam, A.A. and Egwim, E.C., 2014. Evaluation of nutrient and phytochemical constituents of four eggplant cultivars. *Food Science*, *73*, pp.26424-26428.

Pandey, A. and Tripathi, S., 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*, *2*(5) : 115-119.

Saba, A.B., Dina, O.A., Adedapo, A.A. and Akhiromen, I.O., 2003. Effect of aqueous leaf extract of Solanum aethiopicum on isolated guinea pig ileum. *African Journal of Biomedical Research*, *6*(3):1-4.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Latha, L.Y., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1) :1-10.

Solich, P., Sedliakova, V. and Karlíček, R., 1992. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. *Analytica chimica acta*, *269*(2), pp.199-203.

Trease, G.E., Evans, W.C. 2002. Text Book of Pharmacognosy (16th edition) WB Saunders *Harcourt Publishers Ltd*. London, UK. 137-139,230-240.

Tunwagun, D.A., Akinyemi, O.A., Ayoade, T.E. and Oyelere, S.F., 2020. Qualitative Analysis of the Phytochemical Contents of Different Anatomical Parts of Ripe Solanum aethiopicum Linneaus Fruits. *Journal of Complementary and Alternative Medical Research*, pp.14-22.

Van Buren, J.P. and Robinson, W.B., 1969. Formation of complexes between protein and tannic acid. *Journal of Agricultural and Food Chemistry*, *17*(4), pp.772-777.

Watanabe, A., Toshima, H., Nagase, H., Nagaoka, T. and Yoshihara, T., 2001. Structural confirmation of 15-norlubiminol and 15-norepilubiminol, isolated from Solanum aethiopicum, by chemical conversion from lubimin and epilubimin, and their antifungal activity. *Bioscience, biotechnology, and biochemistry*, *65*(8), pp.1805-1811.