WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 131-145. Research Article ISSN 2277– 7105

PHYTOCHEMICAL INVESTIGATION ON THE FRUITS OF SOLANUM XANTHOCARPUM

Henok Brehanu Abebe* and Dr. Hailemichael Tesso Bayou

Adama Science and Technology University, College of Applied Sciences Department of Applied Chemistry.

Article Received on
08 Feb. 2021,

Revised on 01 March 2021,
Accepted on 21 March 2021

DOI: 10.20959/wjpr20214-20108

***Corresponding Author**
Henok Brehanu Abebe
Adama Science and Technology University, College of Applied Sciences Department of Applied Chemistry.

ABSTRACT

The plant used for this study was *Solanum xanthocarpum*, a prickly diffuse, bright green perennial herb, locally known as enbuay. The aqueous extract of the fruit of the plant *S. xanthocarpum* is administered by some traditional healers for the treatment of tuberculosis, locally known as samba nekersa and other diseases. However, these traditional claims have not been supported by scientific research nor the active ingredients have been identified. This research attempted to investigate the phytochemical constituents of the fruits of *S. xanthocarpum.* The phytochemical tests of *S. zanthocarpum* fruits revealed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, tannins and saponins in chloroform, ethyl acetate and methanol extracts. Of 170 gm. powdered fruit of *S. xanthocarpum*, when successively extracted, 1.1 gm, 0.8 gm, 0.6 gm and 17.6 crude extracts were obtained by petroleum ether, chloroform, ethyl acetate, and methanol solvents respectively. Then, isolation and purification of phytoconstituents from methanol extracts by column chromatography led to the isolation of a new compound. The new compound is a steroidal glycoside of furostane parent aglycone skeleton. The structures of this compound have been elucidated by interpretation of its UV-Vs, IR, and NMR spectral data and comparison of these spectral data with literatures.

KEYWORDS: Solanumxanthocarpum, furostane, spirostane, steroidal glycosides, saponin.

1. INTRODUCTION

Plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock since ancient times. Historical accounts of

traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians.[1]

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc.[2]

Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals and the emphasis is inevitably on chromatographic techniques. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom. The continuous improvement in chemical technology allowed the isolation, structural characterization and further synthesis of the pharmacologically active plant constituents. The enormous variety of organic substances that are elaborated by plants with different chemical structure, biosynthesis, turnover and metabolism, natural distribution and biological function, separation, purification and identification of the many different constituents present in the plant are secondary metabolites.[3-6]

The genus *Solanum* belongs to the family *Solanaceae* and comprises more than 1700 species, and, is found in the tropical and temperate regions.[7,8] S. xanthocarpum is a very prickly diffuse, bright green perennial herb, 2-3 m high. Stems are zigzag; prickles compressed, straight, yellow and shining; leaves 5-10 by 2.5-5.7 cm ovate or elliptic, sinuate or sub pinnatified, hairy on both sides, Petiole prickly. Flowers are small, in extra-axillary few flowered cymes. Corolla is purple, lobes deltoid, hairy outside. Fruits are of 1.3 cm diameter berry, yellow or white with green veins, surrounded by enlarged calyx. It grows on all kind of soil but does well on dry and hot temperate region.[9]

*S. xanthocarpum*is one of the ten plants in the Ayurveda (the science of life prevention and longevity – the oldest traditional system of medicine in India).[10-12] The plant has been

reported to possess antispasmodic, cardiotonic, hypotensive, antitumor, antianaphylactic and cytotoxic activities.[11–16] It is also useful in the treatment of asthma, cough, fever, enlargement of liver, spleen, controlling stones in bladder and pain in chest.[16] Various studies indicated that *S. xanthocarpum* possesses antiasthmatic, hypoglycemic, hepatoprotective, antibacterial and insect repellent properties. Various traditional claims like immunomodulation, anti-inflammatory, antiallergic, antianaphylactic and antitumor effects of the plant are still remains to be validated scientifically. More clinical trials should be performed to further validate the claims of local people.[13]

Phytochemical studies of the fruits are reported to contain several steroidal alkaloids like solasodine andsolamargine. Other constituents like caffeic acid, coumarins like esculetin, steroids like carpesterol, diosgenin, campesterol, β-sitosterolandtriterpenes like cycloartenoland cycloartanol were reported from the fruits.[17-22] Fruits of the plant, *S. xanthocarpum* collected from Adama Science and Technology University campus is depicted below in Fig.1.

Fig. 1: Fruits of *S. xanthocarpum* (Image taken by Henok B. Abebe in October, 2015).

Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy and chemical constituents of some of the herbs used in the treatment of some illness would be worthwhile. In Ethiopia, despite the availability of the plant in different parts of the country, no research work is reported up to now, to the best of our knowledge except that we found some traditional medicinal scripture related to the Orthodox “Debteras” reporting the use of the fruit of *S. xanthocarpum* for the treatment of

tuberculosis, locally known as samba nekersa. Hence, this research emphasized on the investigation of the chemical constituents of the fruits of *S. xanthocarpum.*

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Plant material: Fruits of the plant, *S. xanthocarpum* was collected from Adama Science and Technology University campus. The sample was shade dried and powdered into similar mesh size.

2.2. Chemicals and Apparatus

2.2.1. Chemicals and solvents

Some of the chemicals and solvents that were used in this study include: methanol, petroleum ether, silica gel, H_2SO_4, HCl, NaOH, acetone, ethyl acetate, n-hexane, $CHCl_3$ (chloroform), CH_2Cl_2 (dichloromethane), $FeCl_3$ (ferric chloride), $Hg(NO3)_2$ (mercuric nitrate), mercuric chloride ($HgCl_2$), Iodine (I_2), potassium iodide (KI), ammonia (NH_3), benzene (C_6H_6), vanillin, zinc ribbon.

2.2.2. Apparatus and Instruments

Some of the apparatus used in this study were: electronic balance, rota evaporator, glass Column, filter paper, measuring cylinders, pippets, test tubes, reagent bottles, analytical TLC, preparative thin layer chromatography (PTLC), UV-light cabinet, and UV-Vis spectrophotometer.

2.3. Methods

2.3.1. Extraction, TLC analysis and fractionation

170 gm. of dry powdered fruit of *S. xanthocarpum* was extracted successively with petroleum ether (1.5L), chloroform (1.5L), ethyl acetate (1.5L), and methanol (1.5L) for 72 hours each. And the solutions were filtered with filter papers and evaporated under reduced pressure using rotary evaporator and crude extracts were obtained for each solvent used. The yields from each extract were measured with analytical balance and recorded. TLC was used for the analysis of components in each extract and for the determination of solvent system to run the column chromatography. Column chromatography and preparative tin layer chromatography were used for the fractionation and purification, respectively. NMR, IR and UV-Vis spectroscopy were used for the structure determination of isolated compounds. But the dry

crude extracts were first stored in a refrigerator for the upcoming phytochemical analysis, isolation and purification of natural products.

2.3.2. Phytochemical screening tests

2.3.2.1. Test for carbohydrates

Molisch's test

The extracts are combined with drops of molisch's reagent (α-naphtol dissolved in ethanol) in a test tube. And the mixtures were shaken well and a small amount of concentrated sulfuric acid is slowly added down the side of the sloping test tubes without mixing to form a layer. A positive result is indicated by appearance of a purple ring at the interface between the acid and test layer. The results for each extracts are summarized in table 1.

2.3.2.2. Test for proteins and amino acids

Millon's test

To 2ml of each extract 2 ml of millon's reagent (mericuric nitrate in nitric acid) was added. A positive result that indicates the presence of proteins and amino acids is the appearance of white precipitate which turns red up on gentle heating. The results for each extracts are summarized in table 1.

2.3.2.3. Test for oils and fats

Stain test

Small amounts of each extract were spotted on the same filter paper and left to dry. A positive result indicating the presence of fixed oils is that the spotted place remains stained. Hence, only the petroleum ether extract gave a positive result for the stain test. For confirmation a saponification test was done as follows for petroleum extract. To 1 ml of petroleum ether extract 3 drops of sodium hydroxide solution and 1 drops of ethanol was added and heated in a water bath. Froth started to form after 30 minute.

2.3.2.4. Test for alkaloids

Wagner's test

Wagner's reagent was added to a petroleum ether and methanol extract and gave a brown precipitate for the methanol extract which is a positive test for alkaloids.

2.3.2.5. Test for flavonoids

Zinc hydrochloride reduction test

To petroleum ether and methanol extract, 1 ml each in a test tube, zinc metal in HCl was added. A positive result is the turning of the test solution to red color after few minute.

Alkaline reagent test

To 1 ml petroleum ether and 2 ml methanol extract 3 drops of sodium hydroxide solution was added. The formation of yellow color would indicate the presence of flavonoid.

2.3.2.6. Test for saponins

To each 2ml extract in 3 different test tubes 5 ml distilled water was added and shaken. A positive result indicating the presence of saponins is the formation of froth which persists for few minutes. The results are summarized in table 1.

2.3.2.7. Test for phenolics and tanins

Ferric chloride test

Ferric chloride solution was added to petroleum ether and methanol extract and no color change for the petroleum ether extract but a greenish black color for methanol extract which is a positive test for phenolics and tannins.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

Preliminary phytochemical tests were conducted for only 7 classes of phytochemicals by the standard methods described under section 2 of this manuscript. Carbohydrates,Proteins and amino acids, and Saponinswere not detected in petroleum ether extract but were detected in ethyl acetate and methanol extracts. Alkaloids and Phenolics and taninswere not detected in petroleum ether and Ethyl acetate extracts but were detected in methanol extract. Fats and oils were detected in petroleum ether extract but were not detected in ethyl acetate and methanol extracts. Flavonoids were not detected in any of the extracts by the two methods employed in this research. The results of preliminary phytochemical screeningfor all of these 7 classes of phytochemicals are summarized in Table 1.

Table 1: The above preliminary phytochemical screening results are tabulated as follows.

Phytochemicals		Petroleum ether exract	Ethyl acetate extract	Methanol extract
Carbohydrates	Molisch's test	-	+	+
Proteins and amino acids	Millon's test	-	+	+
Fats and oils	Stain test	+	-	-
	Saponification test	+		
Alkaloids	Wagner's test	-	-	+
Flavonoids	Zinc hydrochloride reduction test	-	-	-
	Alkaline reagent test	-		-
Saponins		-	+	+
Phenolics and tanins		-	-	+

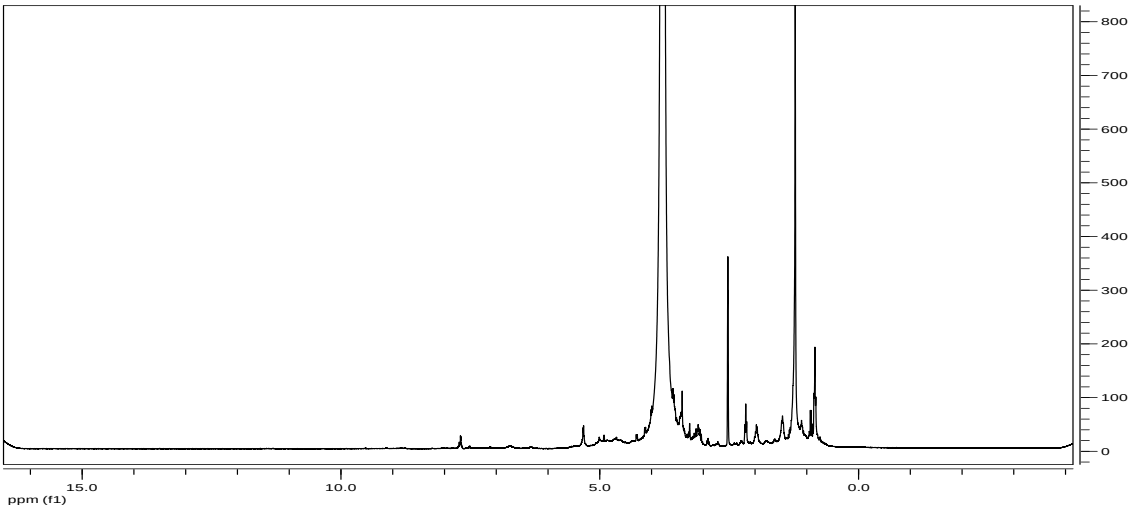
3.2. Isolation of compounds from methanol extract

During the TLC analysis, the methanol extract was unable to move with 100% methanol using a normal phase TLC plate but, gave many spots using a reversed phase TLC plate with 100% methanol. Isolation of compounds from methanol extract was achieved first using column chromatography over silica gel followed by purification of fraction 13 by preparative thin layer chromatography (PTLC) by using methanol: glacial acetic acid (10: 1). First slurry of 120 gm. silica gel was prepared in petroleum ether and poured in to the column. Then the methanol extract (17 gm.) was loaded by dissolving in 50 ml methanol. 23 fractions were collected from the column by eluting with methanol: glacial acetic acid (10: 1). Fraction 13 showed a single spot with some impurity and taken for further purification by preparative TLC (PTLC) with the same solvent system (i.e. 10:1 methanol and glacial acetic acid). The isolated compound was analyzed by NMR, IR and UV spectroscopy at AAU 4 Kilo campus.

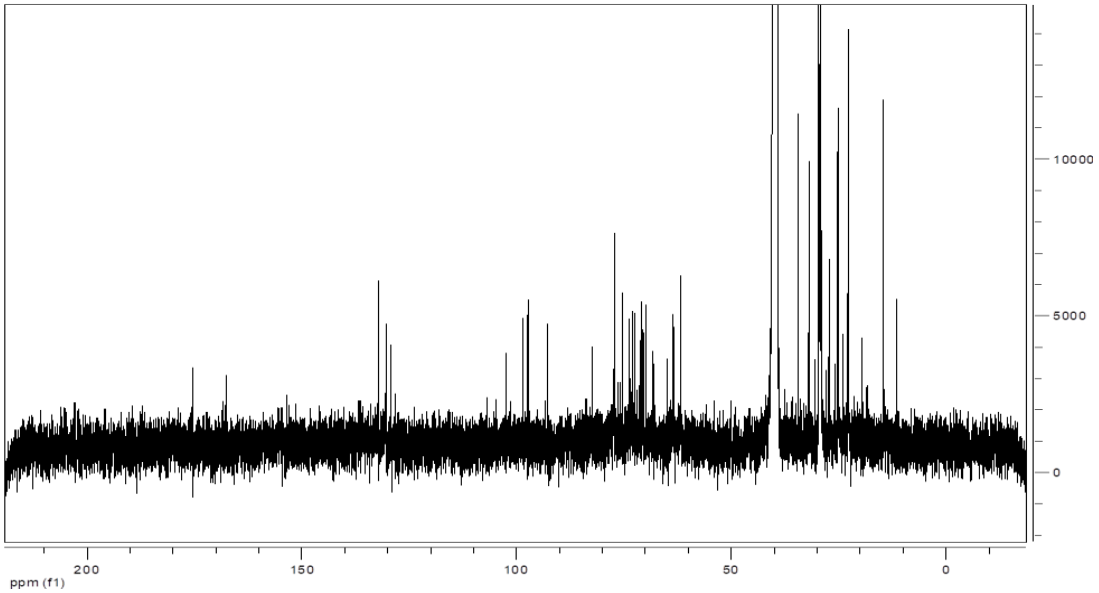
Characterization of compound SX_{-13}

SX_{-13} was obtained as a red solid compound. The UV-Vis spectrum showed absorption maxima at 277. This suggests that the compound is composed of a conjugated diene system that would absorb around 200 nm and this increase to 277 nm is possibly due to the extending conjugation of the conjugated double bonds being next to a carbonyl group. Extensive analysis of the ^{1}H and ^{13}C NMR spectrum of the compound and comparison with literature data led to a frostane type skeleton for the aglycone of the compound. The ^{1}H-NMR spectrum provided key information about the skeleton of the compound. The H_{-16} resonates between

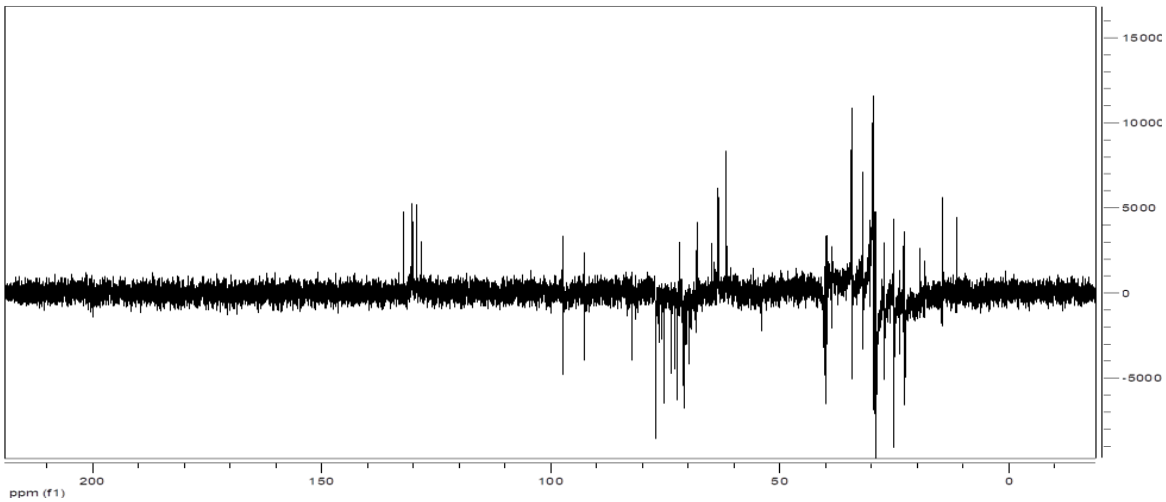
4.1 and 4.5 ppm as a quartate, doublet of doublet, having $^{3}J_{16,15a} = {}^{3}J_{16,15b} = {}^{3}J_{16,17}$ = 6.2 -8.2 in the case of both spirostanes and furostanes.[23] The ^{1}H-NMRspectrum (Table 2, δH and δC in ppm) showed resonance for H_{-16} at 4.112 ppm as doublet of doublet with $^{3}J_{16,15a} = {}^{3}J_{16,15b} = {}^{3}J_{16,17}$ = 7. The ^{1}H-NMRspectrum displayed signals at 0.83(s), 0.911(s) and at 1.20 (s) ppm indicating singlet methyl protons located at the junction of the rings A-E. If there is any substituent on a furostane ring at the junction carbon, then it will result in the non-protonation of these carbons. The presence of multiple down field peaks in the aliphatic protons region in the range from 3-4.9 ppm (almost nine peaks) indicates the protons are connected to an electron withdrawing group most likely oxygen as in cyclic sugars connected by a glycosidic bond or otherwise to other deshielding groups. The presence of sugar moieties is also supported by its ^{13}C NMR spectrum. There are also resonances for olifinic protons at around 5.3 ppm. For ^{1}H-NMR a well-known difficulty with the assignment of proton resonances of oligosaccharides is the overlap of constituent proton resonances in a narrow spectral width (3.2-4.5 ppm). An exception is the anomeric resonances of the sugar moieties which occur largely in a well-defined region (4.4-6.4).[23] So, ^{1}H-NMR spectrum shows anomeric proton resonances at 4.900 and 4.995 ppm. ^{13}C-NMR spectrum showed signals for methyl carbons 11.21, 14.35, 18.17 and 19.26 δC in ppm and 15 methylene carbons from 23.69 to 71.71 ppm. There are four double bond carbons and DEPT-135 suggests they all are methines (CH) and the UV-Vis spectrum shows absorption maxima at 274 nm. This tells the two double bonds are not only conjugated with each other but also with another group like C=O. Therefore, there is no chance for the double bonds to be inside the cholestane ring since a double bond at any position or substituents at junctions cause non-protonated nature of these carbons. The ^{13}C anomeric region (92-108) is straight forward and hence the number of C-signals in this region usually reflects the number of sugar units, considering C_{-22} can appear to confuse in this region which usually can be identified by its none protonated nature.[23] Its ^{13}C NMR spectrum shows multiple methine resonances in region between 68 and 80 ppm and their anomeric carbons at 92 .60 and 97.19 ppm. The chemical shits of the ^{1}H, ^{13}C and DEPT spectra are tabulated in Table 2.



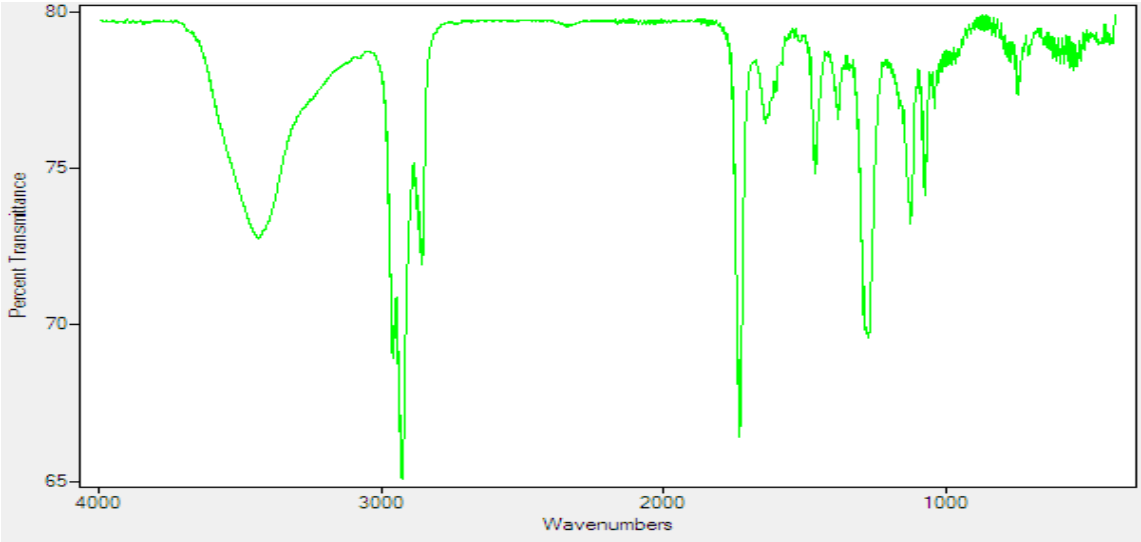
1H NMR spectrum of SX-$_{13}$ in DMSO



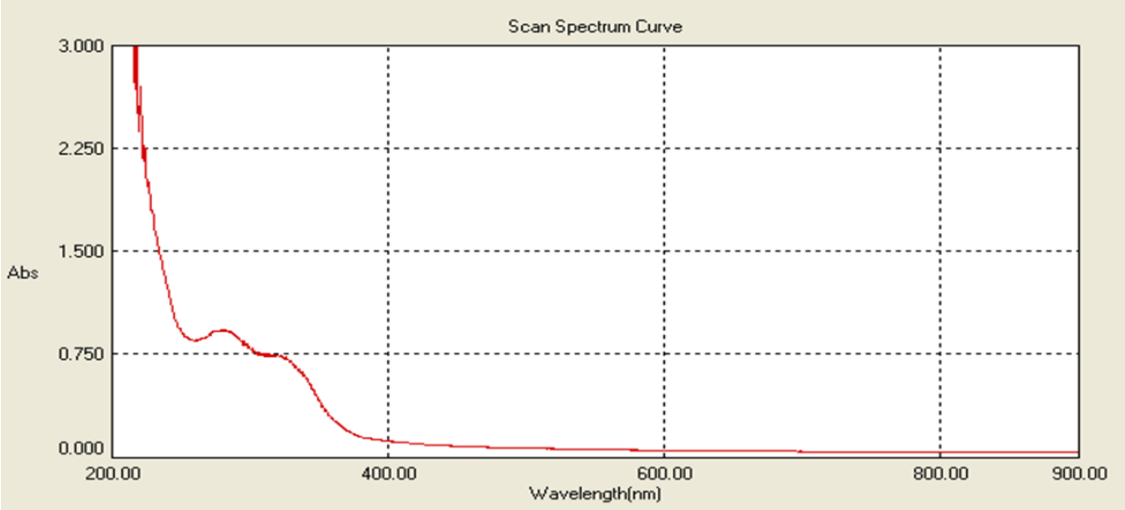
13C NMR spectrum of SX-$_{13}$ in DMSO



DEPT-135 spectrum of SX-$_{13}$



IR spectrum of SX-13



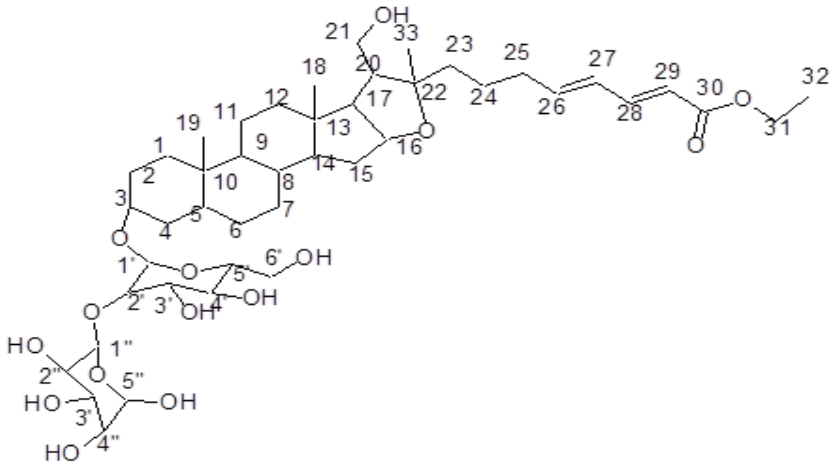
UV-Vis spectrum of SX-13 in methanol

Table 2: ^{1}H, ^{13}C and DEPT spectral data of compound SX-13.

Position	$\delta^{1}H$ (400MHz, DMSO-d6)	$\delta^{13}C$(400MHz, DMSO-d6)	DEPT-135 implications
1	1.45 (t, J=15)	30.20	CH_2
2	1.07 (t, J=15)	24.89	CH_2
3	4.66 (m)	72.70	CH
4	0.89	28.91	CH_2
5	1.96 (m)	34.13	CH_2
6	1.30(q)	29.08	CH_2
7	1.30	29.36	CH_2
8	2.14 (t, J=18)	38.51	CH
9	2.14 (t, J=18)	39.42	CH
10	-	35.51	C
11	1.20	23.69	CH_2
12	1.20	26.94	CH_2

13	-	37.33	C
14	-	53.75	CH
15	-	31.67	CH_2
16	4.11(dd, ^3JH_a= ^3JH_b =^3JH_c =7)	77.06	CH
17	1.95	68.10	CH
18	0.82 (s)	14.36	CH_3
19	0.91 (s)	14.37	CH_3
20	2.87 (t, J=20)	70.67	CH
21	4.26 (d, J=18)	63.12	CH_2
22	-	97.19	C
23	1.45 (t, J=16)	29.27	CH_2
24	1.2 (m)	28.79	CH_2
25	1.30 (q)	29.46	CH_2
26	5.29	128.12	CH
27	5.30	129.13	CH
28	5.33	130.12	CH
29	5.31	132.08	CH
30	-	167.60	C
31	3.5	67.97	CH_2
32	0.84 (t, J=15)	18.26	CH_3
33	1.07 (t, J=15)	19.28	CH_3
1’	4.90 (d, J=7)	92.60	CH
2’	4.26m	77.01	CH
3’	.3.98 m	76.99	CH
4’	4.60 (m)	75.23	CH
5’	4.11 (m)	73.46	CH
6’	4.26 m	63.12	CH_2
1”	4.99m	97.19	CH
2”	3.25m	73.46	CH
3”	3.57	72.70	CH
4”	3.39	70.89	CH
5”	3.42	70.32	CH

Although the complete assignments of the ^1H and ^{13}C chemical shifts, coupling constants and configuration in order to establish the nature and types of the sugar moiety and their connectivity with each other as well as with the furostane ring requires extensive 2D NMR spectral analysis,[23-27] we proposed the structure of compound SX_{-13} based on the above spectral data.



Compound SX-13

4. CONCLUSION

Although plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock since ancient times, research works must proof the traditional claims that the fruit of this plant, *S. xanthocarpum,* aqueous extract can be used for treating bacillus tuberculosis (locally known as samba nekersa). The fruits of *S. xanthocarpum* are the source of natural products; carbohydrates, fixed fats and oils (which are detected in only petroleum ether extract), proteins, amino acids, saponins, phenolic, tannins, flavonoids, alkaloids and glycosides whose presence and/or absence are confirmed by the methods employed in this work. 170 gm. of powdered fruit of *S. xanthocarpum* when successively extracted with petroleum ether, chloroform, ethyl acetate, and methanol gave *a* maximum yield, 17.6 gm., 10.4%, for the methanol extract and none of the other gave a yield that exceeded 1.1gm (the amount obtained from chloroform extract), is an indicative of the plant having more proportion of polar compounds. The presence of these secondary metabolites detected in different solvent extracts of fruits of *S. xanthocarpum*is consistent with the previous reports. Almost entirely, literature on the isolation of natural products from this plant reports that the plant has steroidal saponins and steroidal glyco-alkaloids. And compound SX-13 of the methanol extract reveals this fact. It is a steroidal saponin with a furostaneaglycon skeleton. Although the most common natural steroidal saponin are from spirostane parent skeleton in which case C_{22} connected to two oxygen atoms usually resonates in a narrow spectral width between 108.9 and 110.0 ppm,[23,27] the lack of signals not only even in the vicinity of this narrow band, but also from 103 to 127 ppm in our ^{13}C spectrum led us to the conclusion that our compound (SX-13) is of a furostane parent skeleton in which case C_{22} resonates at 97.19 ppm. Because of the inaccessibility of

spectroscopic instruments, specially NMR, and other constraints, we only isolated and partially characterized one compound and conducted preliminary phytochemical tests. Thus we hope our study titled "phytochemicals investigation of fruits of *S. xanthocarpum"* will help other researchers who are interested to isolate and characterize more constituents from *S. xanthocarpum* plant.

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to Dr. Haelemichael Tesso, associate professor at Adama Science and Technology University, for his fatherly and professional advice in doing the research and writing the report. I thank Dr. Aman kekebo, associate professor at Adama Science and Technology University for giving his constructive comments whenever I needed. And finally I like to take this chance to thank some of senior instructors of ASTU; Dr. Tegene, Dr. Dereje, Dr. yadesa, Dr. teshome, Mr. Bedasa who helped me to become a person I am today and also many thanks to Mr. Guta and Mr. Lemi for their kind collaboration in the laboratory works. Almighty God gave me you all, let all the glory be to him.

REFERENCES

1. Endashaw Bekele, Study on Actual Situation of Medicinal Plants in Ethiopia (Japan Association for International Collaboration of Agriculture and Forestry; December, 2007.
2. Mazen E. and Jehad A., Manual of Phytochemistry. Al Azhar University Faculty of Pharmacy Department of Pharmacognosy, 2010; 7-8.
3. Farnsworth N., Soejarto D., Global importance of medicinal plants. In: Akerele O, Heywood V, Synge H, editors. The Conservation of Medicinal Plants. *Cambridge University Press*, Cambridge, 1991; 25–51.
4. Muralidharan S., Rajan S. and Danaphal S. P., Simultaneous Estimation of Plumbagin and Embelin by Reverse Phase-High Performance Liquid Chromatographic method, 2009*;* 1(1): 135-142.
5. Smit H., Woerdenbag H., Singh R., Meulenbeld G., Labadie R., Zwaving J., Ayurvedic herbal drugs with possible cytostatic activity. *J. Ethanopharmacol*, 1995; 47: 75- 84.
6. Mayer B., Ferrigni N., Putnam J., Jacobsen L., Nichols D., McLaughlin J., Brine S., A Convenient General Bioassay for Active Plant Constituents. *PlantaMed*, 1982; 45(5): 31-34.

7. Okram Mukherjee S. and ThokchomPrasanta S., Phytochemistry of *Solanumxanthocarpum*: an amazing traditional healer, *Journal of Scientific & Industrial Research*, 2010; 732-740.
8. Sachin P., Amit G., Navin S., *Solanumxanthocarpum*(Yellow Berried Night Shade): A review. *Der Pharmacia Lettre*, 2010, 2(4): 373-383.
9. Dixit V., Gupta R., Antispermatogenic/antiandrogenic properties of solasodine (C27H43O2N) obtained from *solanumxanthocarpum* berries on the male genital tract of dog (Canis-familiaris). A Histophysiological approach. *Int J Androl*, 1982; 5(3): 295 307.
10. Heble M., Narayanaswami S., Chadha M., *Am AssocAdvSci Stable*, 1967; 161(13): 1145.
11. Sato Y., Latham J., The Isolation of Diosgenin from *SolanumXanthocarpum. J Am ChemSoc*, 1953; 75(23): 6067-6067.
12. Lu Y., Luo J., Kong L., Steroidal alkaloid saponins and steroidal saponins from *Solanumsurattense*. *Phytochemistry*, 2011; 72(7): 668-673.
13. Sachin P., Amit G. and Navin S., *Solanumxanthocarpum* (Yelow Berried Night Shade): A review. *Der Phrmacialettere*, 2010; 2(4): 372-383.
14. SadafSaleem 1, Muhammad Uzair, Bashir Ahmad, Muhammad I. Qadir 2 &Khizar Abbas, Cytomorphological, Phytochemical, Antimicrobial and Antioxidant Investigations of Different Morphological Parts of Solanumxanthocarpum Schrad & Wendl, 2018, Lat. Am. J. Pharm, 2018; 37(4): 839-848.
15. Shelly Rana, VedPrakash and AnandSagar, Antibacterial Activity of Solanumxanthocarpum Leaf Extract, Int. J. Curr. Microbiol. App. Sci, 2016; 5(4): 323-328.
16. OnilaLugun, SobharaniBhoi, PriyankaKujur, D Vinay Kumar and William R. Surin, Evaluation of Antithrombotic Activities of Solanumxanthocarpum and Tinosporacordifolia, Pharmacognosy Res, 2018; 10(1): 98–103.
17. Kusano G., Beisler J., Sato Y., *Phytochemistry*, 1973; 12(2): 397-401.
18. Singh O., Subharani K., Singh N., Devi N. and Nevidita L., Isolation of steroidal glycosides from *Solanumxanthocarpum* and studies on their antifungal activities. *Nat Prod Res*, 2005; 21(7): 585-590.
19. Divya K., Mayak G., Amit K., Gopal N., Yamini B., Tripathi J., Tiwari S., Comparative Evaluation Phenol and Flavonoid Content of Polyherbal Drugs. *Pharmacologyonline*, 2011; 3: 1365-1373.
20. Nasir E, Ali S., Flora of Pakistan, Pakistan Agricultural Research Council Islamabad, 1985; 168: 1-3.

21. Parveen F., and Rahat A., Isolation of a New Steroidal Glycoalkaloid from *solanumxanthocarpum*, *Journal of Basic & Applied Sciences*, 2014; *10:* 28-32.
22. Poul R. and Datta K., An Ubdated Overview on *solanumxanthocrarpum*Schrad and wendl, *IJRAP*, 2011; 2(3): 730-735.
23. Pawan K., A SYSTEMATIC NMR APPROACH FOR THE DETERMINATION OF THE MOLECULAR STRUCTURES OF STEROIDAL SAPONINS, Central institute of Medicinal and Aromatic Plants, *Lucknow* – 226 015, INDIA.
24. AgrawalP.k., Pathak A.K., NMR spectroscopic approaches for the determination of interglycosidiclincage and sequence in oligosaccharides, *phytochem. Anal.*, 2014; 7: 113-130.
25. Chen S. and Snyder J.k., Diosgenin-bearing molluscidalsaponins from *Allium vineale*: an NMR approach for the structural assignment of oligosaccharide units. *J.Org. chem.*, 1988; 54: 3679.
26. Jacek W., Morzycki, Katarzyna P., Karolina D., Izabella J., Leszek S., Iwona W., ^{13}C NMR study of spirostanes and furostanes in solution and solid state, Institute of Chemistry, University of Bialystok, Pilsudskiego Bialystok Poland, 2005; 15-443: 11-4.
27. Agrawal P., Jain D., Gupta R., Thakur R.., Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins., *Phytochemistry*, 1985; *24*: 2479-2496.