



# Phytochemical screening of *Solanum xanthocarpum* and its xanthine oxidase inhibitory activity

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### ABSTRACT

Pharmacological features and chemical compounds derived from *Solanum xanthocarpum* (*S. xanthocarpum*) are often investigated. *S. xanthocarpum* has been widely used to treat a variety of ailments as traditional medicine. Different parts of the plant are being studied to find more new uses for the herb. Xanthine oxidase inhibition is important in many vascular, renal, and bone disorders as this super enzyme causes an increase in the uric acid formation and oxidative stress. With this perspective and current literature, further studies are required to be conducted to highlight new features that may be derived from the plant. This study aimed to explore the fruits of *S. xanthocarpum* for their phytochemical constituents and their xanthine oxidase inhibitory activity. Fruits from *S. xanthocarpum* were identified and collected. We performed alcoholic extraction in the Soxhlet apparatus and fractionation using column chromatography. Furthermore, we analyzed phytochemical properties using fingerprinting chromatography, NMR (Nuclear magnetic resonance), mass-spectrometry (MS), ultraviolet (UV), and infrared (IR) spectroscopy. The NMR spectrum revealed new chemical moieties which were validated using MS. Later on, we conducted bioassays for xanthine oxidase Inhibitory activity on individual fractions. Three new chemical compounds were discovered (Eicosa -11, 14-dienoic acid, Docosa-7, 10, 13, 16, 19-pentanoic acid, and Glyceryl Tri Palmitoleate). These compounds were fatty acids in nature. Bioassay results obtained were positive and encouraging. Our study findings provide evidence of new chemical compounds that can be derived from *S. xanthocarpum* and can be used to treat renal diseases and gout by inhibiting excessive enzyme catalyzation of xanthine oxidase. The results suggest *S. xanthocarpum* as a possible candidate for a new therapeutic approach.

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### Introduction

The ethnomedicinal plant history is of worldwide significance [1]. Plants are sources for producing phytochemicals, which create abundant treatment options for various diseases. According to the

**Abbreviations:** NMR: Nuclear magnetic resonance; MS: Mass- spectrometry; UV: Ultraviolet; IR: Infrared; XO: Xanthine oxidase inhibitors; HPTLC: High-performance thin layer chromatography; TLC: Thin layer chromatography; v/v: Volume by volume; kg: Kilogram; SX: Solanum xanthocarpum.

World Health Organization, traditional medicine serves 80 percent of the global population [2]. They are used in improving health and lifestyle and treating common illnesses as they are effective, safe, and less complicated. Traditional medicines are also accepted in our culture [3]. Several phytochemicals such as alkaloids, saponins, flavonoids, and terpenoids among others are found in medicinal plants as secondary metabolites [4]. Due to their unrivaled chemical variety, natural substances provide an infinite opportunity for potential drug discovery, whether in pure molecules or through standard extracts [5, 6]. People have turned to ethnopharmacology with the obvious rise in undesirable effects and microbial resistance to chemically produced drugs [7, 8].

Traditional medicines like Indian Nightshade or *Solanum xanthocarpum* (*S. xanthocarpum*) of the Solanaceae family of flowering plants have been widely used for the treatment of various degenerative disorders and infections. *S. xanthocarpum* is found in many countries including Australia, Ceylon, Polynesia, Malaysia, South-East Asia, and India [9]. Punjab along with Mysore, West Bengal, Bihar, and Uttar Pradesh has observed the growth of *S. xanthocarpum* in India [10]. Often, it is spotted growing near arid lands and roadsides as wild plants and was used in curing inflammatory diseases and sore throats [11].

Research has indicated *S. xanthocarpum* to have fatty acids, alkaloids, steroids, and their alkaloids, phenolics, coumarins, tri- terpenoids, glycoalkaloids, and sapogenin in their phytochemical properties. Fruits of these plants contain triterpenes such as cycloartenol, coumarins such as daucosterol, diosgenin, campesterol, steroids carpesterol, aesculin, and aesculetin. Along with these, caffeic acid and many steroidal alkaloids such as solamargine, solanacarpine, solasonine, and solanacarpidine are also found in these fruits [12]. These properties increase the validation of *S. xanthocarpum* scientifically for medicinal use according to existing literature. It has been found that *S. xanthocarpum* can aid in the treatment of various ailments as it produces antifertility, antidiabetic, anticancer, antiasthmatic, antibacterial, antifungal, antiulcer, antioxidant, anti-hyperlipidemic, anti-helminthic, anti-HIV, antinociceptive, hepatoprotective, diuretic, analgesic, and wound healing effects [13, 14].

Superoxide-producing enzyme xanthine oxidase, present in lungs and serum, has shown its efficacy in the treatment of influenza, infections, and genetic disorders such as Xanthinuria which causes concentrations of xanthine to increase in blood. This

condition is caused due to lack of xanthine oxidase [XO], which can cause concerns like a renal failure in health conditions. There is no particular therapy; instead, doctors urge patients to drink plenty of fluids and avoid purine-rich meals [15].

Potential treatments can be developed from natural substances as they contain various xanthine oxidase inhibitors (XOI). It is yet to explore complete natural therapeutic approaches for disorders occurring from XO. Therefore, to address this issue it is important to establish promising XOI through screening and optimization [16-18]. Nephropathy and renal stones are treated with xanthine oxidase inhibitors as they are caused by hyperuricemia [19]. The synthesis of uric acid is directly inhibited through these agents in vivo as they are reported to have polyphenolic compounds and flavonoids as active moieties in plant extracts [20, 21]. In vitro studies also report the activity of flavonoids as inhibitors. This may occur due to the hydroxyl group at C-3 not being present. These results have paved the way for the discovery of natural substances that could be XO inhibitors, as well as renewed interest in the study of traditional medicines [22-24].

A thorough analysis of existing literature gave rise to the rationale of our study. This research is one of the first of its kind to be published as we aimed to primarily isolate and characterize fresh *S. xanthocarpum* phytoconstituents and screen their inhibitory activity of xanthine oxidase.

## Material and methods

### UV Analysis of extract of *S. xanthocarpum*

The plant *S. xanthocarpum* was collected from M/S Universal Biotech, Farash Khana, Delhi, India during the flowering period between March and June 2021. The plant material was authenticated by comparison with the voucher specimen in PRL, The Department of Pharmacognosy and Phytochemistry, Jamia Hamdard University, New Delhi. The herbarium file of the plant was prepared and stored in Phytochemistry Research Lab, Pharmacognosy Department of Jamia Hamdard, New Delhi, India 110062 for future reference.

### Reagents, chemicals, and apparatus

Silica gel F<sub>254</sub> HPTLC (high-performance thin layer chromatography) plates, chloroform, methanol, and ammonia solution were purchased from Merck, Mumbai, India. All the chemicals were of AR grade. Silica gel (200-400 mesh) was loaded within a built-in assembly of the glass column for the stationary

phase and was carried out on the column chromatography. The sample applicator of CAMAG Linovmat IV [Muttentz, Switzerland] equipped with a 100 µl Hamilton (USA) syringe, was used along with the CAMAG TLC (Thin layer chromatography) scanner with a deuterium lamp and Wincat Software [25].

#### *Extraction of plant constituents*

After the collection of mature fruits, they were dried in shade. We manually separated the dried fruits and crushed them using mortar and pestle. Soxhlet extraction was done using methanol as solvent and the sample was evaporated in the rotary evaporator pressure. The extracted yield was 25% with a weight of 0.5 kg from the total of 2 kg. The extract produced dark brown color.

#### *Isolation of phytoconstituents by column chromatography*

The reduced mass was placed in a minimum quantity of methanol and adsorbed into silica gel (200- 400 mesh) for slurry preparation. The silica gel column was covered with hexane before being dried, packaged, and chromatographed. The chromatography was carried out as a stationary phase on an indigenous assembly using a silica gel-loaded glass column (200-400 mesh). The lower end of the column was plugged with non-absorbent cotton and was covered with a piece of filter paper. After half-filling the column with solvent, small amounts of silica gel (200- 400 mesh) were added and allowed to settle down gently until the column's required length was attained. The solvent was continuously run through the column to allow air bubbles to escape. The silica gel slurry of the extract was packed in the column and then eluted successively in order of increasing polarity with different solvents. The column was developed and eluted using a range of solvents in various combinations, including ethyl acetate: hexane and ethyl acetate: methanol. The completion of elution of the component(s) was ascertained when evaporating a small fraction of eluant left no residue.

#### *Chromatographic procedure*

Various solvent compositions were explored as mobile phase, including Toluene-ethyl acetate (93:7v/v), 1propanol-water-formic acid (90:9:1), and Toluene-ethyl acetate- diethylamine (70:20:10). Chloroform: methanol: ammonia solution (7:2.5:0.5) was then deemed as the best solvent. Band separation, band shape, band compactness, and development time were used to determine the suitability of the solvent mixture. Various TLC [Thin layer chromatography] chamber saturation times

were also tested. Different volumes of samples (1-5 l) were spotted on TLC plates before development. A stock solution of extract and different fractions (100µg/ml) was prepared in methanol. Using triplicate, different volumes of standard stock solution (1-5 l/band) were spotted onto a TLC plate, and calibration curves were generated by plotting peak areas versus concentrations ranging from 100 to 500 ng/band. With the use of correlation coefficients and regression equations, calibration curves were evaluated [26].

The test samples (4µl/band) were applied in triplicate and chromatograms were obtained under the same conditions as that of standard solasodine. The area under the peak corresponding to that of the standard was recorded and the content of the same was calculated from the regression equation obtained from the calibration curve.

#### *Extraction of xanthine oxidase*

The chemicals used to extract were xanthine oxidase, sodium buffer, xanthine substrate, extract, and fractionated compounds. To 0.12m xanthine substrate, 80m sodium phosphate buffer was added, and 0.1U xanthine oxidase enzyme was added to the test sample. The solution was combined and incubated at 25oC in an incubator for 25 minutes before measuring absorbance at 295nm. The operation was repeated for control, and absorbance at 295nm wavelengths was meticulous, with single values obtained for each extract separately [15].

## **Results**

#### *Extraction of xanthine oxidase*

**Table 1** shows the different percentage concentrations [µg/ml] of ethyl acetate and methanol and their absorbance that was noted. **Table 2** shows the absorbance of different concentrations [µg/ml] of ethyl acetate fractions. **Figure 1** depicts the absorbance marked on the y-axis with a value  $1E-04x + 0.0057$  and an R2 value of 0.8342 at IC50-1200 µg/ml. **Table 3** shows the absorbance observed at different concentrations [µg/ml] of methanol. **Figure 2** depicts absorbance and concentrations at IC50 1150 µg/ml.

#### *UV analysis of extract of S. xanthocarpum*

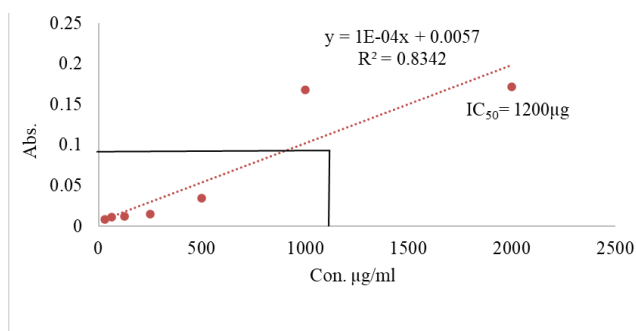
$$R_f \text{ value} = \frac{\text{Distance from Baseline traveled by Solute}}{\text{Distance from Baseline traveled by Solvent (Solvent Front)}}$$

**Table 1.** Absorbance at 295 nm (with enzyme).

S.No.	Concentration (50 µg/ml)	Absorbance (nm)
1	60% Ethyl acetate	0.82
2	80% Ethyl acetate	0.76
3	2% Methanol	0.98
4	5% Methanol	0.71
5	10% Methanol	0.74
6	13% Methanol	0.97
7	15% Methanol	0.75
8	20% Methanol	0.71
9	Extract	0.78
10	Control (Allopurinol)	0.83

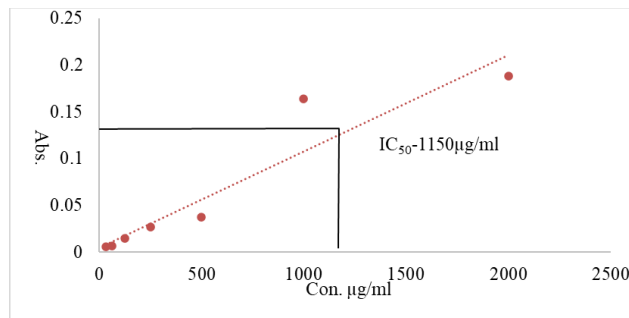
**Table 2.** Absorbance for ethyl acetate fraction.

S.No.	Concentration (µg/ml)	Absorbance (nm)
1	2000	0.172
2	1000	0.168
3	500	0.0345
4	250	0.0153
5	125	0.0121
6	62.5	0.0112
7	31.25	0.0087

**Figure 1.** The absorbance of ethyl acetate fraction.**Table 3.** The absorbance of methanolic fractions

S.No.	Concentration (µg/ml)	Absorbance (nm)
1	2000	0.188
2	1000	0.164
3	500	0.0371
4	250	0.0267
5	125	0.0143
6	62.5	0.0063
7	31.25	0.0057

Rf value as well as the melting point of *S. xanthocarpum* in the different fractions are indicated in **Table 4**.

**Figure 2.** The absorbance of methanolic fractions.

### Phytochemical screening

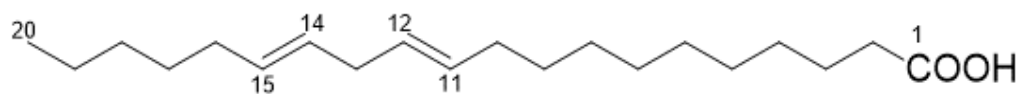
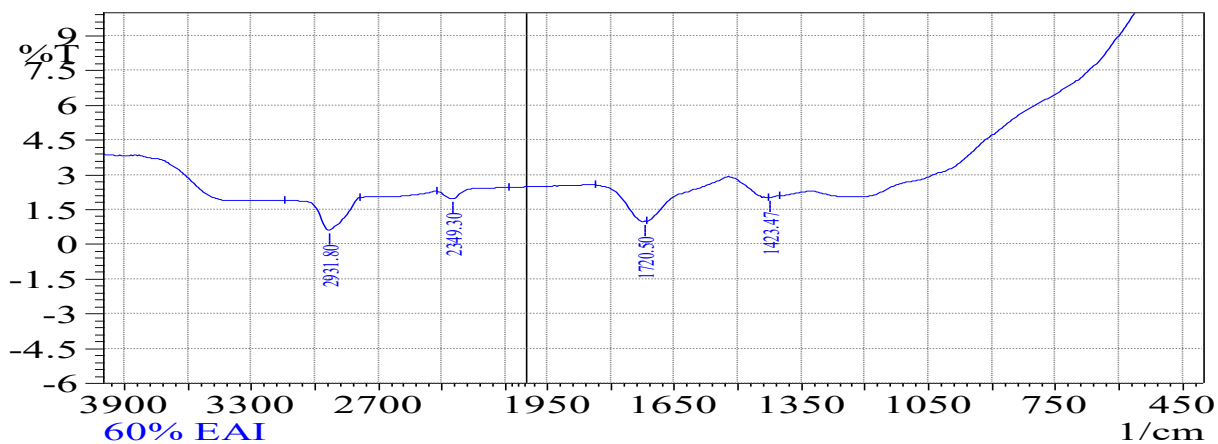
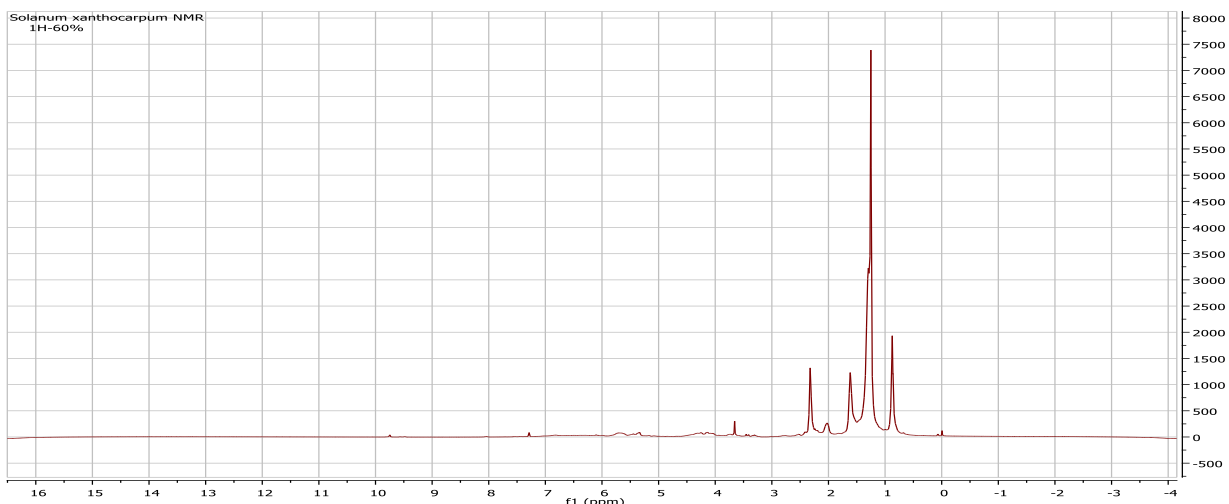
Isolation of compounds derived from *S. xanthocarpum*

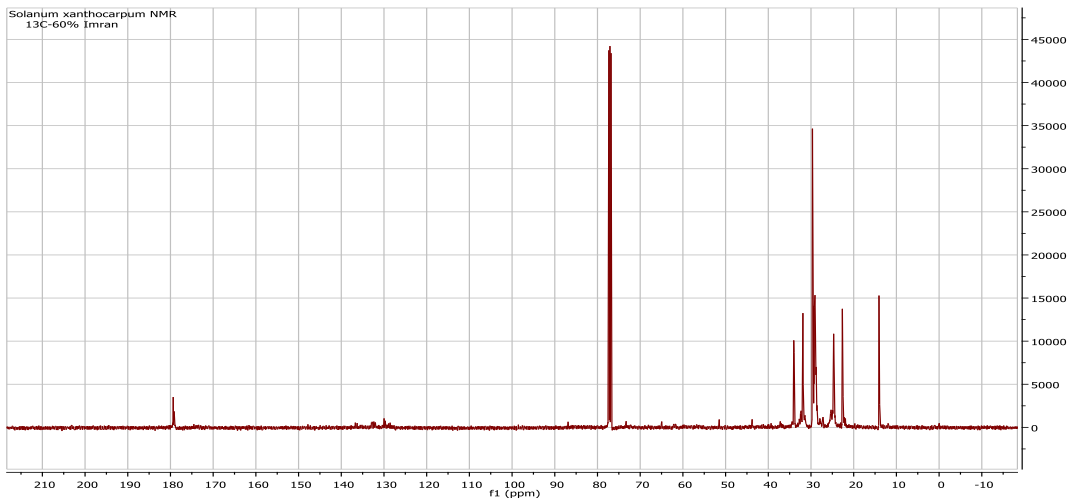
### Compound SX1

Hexane-ethyl acetate (40:60 v/v) was the eluent through which the compound was obtained in its physical state of semisolid sticky mass. The color obtained was light brown with a melting point of 51°C. The Rf value of Hexane-ethyl acetate (40:60 v/v) was 0.78, and the UV [Ultraviolet]  $\lambda$  max of Methanol was 245 and 285 nm. Its FTIR (Fourier transform infrared spectroscopy) spectrum exhibited absorption bands in 1720 and 1640  $\text{cm}^{-1}$  characterizing the presence of a carboxyl group and vinylic linkage respectively. The molecular weight of SX1 was measured as 308 based on  $^{13}\text{C}$  NMR (Nuclear magnetic resonance) and mass spectra, corresponding to the chemical formula  $\text{C}_{20}\text{H}_{36}\text{O}_2$ . The presence of three double bond equivalents was indicated by the formula, two of which were adjusted in two vinylic linkages and the remaining ones in the carboxyl group. Its ES-MS spectrum displayed a pseudo-molecular ion peak at  $309[\text{M}+\text{H}]^+$ . The two fragment's ion peaks at  $m/z$  195 $[\text{C}_{12}\text{H}_{19}\text{O}_2]^+$  and 109 $[\text{C}_8\text{H}_{13}]^+$ , arising due to C13-C14 fission supported the presence of an 11, 14 conjugated vinylic system.  $^1\text{H}$  NMR Spectrum of SX1 showed typical signals of unsaturated fatty acid. Two broad multiples at  $\delta$  5.51 and 5.40, each integrating two protons, were ascribed to H-12/14 and H-11/15 vinylic protons. Another two proton multiples at  $\delta$  2.46 were assigned to H2-13 protons. A two-proton triplet at 1.50 ( $J = 6.5$  Hz) was attributed to H2-2 protons vicinal to the carboxylic functionality. The remaining methylene protons resonated as a broad signal at  $\delta$  1.29. A triplet signal at  $\delta$  0.95 ( $J = 7.0$ Hz) integrating for three protons was ascribed to primary methyl protons H3-20. The  $^{13}\text{C}$  NMR spectrum of SX1 displayed diagnostic signals for carboxylic carbon at  $\delta$  179.1(C-1), vinylic carbon at  $\delta$  132.4(C-15/11), and 130.2(C-12/14), and primary methyl carbon at  $\delta$  12.5(C-20).The methylene carbon resonated at  $\delta$  35.1 (C-13), 32.4 (C-10/16), 30.1 (C-2), 29.5 (8 \* CH<sub>2</sub>), 25.1 (C-3) and 23.4 (C-19).

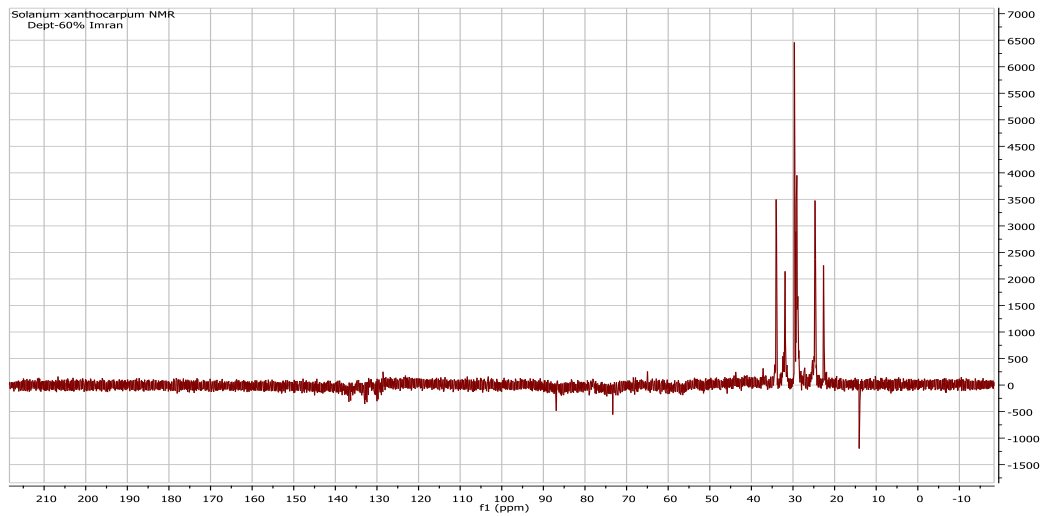
**Table 4.** Rf value as well as the melting point of *S. xanthocarpum* in different fraction.

S.No.	Concentration of fraction	Solubility of compound	Rf calculated	Melting point (°C)
1	40% Ethyl acetate	Chloroform/Methanol	0.80	47
2	60% Ethyl acetate	Chloroform/Methanol	0.78	51
3	80% Ethyl acetate	Chloroform/Methanol	0.80	45.5
4	100% Ethyl acetate	Chloroform/Methanol	0.88	54
5	2% Methanol	Chloroform/Methanol	0.88	52
6	5% Methanol	Chloroform/Methanol	0.50	43
7	7% Methanol	Chloroform/Methanol	0.54	48.5
8	10% Methanol	Chloroform/Methanol	0.50	51
9	13% Methanol	Chloroform/Methanol	0.83	53
10	17% Methanol	Chloroform/Methanol	0.74	55
11	20% Methanol	Chloroform/Methanol	0.80	56

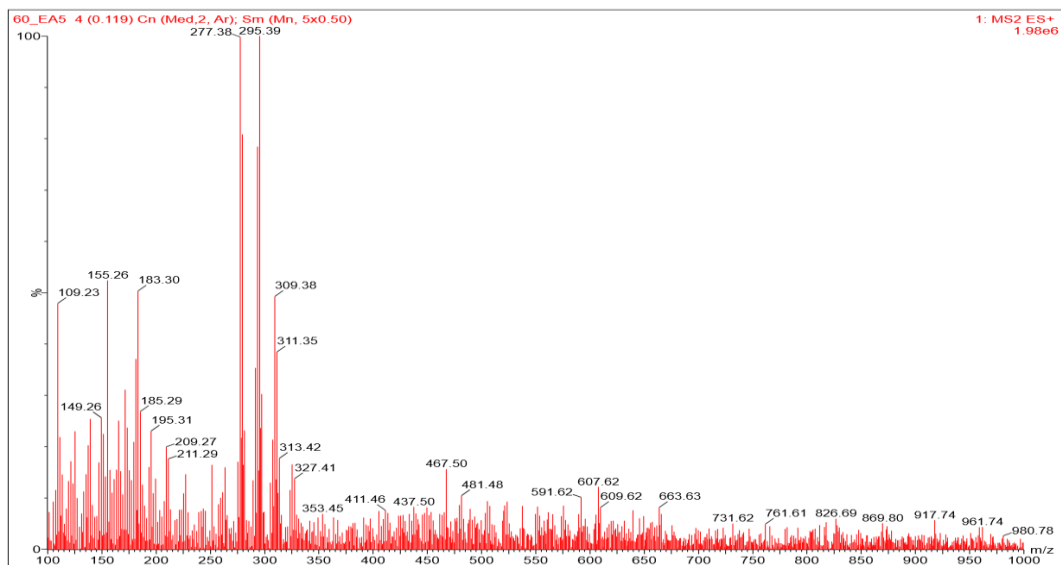
**Figure 3.** Structure of Eicosa-11, 14-dienoic acid (SX1).**Figure 4.** FTIR spectrum of ethyl acetate fraction of Solanum xanthocarpum (SX1).**Figure 5.** <sup>1</sup>H NMR spectra of ethyl acetate fraction of Solanum xanthocarpum (SX1).



**Figure 6.** 13 C NMR spectra of ethyl acetate fraction of Solanum xanthocarpum (SX1).



**Figure 7.** DEPT NMR spectra of ethyl acetate fraction of Solanum xanthocarpum (SX1).



**Figure 8.** MASS spectra of ethyl acetate fraction of Solanum xanthocarpum (SX1).

Based on our findings, the structure of compound SX1 was determined to be eicosa -11, 14-dienoic acid. This is an important  $\omega$ -6 fatty acid from nature. **Figure 3** depicts the structure of the compound. **Figure 4** depicts the FTIR spectrum. **Figure 5** depicts the <sup>1</sup>H NMR spectra of the ethyl acetate fraction. **Figure 6** depicts <sup>13</sup>C NMR spectra of ethyl acetate fraction. **Figure 7** depicts the DEPT NMR spectra of ethyl acetate fraction. **Figure 8** depicts Mass spectra of ethyl acetate fraction.

#### Compound SX2

Ethyl Acetate: Methanol (98:2 v/v) was the eluent through which the compound was obtained in the physical state of semisolid sticky mass. The melting point observed was 52°C and the compound was dark brown. The R<sub>f</sub> value observed for Ethyl acetate-Methanol (98:2 v/v) was 0.88. UV  $\lambda$  max of Methanol was 239 and 276 nm. FTIR spectra exhibited absorption bands for the carboxylic group (1730 cm<sup>-1</sup>), unsaturation (1540 cm<sup>-1</sup>) and aliphatic moiety (1035, 802 cm<sup>-1</sup>). The molecular weight of SX2 was determined to be 330 based on <sup>13</sup>C/DEPT spectra and mass data that matched the chemical formula C<sub>22</sub>H<sub>32</sub>O<sub>2</sub>. ES-MS spectrum had a base peak at m/z 329 [M-1]. The formula indicated the presence of six double bond equivalents, five of which were adjusted in the vinylic linkage and the other in the carboxylic group. SX2's <sup>1</sup>H NMR spectra displayed two downfield multiplets at 5.35 and 5.09, each integrating for four protons attributed to H-20, H-16, H-14, H-10, H-8, H-19, H-17, H-15, H-11, and H-7 vinylic protons. At  $\delta$  2.34, the methylene proton adjacent to double bonds resonated an eight-proton signal. The H<sub>2</sub>-2 methylene proton adjacent to the carboxylic group was assigned a two-proton triplet at 2.03 (J = 6.5 Hz). The remaining methylene protons resonated at 1.95 (2 \* CH<sub>2</sub>) and 1.29 (4 \* CH<sub>2</sub>). A three-proton triplet was observed at  $\delta$  0.90 (J = 7.0 Hz). SX2's <sup>13</sup>C NMR spectra revealed significant signals for carboxylic carbon at 172.6 (C-1); ten vinylic carbon between 139.2 and 122.6; and a primary methyl carbon at 14.3 (C-22).

Based on the above explanation, the structure of SX2 was determined to be DOCOSA-7, 10, 13, 16, 19-Pentenoic acid. It is a beneficial  $\omega$ -3 fatty acid found in nature. **Figure 9** depicts the structure of the compound. **Figure 10** depicts the FTIR spectra of the methanolic fraction. **Figure 11** depicts the <sup>1</sup>H NMR spectra of the methanolic fraction. **Figure 12** depicts the <sup>13</sup>C spectra of a methanolic fraction. **Figure 13** depicts the DEPT spectra of the methanolic fraction. **Figure 14** depicts the MASS spectra of a methanolic fraction.

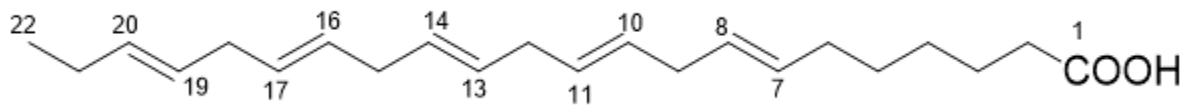
#### Compound SX3

Ethyl Acetate: Methanol (87:13 v/v) was the eluent used to obtain compound SX3 in its physical state of semisolid sticky mass. The color observed was light yellow-brown with a melting point of 53°C and an R<sub>f</sub> value of 0.74. The UV  $\lambda$  max of Methanol was 238 and 275 nm. The FTIR spectrum displayed absorption bands for the hydroxyl group (3390 cm<sup>-1</sup>); the unsaturation (1462 cm<sup>-1</sup>) group, the carboxylic group (1722 cm<sup>-1</sup>), and the aliphatic chain (1037 cm<sup>-1</sup>). The molecular weight of SX3 was determined to be 800 depending on <sup>13</sup>C/DEPT and mass spectra, with the corresponding molecular formula C<sub>51</sub>H<sub>92</sub>O<sub>6</sub>. The formula indicated the presence of six double bond equivalents, three of which were adjusted in three ester linkages and the remaining three in vinylic linkages of fatty acid chains. The fragments ion peak at m/z 547 [C<sub>35</sub>H<sub>63</sub>O<sub>4</sub>]<sup>+</sup> and 238 [C<sub>16</sub>H<sub>30</sub>O]<sup>+</sup> supported the presence of palmitoleic fatty acids in ester formation with glycerol as a homogenous glyceride.

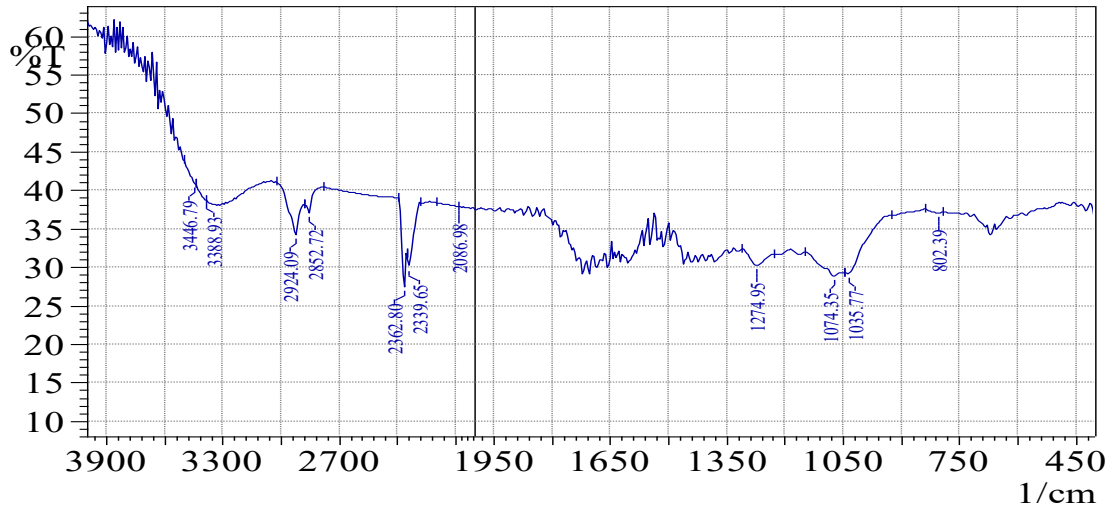
<sup>1</sup>H NMR spectrum of SX3 exhibited a six -proton broad multiplets at  $\delta$  5.61, assigned to vinylic protons H-9',9'',9'''/10',10'',10'''. Another broad signal centered at  $\delta$  5.34 was ascribed to H-2 oxygenated methane proton. A four proton broad multiplets at  $\delta$  4.32 were attributed to H<sub>2</sub>-1 and H<sub>2</sub>-3 oxygenated methylene protons. The protons of primary methyl group Me-16', 16'', and 16''' appeared as a broad triplet at  $\delta$  2.13 and 1.30. The <sup>13</sup>C NMR spectrum of SX3 showed carbon signals of atypical homogenous glyceride prominent signals from the ester carbons at  $\delta$  170.4 (C-2), 168 (C-1/3), vinylic carbons at  $\delta$  139.4 (C-9'/10'), 125.1.0 (C-9''/10''), and 121.0 (C-9'''/10'''); and primary methyl carbons at  $\delta$  15.5 (C-16'/16''/16'''), were observed. Based on the above discussion, the structure of SX3 was determined to be Glycerol Tri Palmitoleate. **Figure 15** depicts the structure of the compound. **Figure 16** provides the FTIR spectra of a methanolic fraction. **Figure 17** depicts the <sup>1</sup>H NMR spectra of the methanolic fraction. **Figure 18** depicts the <sup>13</sup>C NMR spectra of the methanolic fraction. **Figure 19** depicts the DEPT spectra of the methanolic fraction. **Figure 20** depicts the MASS spectra of a methanolic fraction.

#### Discussion

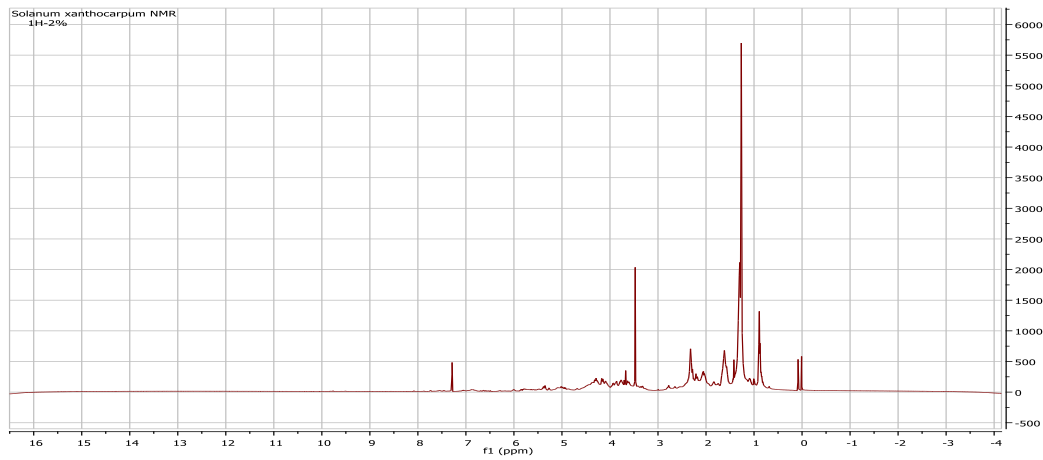
Xanthine oxidase is a member of the molybdenum-protein complex with one molybdenum, one flavin adenine dinucleotide, and two iron-sulfur ferredoxin centers within two separate subunits as its structure. This homodimer with two different sites for substrate binding that are present in this enzyme which is useful in catalyzing hypoxanthine oxidation activity. The breakdown products of this reaction are uric acid and xanthine [27-29]. In humans, uric acid is often not metabolized further and is eliminated



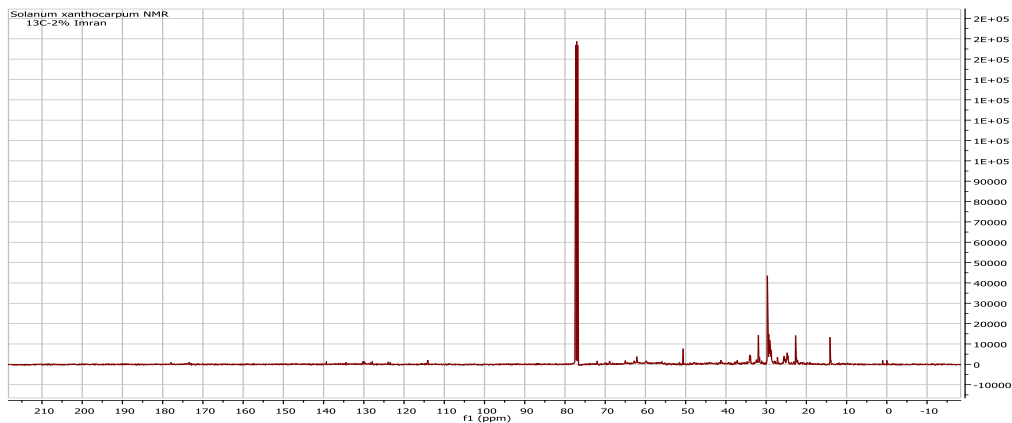
**Figure 9.** Structure of Dodecapentenoic acid (SX2).



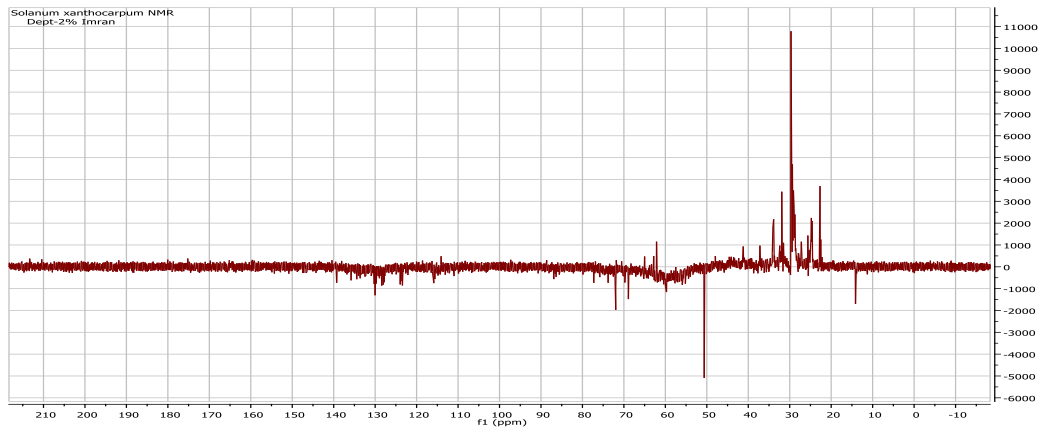
**Figure 10.** FTIR spectra of methanolic fraction of *S. xanthocarpum* (SX2).



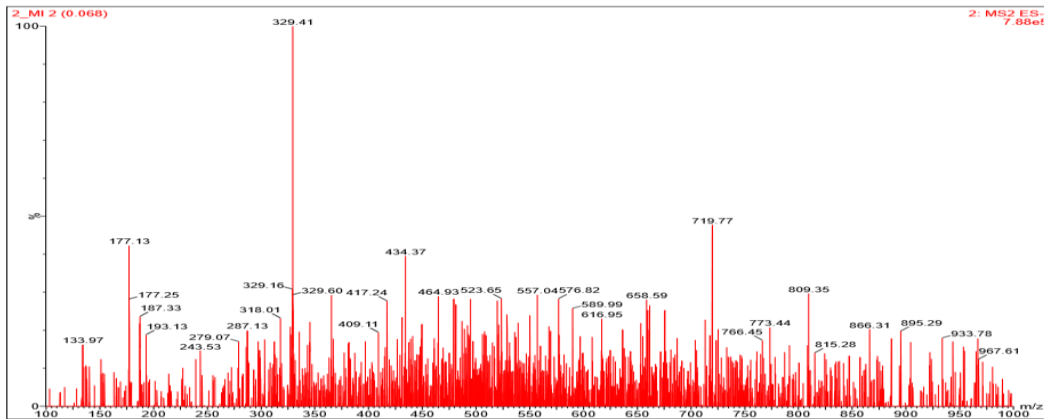
**Figure 11.** <sup>1</sup>H NMR spectra of methanolic fraction of *S. xanthocarpum* (SX2).



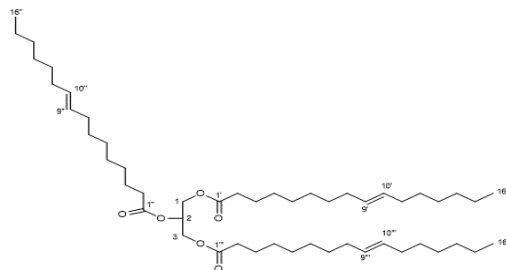
**Figure 12.** <sup>13</sup>C spectra of a methanolic fraction of *S. xanthocarpum* (SX2).



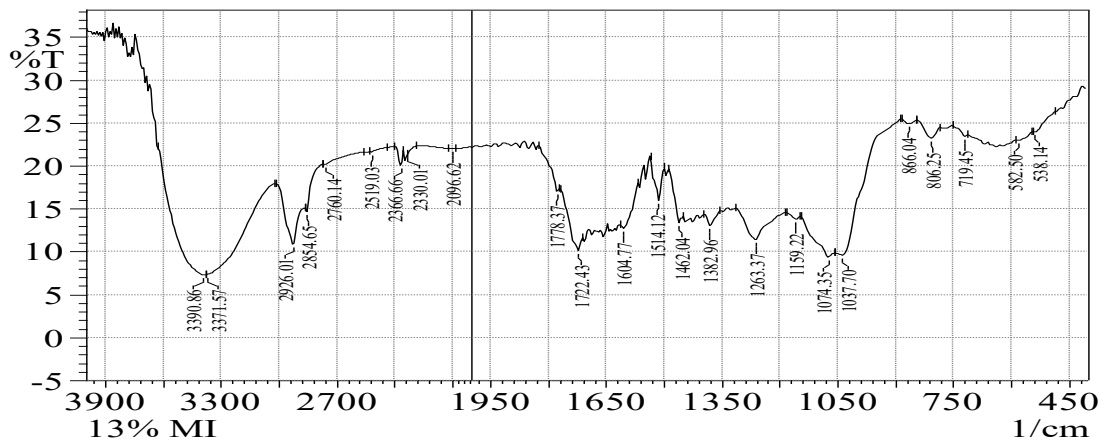
**Figure 13.** DEPT spectra of a methanolic fraction of *S. xanthocarpum* (SX2).



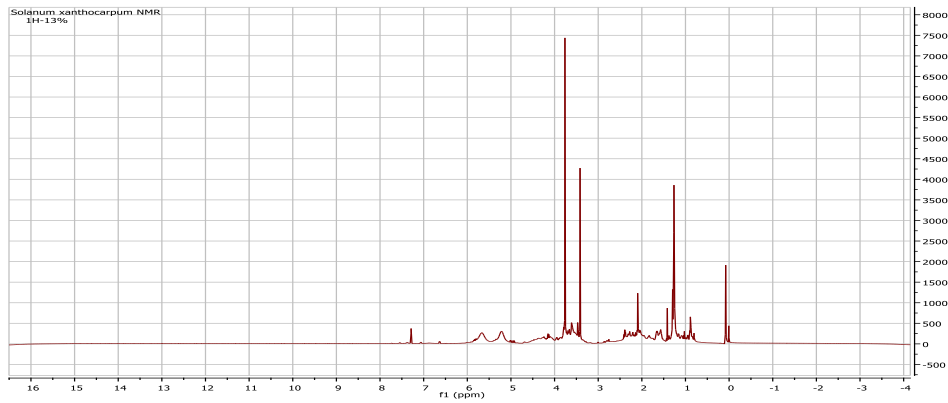
**Figure 14.** MASS spectra of a methanolic fraction of *S. xanthocarpum* (SX2).



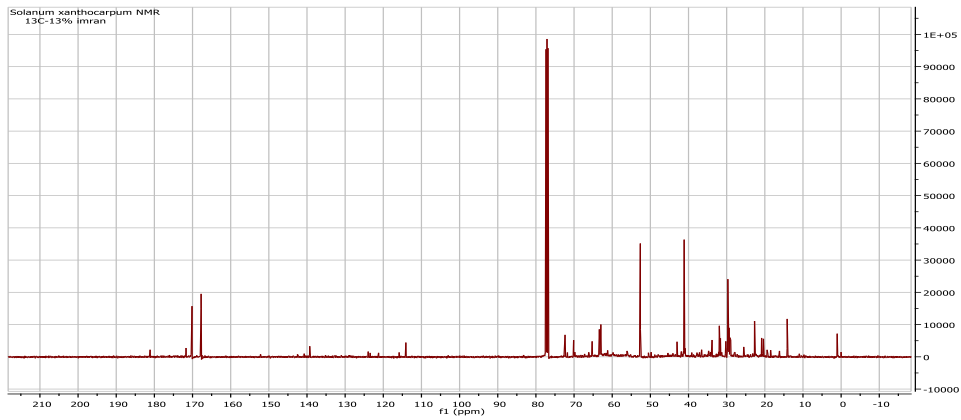
**Figure 15.** Structure of glyceryltripalmitoleate (SX3).



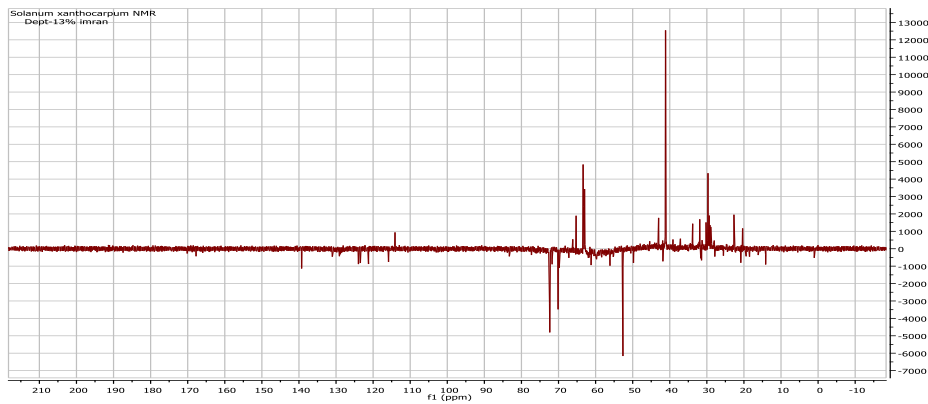
**Figure 16.** FTIR spectra of a methanolic fraction of *S. xanthocarpum* (SX3).



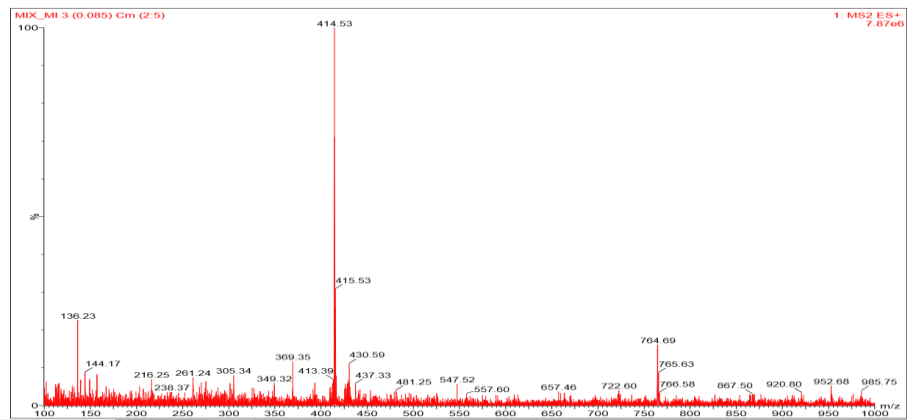
**Figure 17.** 1H NMR spectra of methanolic fraction of *S. xanthocarpum* (SX3)



**Figure 18.** 13C NMR spectra of methanolic fraction of *S. xanthocarpum* (SX3)



**Figure 19.** DEPT spectra of methanolic fraction of *S. xanthocarpum* (SX3)



**Figure 20.** Mass spectra of a methanolic fraction of *S. xanthocarpum* (SX3)

through the kidneys and intestines. [30] As a consequence of oxidative stress and xanthine oxidase overactivity, health disorders such as rheumatoid or acute arthritis or gout may emerge, as they provide key features of vascular diseases. [31-33] Research studies have previously indicated the link between vascular diseases of the heart, hypertension, and diabetes with uric acid [34-37]. Xanthine oxidase inhibitors are therefore very essential in the treatment of such diseases as it decreases circulatory uric acid levels along with oxidative vascular stress [38]. Febuxostat, Allopurinol, and Oxypurinol are three among the most widely used xanthine oxidase inhibitors. [39] They act by preventing uric acid synthesis through purines and are observed to lower their production. These inhibitors also tend to increase uric acid secretion [40, 41].

*S. xanthocarpum* was described as constringent, bitter, pungent, and digestive in historical Ayurvedic texts, although it has been shown to have pharmacological properties [12]. Various studies have been published consisting of new and unique ways of using this herb. It has been observed that fruits of *S. xanthocarpum* have more production of alkaloids compared to other parts of this species. [42] The biological properties and composition of chemical components of this herb were often studied but literature studies of *S. xanthocarpum* and its properties in producing inhibition to xanthine oxidase are not yet addressed. To bridge the gap between the existing treatment options and new findings on xanthine oxidase inhibitors, this study provides an addition to further knowledge.

Our research study is the first of its kind that has shed light on three new phytoactive compounds from *S. xanthocarpum*. Fatty acid compounds Eicosa-11, 14-dienoic acid (SX1), Dodecapentenoic acid (SX2), and Glyceryltripalmitoleate (SX3) were identified in our study. These novel compounds exhibit xanthine oxidase inhibitory activity. Chromatographic profiles were developed for its extract and fractions. The chromatographic profiles of *S. xanthocarpum* extracts and their fractions were also developed. When multiple dilutions were evaluated for xanthine oxidase inhibitory activity, ethyl acetate and methanol fractions were observed to be most potent. As a result, it might be developed to protect the kidneys and treat gout. Fatty acid components of *S. xanthocarpum* were isolated and explored as a consequence of our efforts. Our findings support its usage in renal protection and gout. The exploration of phytochemical compounds is an important step in the development of new strategic therapy and treatments, these compounds can also be studied in other areas of research.

## Conclusion

New applications for prophylaxis, diagnosis, and treatment of various diseases or conditions may be explored in context with natural compounds obtained from plants and herbs. These compounds exhibit biological and pharmacological activities that are the potential in creating new research opportunities. *S. xanthocarpum* had been considered for similar reasons as its widespread pharmacological actions provide new ideas in determining new uses. Our study provides evident results of fatty acid compounds that were derived from *S. xanthocarpum* for its new properties of inhibition of xanthine oxidase. These findings contribute additional value to *S. xanthocarpum* and its biologically active compounds for pharmacological and potential therapeutic uses.

## Contribution of authors

Mohd. Imran conducted the study and produced results along with the help of other authors; Haya Majid., Writing - original draft; Mohammad Ali Khan Data curation (Equal) Methodology (Equal); Abdul Qadir, Writing - review & editing; Proofreading the article; All authors have read and agreed to the published of the manuscript.

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## Conflict of interest

There are no conflicts to declare.

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