



(RESEARCH ARTICLE)



## Phytochemistry, GS-MS analysis, and heavy metals composition of aqueous and ethanol stem bark extracts of *Ximenia americana*

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### Abstract

Medicinal plants employed in the management of diseases has been attributed to their phytochemical compositions. The present study aimed to investigate the phytochemicals, bioactive, and heavy metals components present in aqueous and ethanol stem bark extracts of *Ximenia americana*. The phytochemical compositions were qualitatively, and quantitatively determined, followed by the identification of bioactive compounds present. The heavy metals composition was also determined. The result revealed the presence of saponins in the aqueous (30.67% ±0.39), and ethanol (19.67% ±0.78) extract. However, alkaloids (14.61% ±0.46) were detected only in the aqueous extract while steroids (7.00% ±1.16), and glycosides (0.38% ±0.03) were in the ethanol extract only. A total of 17 and 26 compounds were identified in the aqueous and ethanol extract respectively. Chromium and lead had a concentration of 0.184 ppm ±0.080 and 0.886 ppm ±0.210 respectively in the aqueous and ethanol extract. Cadmium had a concentration of 0.001 ppm ±0.000 in both aqueous and ethanol extract. Conclusively, *X. americana* contains bioactive components that could be utilized in the production of novel drugs by isolation of these bioactive compounds.

**Keywords:** GC-MS; Heavy metals; Phytochemical composition; *Ximenia americana*

### 1. Introduction

Medicinal plants are made up of components that can be applied as a whole or part for management of ailments or utilized as a precursor for the manufacture of therapeutics. The application of plants for various purposes has been for millennia by all populations globally typically as a medicinal purpose in folkloric medicine [1]. They are the basis of many modern medicines, nutraceuticals, food supplements, and pharmaceuticals. In previous studies on the efficacy of medicinal plants subsequently opened door to the manufacture of plant-based medicines worldwide [2]. Herbal medicine forms an important aspect of traditional folkloric medicine involving the application of plants or their parts (leaves, roots, flowers, stem, seeds) in the form of crude drugs such as powder, decoction, tincture, poultice, and other herbal preparations for the treatment of various ailments [3]. Different plants are used as herbs in ethnomedicine for the management of different ailments worldwide including *Ximenia americana*.

*X. americana* is a medicinal indigenous to Nigeria and West African where the plant parts are prepared in different forms targeting different ailments in ethnomedicine, notably in rural areas [4]. In another study, *X. americana* was reported to serve purposes including food, medicine, and also utilized industrially as a raw material [5]. Different studies have reported extracts of *X. americana* from different solvents possess hepatoprotective and hypoglycemic [6], gastroprotective [7], anti-inflammatory, and antioxidant [8] and aphrodisiac [9] effects. Several studies showed that *X. americana* was used for inflammations in general for healing, urinary tract infection, diarrhea, anti-parasitic, mental illness, leprotic ulcers, antiseptic, diuretic, ovarian and prostatic inflammations, pains, bloodshed, itching, burning, gastritis, fracture, inflammation, analgesic, antipyretic, cancer, hepatoprotective, ulcers, skin infections, purgative

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backache, hemorrhage, rash, toothache, and menstrual colic [10]. Bark and leaves prepared in different forms such as infusion, decoction, tincture, and syrup, are used in ethnomedicine [11]. Other investigations revealed showed that the components of *X. americana* are associated with several pharmacological activities against fungi, cancer, oxidative stress and also utilized as a pesticide, and analgesic [12].

Different phytochemicals were reported to be found in *X. americana* attributed to the pharmacological effects of the plant, making it an important source of bioactive constituents that can be used to develop novel agents [13]. Thus, the present study aimed to investigate the phytochemicals, bioactive, and heavy metals components present in aqueous and ethanol stem bark extracts of *X. Americana*.

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## 2. Material and methods

### 2.1. Materials

#### 2.1.1. Plant material

*Ximenia americana* was collected from Girei Local Government Area of Adamawa state, Nigeria. The stem bark was dried and ground into powder.

#### 2.1.2. Chemicals and reagents

All other chemicals and reagents were of Anarlar.

#### 2.1.3. Equipments

Oven: Uniscope SM9053, Water bath: Jinyi HH-S6, Gas chromatography-mass spectrophotometer: Agilent 19091-433HP, Atomic absorption spectrophotometer: Buck Scientific AAS210.

### 2.2. Methods

#### 2.2.1. Extract preparation

*X. americana* bark powder (500 g) was macerated with 1.5 L of distilled water, and 1.5 L of ethanol in a glass jar for 2 days at room temperature. The extract was filtered and concentrated to dryness at 40 °C under reduced pressure [14].

#### 2.2.2. Qualitative phytochemical Analysis

The presence of alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids was detected according to the methods previously described [14].

#### 2.2.3. Quantitative phytochemical Analysis

The phytochemicals present in aqueous (AQ) and ethanol (ET) stem bark extracts of *X. americana* were quantified by the following procedures.

#### 2.2.4. Total alkaloids content

Total Alkaloids were quantified by the gravimetric method described previously [15].

#### 2.2.5. Total saponins content

Total Saponins quantitation was carried out according to a previously described method [16].

#### 2.2.6. Total steroids content

The steroid content was quantified by the method described previously [15].

#### 2.2.7. Total glycosides content

Total Glycosides were quantified according to method described previously [17].

### 2.2.8. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out with a combination of a Gas chromatography-mass spectrophotometer (Agilent 19091-433HP, USA). The system was fitted fused with a silica column. A column flow velocity of 1.6 mL/min was set for the carrier gas (Helium). Ion-source temperature was set to 250°C while the pressure was 8.6 psi. A split mode injection (1 µL) at 250°C was used. The initial temperature of the column was set at 100°C, and gradually increased to 180°C at 20°C/min, then 10°C/min to 280°C. The total elution time was 16 min. The National Institute of Standards and Technology (NIST) database was used for the identification and comparison of the unknown spectrum of the detected compounds with that of known standards.

### 2.2.9. Determination of Mineral Composition

A gram of the samples was ashed at 500°C for 1 h, which was dissolved in 25 mL of 10% HCl and made up to 100 mL. Chromium (Cr), cadmium (Cd), and lead (Pb) contents were quantified by the method previously described [18] using Atomic Absorption Spectrophotometer (AAS) (Buck Scientific AAS210).

### 2.3. Statistical Analysis

Data obtained in the present study were expressed as mean  $\pm$  standard error of triplicate determinations' mean ( $\pm$  SEM) evaluated with Statistical Package for the Social Sciences (SPSS) version 22 Software.

## 3. Results and discussion

The phytochemicals present in AQ and ET extracts of stem bark of *X. americana* are shown in Table 1. Alkaloids were detected in the AQ extract though absent in the ET extract. Saponins were detected in both the AQ extract and ET extracts. Steroids and glycosides were absent in the AQ extract but detected in the ET extract. However, terpenoids and flavonoids were absent in both extracts of stem bark of *X. americana*.

Phytochemical screening of *X. americana* identified of different phytochemicals attributed for the biological activity of the plant. Although some of these phytochemicals were not detected in the AQ extract, their detection in the ET extract might be due to a difference in solvent polarity employed for the extraction [19].

**Table 1** Qualitative determination of the phytochemical composition of AQ and ET extracts of *X. americana*

Phytochemical	Aqueous extract	Ethanol extract
Alkaloids	++	-
Saponins	+++	++
Steroids	-	+
Glycosides	-	+
Terpenoids	-	-
Flavonoids	-	-

+ = Present, - = Absent.

Phytochemicals are utilized by plants for protection against pathogens of different kinds or insects through different mechanisms of action which include damage to membranes and vital metabolic enzymes [20]. Alkaloids have been implicated in many studies to document their application in traditional medicine as they are attributed to antibacterial, antiviral, anticancer, antifungal, and antimalarial activities [21]. In a previous study, alkaloids were reported to be utilized in traditional medicine attributed to their pharmacological effects [20]. In another study, they were reported to possess antimicrobial properties and can intercalate with microbial DNA [22]. The alkaloids detected in the present study might be responsible for the antimicrobial effect of *X. americana*. Saponins were also reported to exert antimicrobial effects and also active against some biological disorders [23]. Saponins exerts several pharmacological effects on cancer, virus, bacteria and fungi, which is attributed to their amphiphilic properties [24]. Saponins are associated with beneficial effects on blood lipids, lowering risk for cancer, and improving glycemic response as well as possessing antioxidant effects [25].

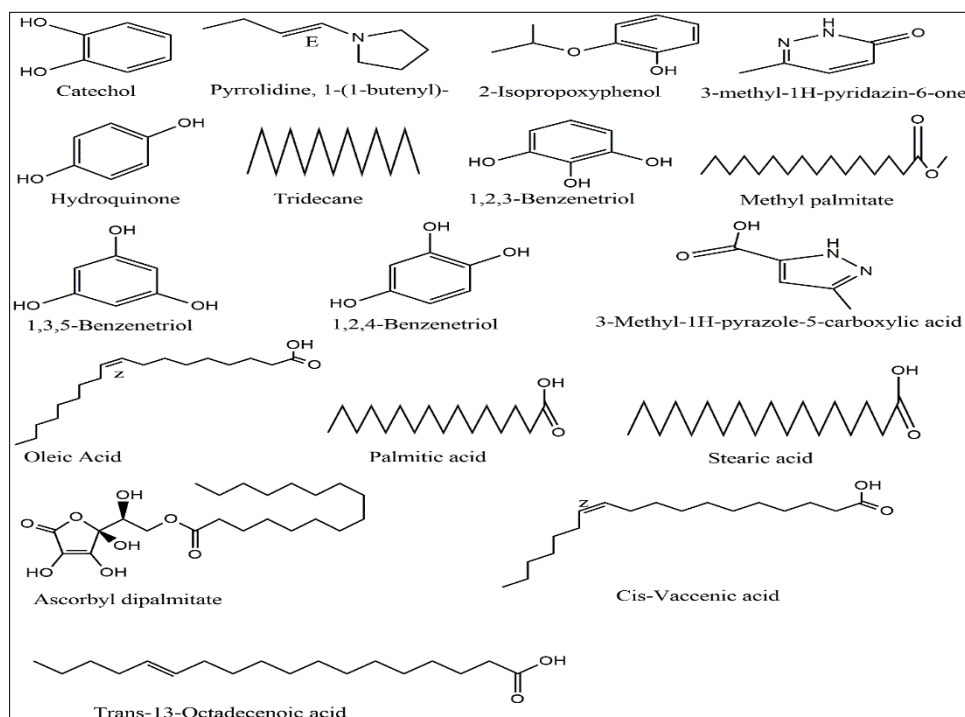
The quantitative phytochemical composition of AQ and ET extracts of stem bark of *X. americana* is presented in Table 2. Alkaloids were quantified up to 14.61%  $\pm$ 0.46, while saponins were in higher (30.67%  $\pm$ 0.39) concentration in the AQ extract but 19.67%  $\pm$ 0.78 in the ET extract. Steroids and glycosides which were present in the ET extract only had a concentration of 7.00%  $\pm$ 1.16 and 0.38%  $\pm$ 0.03 respectively.

**Table 2** Quantitative determination of the phytochemical composition of AQ and ET extracts of *X. americana*

Phytochemical	Concentration (%)	
	Aqueous extract	Ethanol extract
Alkaloids	14.61 $\pm$ 0.46	-
Saponins	30.67 $\pm$ 0.39	19.67 $\pm$ 0.78
Steroids	-	7.00 $\pm$ 1.16
Glycosides	-	0.38 $\pm$ 0.03
Terpenoids	-	-
Flavonoids	-	-

Concentration values are in triplicates determinations ( $\pm$  SEM).

In a similar study, alkaloids and saponins were detected while steroids were absent in aqueous and ethanol stem bark extract of *X. americana*. However, glycosides were detected in the ET extract, though absent in the AQ extract [26]. The present study agrees with this study as glycosides were detected only in the ET extract. In a previous study [27], glycosides and saponins were detected in the AQ extract of *X. americana* with alkaloids being absent, which disagrees with the present study. The present study also agrees with the result reported previously [28] for the detection of saponins and alkaloids in the AQ extract of *X. americana*, where glycosides were absent. In another study to determine the phytochemical composition of alcohol (methanol) extract *X. americana*, saponins were detected up to 50.86% while steroids were detected up to 28.41% with the absence of alkaloids and flavonoids [29]. The present study agrees with this result for the detection of saponins (19.67%  $\pm$ 0.78) and steroids (7.00%  $\pm$ 1.16) in alcohol. The difference in the quantity of the phytochemicals detected might be attributed to the slight difference in the polarity of ethanol and methanol, though both are alcohols [19].



**Figure 1** Structures of compounds identified in the AQ extract of *X. americana*

The various bioactive compounds identified in the AQ extract of stem bark of *X. americana* with their retention time, peak area, molecular weight, and formula are shown in Table 3. A total of 17 compounds were identified in the AQ stem bark extract *X. americana*. Figure 1 shows the various molecular structures of the compounds identified in the AQ extract where most of the compounds identified were aromatic and aliphatic compounds compose of benzene rings and long chain fatty acids respectively. The chromatogram of the GCMS analysis is also shown in Figure 2, displaying the various peaks at different retention times.

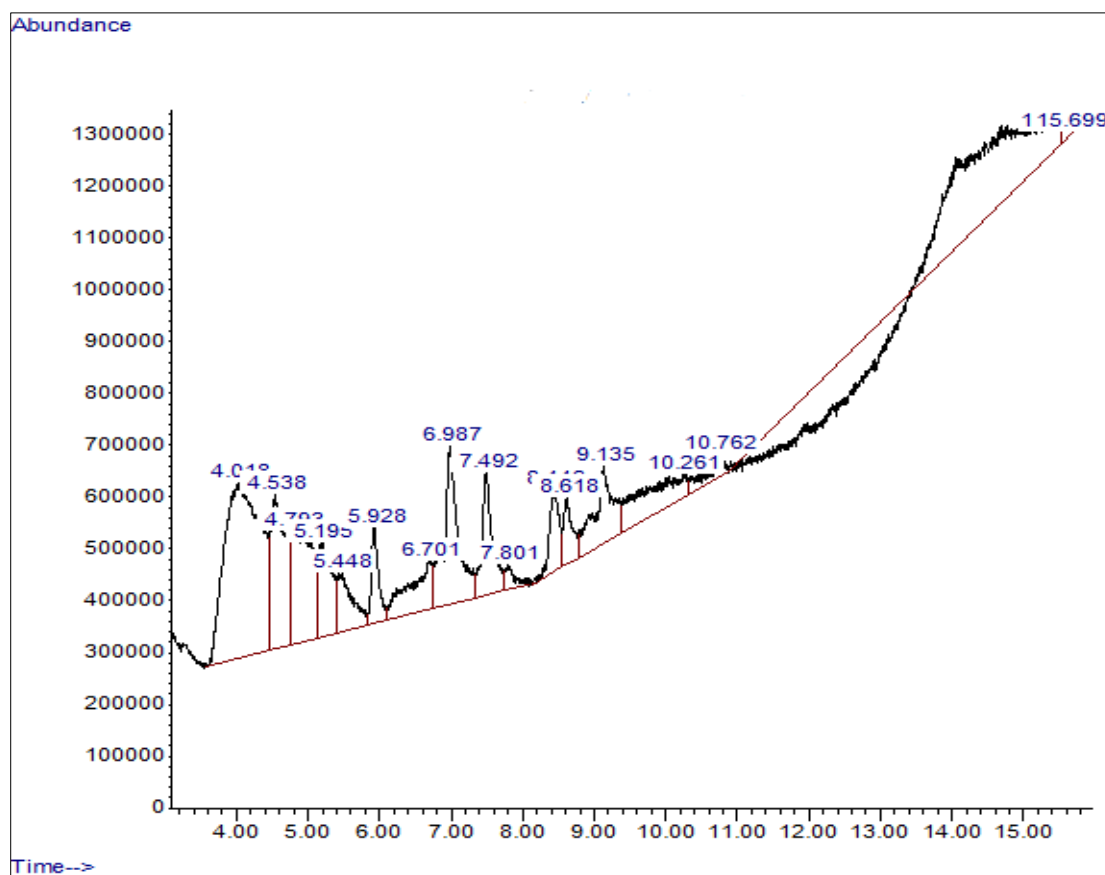
**Table 3** Bioactive compounds identified in the AQ extract of *X. americana*

S/N	Name of compound	Retention Time	Peak Area (%)	Molecular weight	Formular
1	Catechol	4.019	24.59	110.11244	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>
2	Pyrrolidine, 1-(1-butenyl)-	4.540	8.84	125.2138	C <sub>8</sub> H <sub>15</sub> N
3	2-Isopropoxyphenol	4.792	9.48	152.19308	C <sub>9</sub> H <sub>12</sub> O <sub>2</sub>
4	3-methyl-1H-pyridazin-6-one	5.192	4.84	110.11544	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O
5	Hydroquinone	5.450	3.33	110.11244	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>
6	Tridecane	5.925	2.69	184.36532	C <sub>13</sub> H <sub>28</sub>
7	1,2,3-Benzenetriol	6.703	4.49	126.11184	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
8	Methyl palmitate	6.989	9.03	270.45576	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
9	1,3,5-Benzenetriol	7.493	4.95	126.11184	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
10	1,2,4-Benzenetriol	7.802	0.83	126.11184	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
11	3-Methyl-1H-pyrazole-5-carboxylic acid	8.443	2.92	126.11484	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>
12	Oleic Acid	8.620	2.25	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
13	Palmitic acid	9.135	5.52	256.42888	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
14	Stearic acid	10.262	5.60	284.48264	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
15	Ascorbyl dipalmitate	10.760	1.46	652.95312	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>
16	cis-Vaccenic acid	15.463	8.45	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
17	Trans-13-Octadecenoic acid	15.698	1.08	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>

The bioactive compounds detected in the ET of *X. americana* including their retention time, peak area, molecular weight, and formula are shown in Table 4. A total of 26 compounds were identified is thought to contribute to the various phytochemical activities associated with the plant extract

Figure 3 shows the various molecular structures of the compounds identified in the ethanol extract of stem bark of *X. americana*. The majority of the compounds detected were aliphatic compounds composed of long-chain fatty acids. The chromatogram of the GCMS analysis is also shown in Figure 4, displaying the various peaks of the sample at different retention times.

The GSMS analysis showed that much extraction of *X. americana* was achieved with ethanol than with the aqueous solvent. This might be attributed to the polarity of the solvents used during extraction as alcohol has lower polarity compared to water [30]. Thus, more bioactive compounds were extracted from the ET extract than from the AQ extract. Catechol is the bioactive compound detected higher than the other compounds in the AQ extract of *X. americana* with a peak area of 24.59%. 2-Isopropoxyphenol was also detected with a peak area of 9.48% while the dominant fatty acid methyl palmitate had a peak area of 9.03% in the aqueous extract. Pyrrolidine, 1-(1-butenyl)-, and cis-vaccenic acid had peak areas of 8.84% and 8.45% respectively. In the ethanol extract, stigmasterol and octadecanal had the highest peak areas of 48.01% and 11.11% respectively. Tetradec-13-enal and catechol had peak areas of 6.32% and 4.32% in the ethanol extract.



**Figure 2** GS-MS Chromatogram of compounds identified in AQ extract of *X. americana*

Catechol was reported to possess antimicrobial activity against *Staphylococcus epidermidis* [31]. Catechol exerts anticancer effects against pancreatic cancer cells through suppression of epithelial-mesenchymal transition proteins and enhancing the chemo- and radio-sensitivity of the cells [32]. Catechol also prevents cellular proliferation, and promoted apoptosis in human pancreatic cancer cells in a dose dependent manner [32]. Methyl palmitate (MP) detected in the AQ extract was previously reported to exert pharmacological activities against oxidative stress, alpha reductase enzyme, and hypercholesterolemia [33]. MP was reported as an inflammatory cell inhibitor by decreasing the plasma levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) [34]. MP was reported to act as a neuromodulator by activating the protein kinase C pathway subsequently leading to decrease in blood flow in brainstem partly attributed to the vasorelaxative effect of MP [35]. Stigmasterol was previously reported to exert anti-inflammatory effect by stabilizing the membranes of human red blood cells by lowering hemolysis with amplification of the membrane stability in a dose-dependent manner [36]. In another study, administration of stigmasterol to streptozotocin-induced diabetic rats led to a decrease in the levels of blood glucose urea, and creatinine, demonstrating its antidiabetic effects [37].

GCMS analysis also indicated saturated (palmitic acid) and unsaturated (oleic acid) are present in both the AQ and ET extract of *X. americana*. These unsaturated (oleic) and saturated (palmitic acid) fatty acids were previously reported in a similar study on alcohol extract of *X. americana* [26]. In a previous study for GCMS analysis of *X. americana* in different solvent fractions, oleic acid and palmitic acid were detected in the highest amount in the chloroform fraction [38]. Palmitic acid was previously reported to possess pharmacological effect against oxidative stress, inflammation hypercholesterolemia, and cancer [39].

Table 5 shows the various concentrations of heavy metals in both aqueous and ethanol of *X. americana*. Chromium was observed in a higher concentration than all the other heavy metals in both the aqueous ( $0.184 \pm$ ) and ethanol ( $0.886 \pm$ ) extracts of *X. americana*. Cadmium was detected in the least concentration ( $0.001 \pm$ ), followed by lead with aqueous and ethanol concentrations of  $0.100 \pm$  and  $0.076 \pm$  respectively.

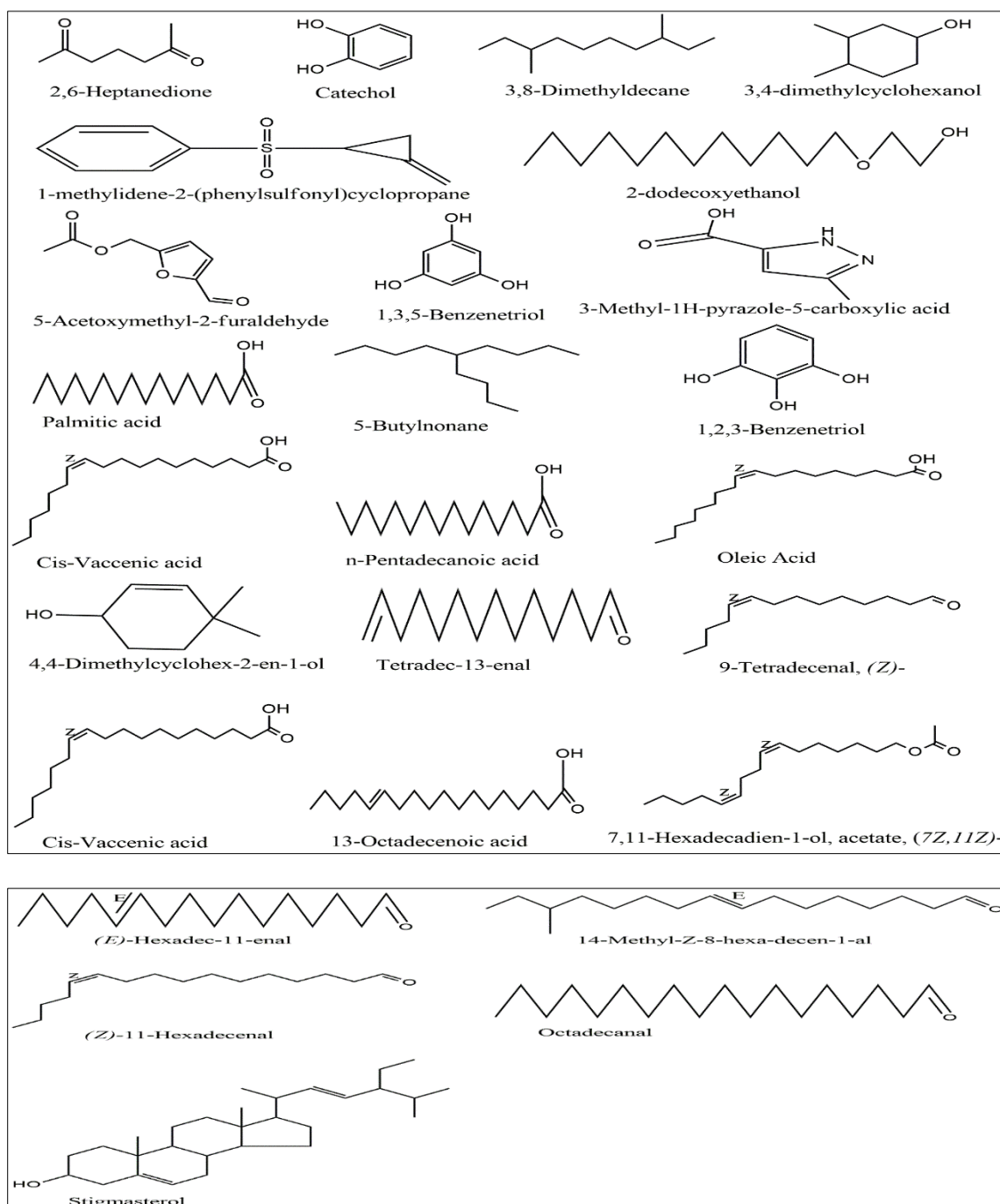
**Table 4** Bioactive compounds identified in ET extract of *X. americana*

S/N	Name of compound	Retention Time	Peak Area (%)	Molecular weight	Formular
1	2,6-Heptanedione	3.281	0.22	128.17108	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>
2	Catechol	4.019	4.32	110.11244	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>
3	3,8-Dimethyldecane	4.523	2.38	170.33844	C <sub>12</sub> H <sub>26</sub>
4	1-methylidene-2 (phenylsulfonyl)cyclopropane	4.815	1.87	106.16	C <sub>8</sub> H <sub>10</sub>
5	3,4-dimethylcyclohexanol	5.181	1.58	128.21444	C <sub>8</sub> H <sub>16</sub> O
6	2-dodecoxyethanol	5.913	1.47	230.391	C <sub>14</sub> H <sub>30</sub> O <sub>2</sub>
7	5-Acetoxymethyl-2-furaldehyde	6.222	0.09	168.14912	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>
8	1,3,5-Benzenetriol	6.686	0.23	126.11184	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
9	3-Methyl-1H-pyrazole-5-carboxylic acid	6.823	0.16	126.11484	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>
10	Palmitic acid	6.978	1.11	270.45576	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
11	5-Butylnonane	7.487	1.81	184.36532	C <sub>13</sub> H <sub>28</sub>
12	1,2,3-Benzenetriol	7.796	0.12	126.11184	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
13	cis-Vaccenic acid	8.431	1.50	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
14	n-Pentadecanoic acid	8.608	1.21	242.402	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>
15	Oleic Acid	8.929	2.07	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
16	4,4-Dimethylcyclohex-2-en-1-ol	9.129	2.40	126.19856	C <sub>8</sub> H <sub>14</sub> O
17	Tetradec-13-enal	10.039	6.23	210.35984	C <sub>14</sub> H <sub>26</sub> O
18	9-Tetradecenal, (Z)-	10.251	1.84	210.35984	C <sub>14</sub> H <sub>26</sub> O
19	cis-Vaccenic acid	10.525	1.08	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
20	13-Octadecenoic acid	10.748	3.09	282.46676	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
21	7,11-Hexadecadien-1-ol, acetate, (7Z,11Z)-	11.160	1.17	280.45088	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
22	(E)-Hexadec-11-enal	11.687	2.23	238.4136	C <sub>16</sub> H <sub>30</sub> O
23	14-Methyl-Z-8-hexa-decen-1-al	11.927	1.30	252.44048	C <sub>17</sub> H <sub>32</sub> O
24	(Z)-11-Hexadecenal	12.311	1.38	238.4136	C <sub>16</sub> H <sub>30</sub> O
25	Stigmasterol	14.519	48.01	412.69952	C <sub>29</sub> H <sub>48</sub> O
26	Octadecanal	15.555	11.11	268.48324	C <sub>18</sub> H <sub>36</sub> O

**Table 5** Heavy metals composition of AQ and ET extracts of *X. americana*

Heavy metal	Concentration (ppm)	
	Aqueous extract	Ethanol extract
Chromium (Cr)	0.184 ±0.080	0.886 ±0.210
Cadmium (Cd)	0.001 ±0.000	0.001 ±0.000
Lead (Pb)	0.100 ±0.020	0.076±0.008

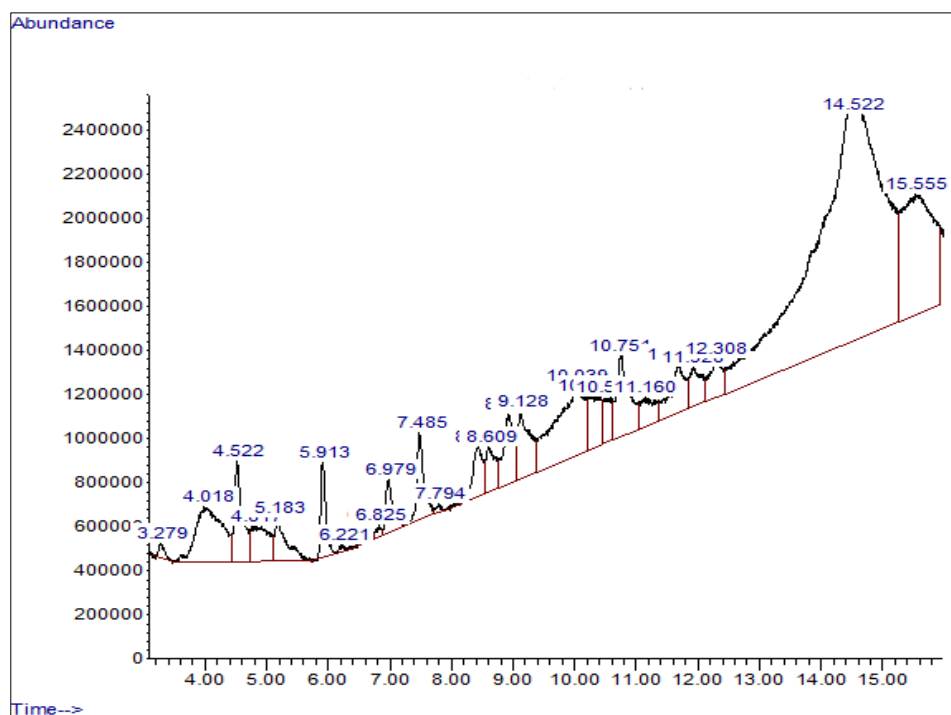
Concentration values are in triplicates determinations (± SEM).



**Figure 3** Structures of compounds identified in ET extract of *X. Americana*

Heavy metals are associated with functional roles vital for the normal functions of the cell. However, their presence in higher doses can cause harm to the body as they interfere with various processes and some including cadmium, chromium, and lead can be fatal. Some heavy metals are carcinogenic acting by interfering with the activities of proteins involved in signaling and regulation of DNA repair, methylation, and cell growth and differentiation processes [40]. Thus, continuous exposure might lead to carcinogenesis. Chromium is a raw material for industries contributing to environmental pollution which has adverse effects on biological and ecological species [41]. Other activities such as sewage disposal and fertilizers use may subsequently lead to the release of chromium into the environment [41]. Thus, chromium is released into the environment and subsequently absorbed by plants. Exposure to chromium, notably through inhalation can lead to irritation of the lining of the nose and nose ulcers. Other effects include anemia, irritations and ulcers of the small intestine and stomach, and damage to male reproductive functions. Exposure to high doses of chromium or its compounds might cause adverse cardiovascular, respiratory, hematological, gastrointestinal, renal, hepatic, and neurological effects and fatal [42].





**Figure 4** GS-MS Chromatogram of compounds identified in ET extract of *X. americana*

Like other heavy metals, cadmium is also disposed into the environment by industrial processes and human activities. Cadmium can remain in soils for a long time and end up being absorbed by plants which are subsequently ingested by humans. Exposure to cadmium by inhalation might lead to adverse damage to the lungs and respiratory irritation while exposure to higher dose might lead to gut upset which subsequently result in vomiting and diarrhea. Continuous exposure for a longer time leads to its deposit in bones and lungs causing damage [43]. Lead exposure is through industrial processes and from vehicles and subsequently ending up in the food chain in water or plants [44]. Exposure to lead may lead to brain damage with memory and learning process being the target and also lead to neurological toxicity [45]. Exposure to lead to damage to the kidney, nerves, muscles, and brain [46].

The regulatory accepted concentration of Cr, Cd, and Pb, in plants, are 1.30, 0.02, and 2 ppm respectively [47]. In the present study, Cr was below the accepted concentration in both aqueous (0.184 ppm  $\pm$ 0.080) and ethanol (0.886 ppm  $\pm$ 0.210) extract. Pb was observed to be below the regulatory limit for both aqueous (0.100 ppm  $\pm$ 0.020) and ethanol (0.076 ppm  $\pm$ 0.008) extract. The Cd level was also below the regulatory limit in both aqueous (0.001 ppm  $\pm$ 0.000) and ethanol (0.001 ppm  $\pm$ 0.000) extract. The detection of Cr and Pb in the sample might be due to the plant sample collection location being close to rivers which might be carrying these metals and easily dumping them in the surrounding, subsequently being absorbed by plants [44].

#### 4. Conclusion

This research established preliminary information on the phytochemistry and heavy metals composition of aqueous and ethanol extracts of *X. americana*. Consequently, 17 and 26 compounds were identified in the aqueous and ethanol extract respectively. The presence of these bioactive compounds provides credence for the use of the plant as a therapeutic in various forms of preparation in traditional folkloric medicine. Thus, *X. americana* contains bioactive components that could be utilized in the production of novel drugs by isolation of these bioactive compounds.

#### Compliance with ethical standards

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*Disclosure of conflict of interest*

Authors declare no conflict of interest.

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