

## UPLC-ESI-MS\MS Phytochemicals Profiling of n-Butanol Fraction of *Xanthium strumarium* Fruit Extract

Mohammed A. Ezghayer<sup>1\*</sup>, Omar Hussein Ahmed<sup>2</sup> and Mostafa F. Tawfeeq<sup>3</sup>

<sup>1</sup>Pharmacognosy Department, faculty of pharmacy, Tikrit university, Tikrit, Iraq.

<sup>2</sup>Pharmaceutical chemistry Department, faculty of pharmacy, Tikrit university, Tikrit, Iraq.

\*Corresponding Author E-mail: moh.cognosy@tu.edu.iq

<https://dx.doi.org/10.13005/bpj/2920>

(Received: 16 February 2024; accepted: 19 March 2024)

The work aimed to explore the phytochemicals of various fractions of *Xanthium strumarium* fruit extract since this plant is found to be toxic for both humans and animals in Iraq. The extracts underwent phytochemical screening, which indicated the occurrence of flavonoids, alkaloids, phenols, coumarin, saponin, tannins, and terpenoids. After extraction, fractionation was done using hexane, chloroform, and N-butanol respectively, for identifying the phytochemicals found in each fraction, UPLC-electrospray ionization–tandem mass spectroscopy was used which revealed the presence of 6 sterol and terpenes compound in hexane fraction, Nine compounds were detected in the chloroform fraction, among which 2-acetyl-atractyligenin and Artemisinin were the primary components. Additionally, this particular plant has been found to contain these compounds for the first time. Furthermore, Pungiolide C, dihydroartemisinin, and atractylenolide II were also detected for the first time in this plant. For the n-Butanol fraction, eight diterpene glycosides were recognized, with Atractyloside and Carboxyatractyloside and their desulphated derivatives as the major compounds responsible for the plant's toxicity. The butanol fraction also showed the presence of eight phenolic compounds, among which caffeoylquinic acid derivatives and flavonoid Syringetin were the major compounds in this fraction.

**Keywords:** Artemisinin; Carboxyatractyloside; UPLC-ESI-MS/MS; *Xanthium strumarium*.

*Xanthium strumarium* L. is categorized as a type of weed which falls under the Asteraceae family. It is often encountered and seen commonly in roadside ditches, hedges, and rice fields.<sup>1</sup> The plant's aerial parts, which refer to the portions above the ground, are believed to contain different types of harmful alkaloids that haven't been identified yet. These aerial parts also contain sesquiterpene lactones like xanthinin, xanthumin, and xanthatin (deacetyl-xanthinin), as well as toxic principles such as a sulphated glycoside called xanthostrumarin, atractyloside, and carboxyatractyloside, and phytosterols such as xanthanol<sup>2</sup>. Caffeoylquinic

acids; and tocopherol.<sup>3</sup> Carboxyatractyloside (CAT), a kaurene glycoside formerly known as xanthostrumarium, has been discovered as the principal poisonous component extracted from the plant<sup>4</sup>.

CAT is water soluble, but it cannot be boiled away (decocted), and it is not simply rinsed away. Instead, eliminating the prickles—which can be partially done by stir-frying alone—is the greatest strategy to eliminate the poisonous component.<sup>5</sup> *Xanthium* fruits predominantly contain a group of derivatives known as caffeoylquinic acid, which belong to the phenolic acid class. The therapeutic

applications of a fruit may depend on the type and level of caffeoylquinic acid derivatives present in it. Consequently, it is essential to verify the chemical composition and accurately identify each caffeoylquinic acid to evaluate the overall quality. The aforementioned factors are critical in determining the utility of *Xanthium* fruits for their medicinal properties<sup>6</sup>.

The aim of the study was to employ ultra-performance liquid chromatography equipped with electrospray ionization (ESI) tandem mass spectrometry, for the characterization of the toxic phytochemicals and chemical constituents present in the fruits of *X. strumarium* that grow naturally in Iraq.

## MATERIALS AND METHODS

### Collection of the plant material

Fruits of *Xanthium strumarium* were gained from Sinjar city, located near Mosul in Iraq, and were authenticated at Tikrit University's College of Agriculture. Following cleaning, the fruits were dried at room temperature in a shaded area. They were then crushed into a powder and preserved for later use.

### Method of Work

A quantity of 100 grams of fruit powder that has been air-dried is measured. Petroleum ether is used to defat the powder by overnight maceration to remove any waxy material and chlorophyll. The powder is then extracted for six hours using the Soxhlet, and the solvent used is (600 ml) 90% ethanol. The rotary evaporator is utilized to dry the extract. After the drying process, the weight of the extract is measured, and it is determined that the extraction yield is 31 grams. The extract is then dissolved in 100 milliliters of distilled water. Using a separatory funnel, the upper layers are partitioned with 100 milliliters of n-hexane three times, and then mixed and dried using rotary evaporators. To obtain a chloroform fraction, the remaining aqueous portion is partitioned with 100 ml of chloroform for three successive times, the lower chloroform layers are combined and dried. To obtain the butanol fraction, the aqueous portion from the previous step is partitioned with butanol three successive times then combined and dried by using a rotary evaporator.

Several qualitative phytochemical screening tests were conducted. These tests offer

crucial information about the kinds of secondary metabolites present in the plant and help design an appropriate method for the isolation of these metabolites to determine the chemical composition of the crude extract. Crude fruit extracts were subjected to a preliminary phytochemical analysis, which identified the presence of flavonoids, alkaloids, phenols, coumarin, saponin, tannins, and terpenoids.

### Method for UPLC

UPLC-ESI-MS/MS technique was employed in the experiment using a triple quadrupole instrument called XEVO TQD. The instrument was manufactured by Waters Corporation in Milford, MA01757 U.S.A. The ESI-MS was operated in negative ion acquisition mode and the Column used is ACQUITY UPLC-BEH C18 1.7  $\mu\text{m}$  - 2.1  $\times$  50 mm. The rate of flow was set at 0.2 mL/min. The solvent system was comprised of two solutions: water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B).

To prepare the sample solution, HPLC analytical grade methanol was used and then filtered using a membrane disc filter (0.2  $\mu\text{m}$ ) before being subjected to LC-ESI-MS analysis. Before injecting into the UPLC apparatus, the sample mobile phase was prepared through sonication and filtering with a 0.2- $\mu\text{m}$  filter membrane disc. A sample injection quantity of 10  $\mu\text{L}$  was used. Negative ion mode analysis was conducted using the following parameters: 150  $^{\circ}\text{C}$  as source temperature, 30 eV for the cone voltage, 3 kV for the capillary voltage, 440  $^{\circ}\text{C}$  for the desolvation temperature, 50 L/h for the cone gas flow, and 900 L/h for the desolvation gas flow.

ESI mass spectrum showed m/z range of (100–1000). The peaks and spectra were analyzed using the software Maslynx 4.1, and the tentative identification of the peaks was made by comparing their retention times (Rt) and MS spectra with reported data<sup>7</sup>.

## RESULTS

### Butanol Fraction

By using the obtained data from UPLC-ESI-MS/MS that are mass fragmentations pattern, LC Rt, and molecular weights and comparing it with previous research and phytochemical mass

libraries such as Respect, MONA, GNPS, CFM-id, and PubChem. sixteen phytochemicals have been tentatively identified in the butanol fraction. Figure 1 represents the total ion chromatograms (TICs) of the compounds extracted from the butanol fraction.

The fruit extract of *Xanthium strumarium* was analyzed using UPLC-ESI-MS/MS and the n-butanol fraction was found to contain a number of phytochemicals, as listed in Table 1. It was observed that the plant contained a significant amount of atractyloside derivatives, which could be a possible explanation for the plant high toxicity.

From the above data the major compounds in butanol fraction are Syringetin dimer in peak 29 and 4-desulfated carboxyatractyloside in peak 23 and various atractyloside derivatives.

For Carboxyatractyloside M-H is 769 and the product ions are [M-SO<sub>3</sub>-H] m/z 689, [M-SO<sub>3</sub>-COO-H] m/z 645, which is 4-desulfated atractyloside and [M-2SO<sub>3</sub>-H] m/z 609 which is 3,4-didesulfated carboxyatractyloside.

For atractyloside M-H is 725 and the product ions are [M-SO<sub>3</sub>-H] m/z 645 which is 4-desulfated atractyloside, [M-2SO<sub>3</sub>-H] m/z 564 corresponding to 3,4-didesulfated atractyloside, also the fragment [ATR + 2H]<sup>2+</sup> m/z 362 result from the presence of tow ionization site as shown in Fig.3.

4-desulfated carboxyatractyloside M-H 689 produced the same product ions as that of carboxyatractyloside and atractyloside which reflect they are derivatives<sup>10</sup>

For Syringetin Loss of methyl groups from the 3-methoxy and 6-methoxy of the molecules result in the product ion of m/z 330 and m/z 315 respectively<sup>25</sup> as in Fig.5. Presence of caffeoylquinic acid derivatives is also common in *Xanthium* genus.

#### Chloroform fraction

In chloroform fraction 9 compounds among which terpenoids and sterols were tentatively identified in the same manner in butanol fraction.

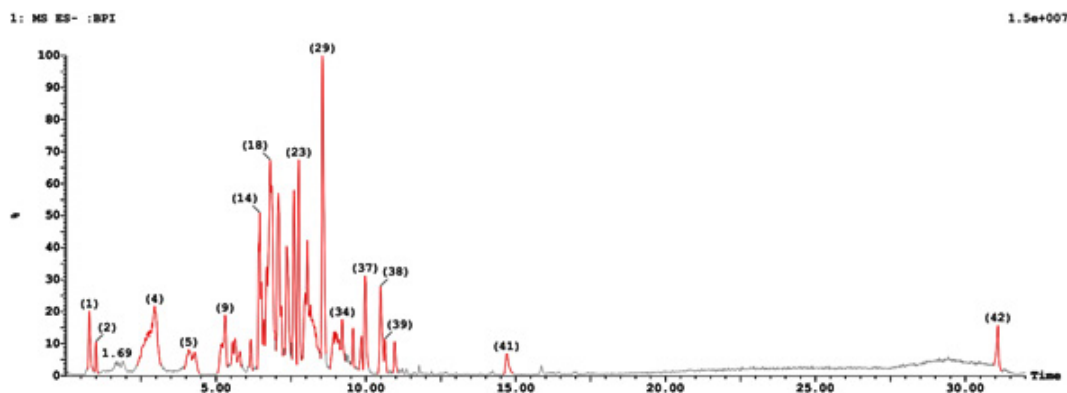


Fig. 1. TIC of butanol fraction of *Xanthium strumarium* fruit extract

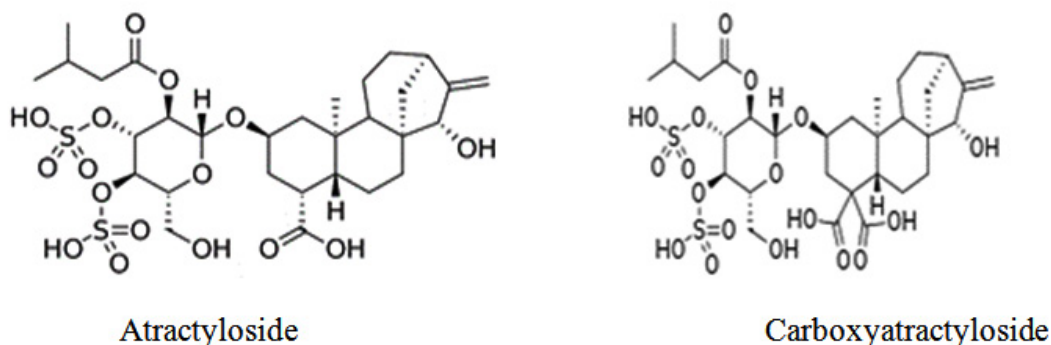


Fig. 2. Structure of Atractyloside and Carboxyatractyloside

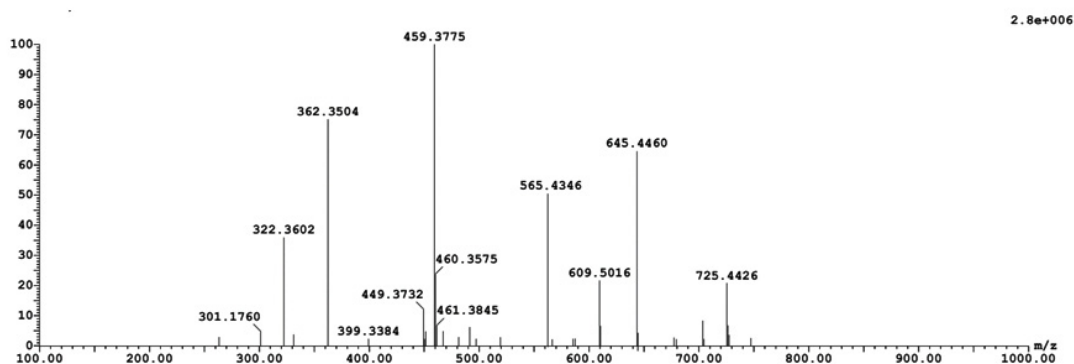
In this fraction 2-acetyl-atractyligenin is the major compound and account for the toxic effect of this fraction along with xanthatin a sesquiterpene lactone. and its derivatives.

From the TIC of chloroform fraction in Fig.7 , Artemisinin dimer at peak 53 is major compound, and the mass spectrum of the compound Fig.8 the precursor ion [M+H] m/z 283 and [M+H]<sub>2</sub> m/z 564 shows two ionization site and the

fragment [M+H-H<sub>2</sub>O] m/z 265 and [M+H-2H<sub>2</sub>O] m/z 247.

#### Hexane fraction

Hexane fraction revealed the presence of 6 compounds (table 3) among which are sterols and terpenes. It is evident that hexane fraction does not contain toxic metabolites. It is also not uncommon to contain hexadecenoic acid since the fruit contains seeds inside that rich in fatty acid.



**Fig. 3.** Mass spectrum of Atractyloside

**Table 1.** The compounds discovered in the butanol fraction of the extract of *Xanthium strumarium* fruits are identified by their peak assignments. fragmentation pattern with Negative ion mode for the precursor ions and their diagnostic daughter ions are used for this purpose

Peak no.	Identified compound	Precursor ion [M-H]	MS\MS m/z ESI-	Rt.min	Ref.
Diterpene glycosides					
21	Carboxyatractyloside	769	689,645,609	7.36	8,9
24	Atractyloside	725	645,565,362,322	7.98	10,11
23	4-desulfated carboxyatractyloside	689	645, 609, 344,362	7.76	12
25	3,4-didesulfated carboxyatractyloside	609	459, 565, 362	8.05	12,13
32	Atractyloside III	565	481, 463, 319	9.03	14
34	Atractyloside V	659	481, 369,329	9.21	14
33	Atractyloside Methyl Ester	739	659,565,369,329	9.10	10,15
34	Atractyligenin-2-O- $\beta$ -D-glucoside	481	369,329,317,301,145	9.21	16
Phenolic compounds					
29	Syringetin dimer	691	346,330,316	8.55	17
14	Quercetin glucuronide	477	301, 179, 151	6.46	17
13	Ononin	429	267,252,224	6.16	18,19
18	dicafeoylquinic acid	515	353,299, 191, 173	6.80	6,20
3	cafeoylquinic acid	353	191,179,173	2.95	21,22
4	cafeoylquinic acid dimer	708	353,191,179	2.95	20,22
12	Methylchlorogenate	368	191,163,295	5.79	23
20	Tricafeoylquinic acid	677	515,497,353	7.18	17,24

From the above data the major compounds in butanol fraction are Syringetin dimer in peak 29 and 4-desulfated carboxyatractyloside in peak 23 and various atractyloside derivatives.

## DISCUSSION

This is the first study that identify eight toxic atractyloside derivatives in this species in the butanol fraction of the extract and since most

of these derivatives are polar, they all eluted in the first ten minutes as in Fig.1.

The negative ionization mode for ESI gave more clear data for the butanol fraction than the positive ionization mode. As seen in Table 1 all

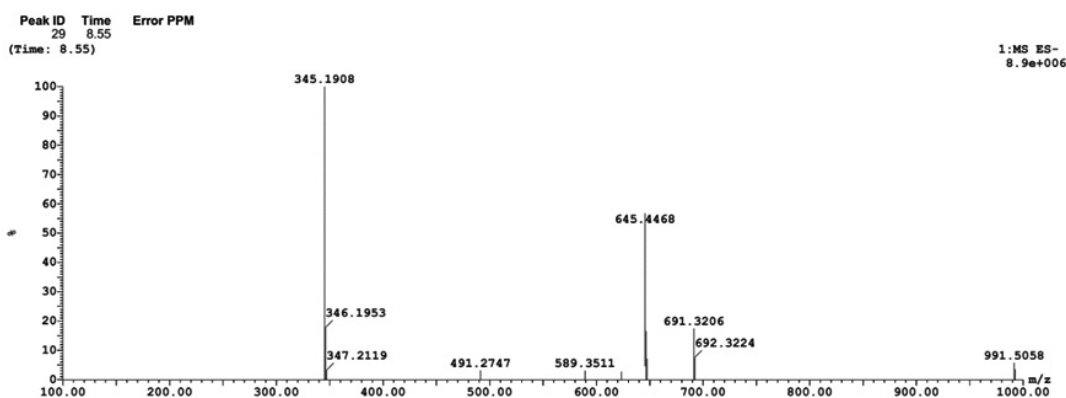


Fig. 4. Mass<sup>1</sup> spectrum of Syringetin dimer

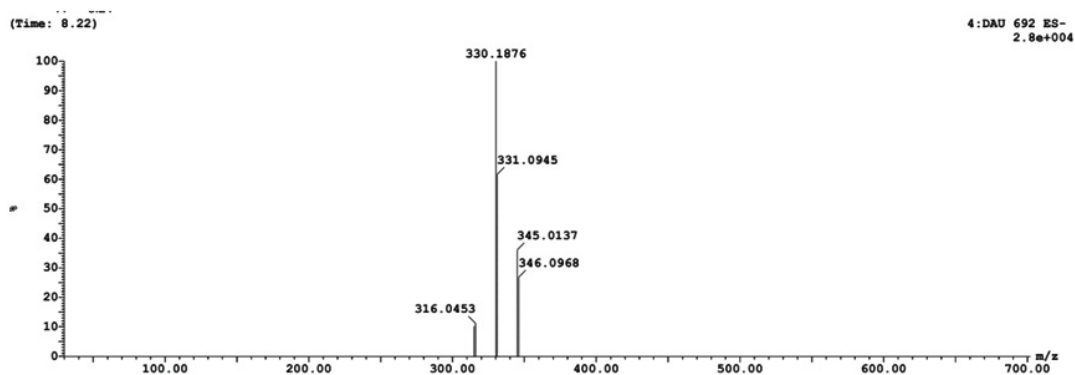


Fig. 5. Mass<sup>2</sup> spectrum of Syringetin dimer

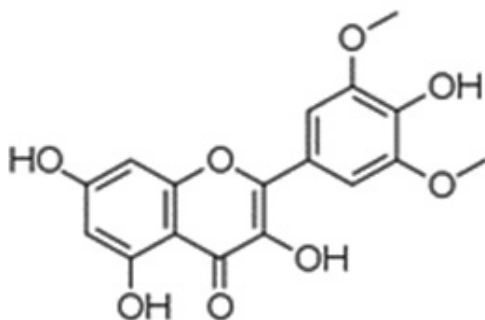


Fig. 6. Syringetin M-H 345

the atractyloside derivatives differ only in loss of sulfated, carboxyl, and disulfated groups and are consistent with the fragmentation pattern of that derivative.

It is not uncommon for the plant extract to contain dimers of certain phytochemicals, and this can be seen in the first mass chart as a doubling of  $m/z$  as it contains two ionization sites while in the second mass normal fragments are seen and consistent with that compound as in Fig. 4 and Fig.5 of Syringetin dimer.

In chloroform fraction, we can see that most of the identified compounds are aglycone derivatives of compounds in butanol fraction, and this further supports the identification of these phytochemicals.

Artemisinin is identified for the first time in this plant and being a major compound may facilitate the utilization of this plant as source for Artemisinin.

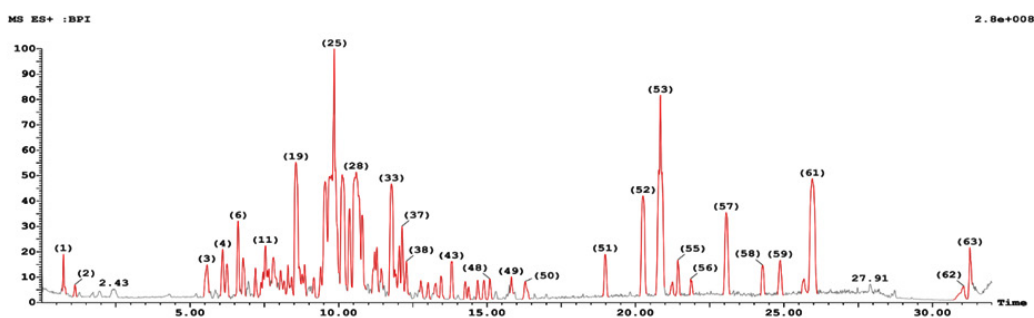


Fig. 7. Total ion chromatogram (TIC) of chloroform fraction of *Xanthium strumarium* fruit extract

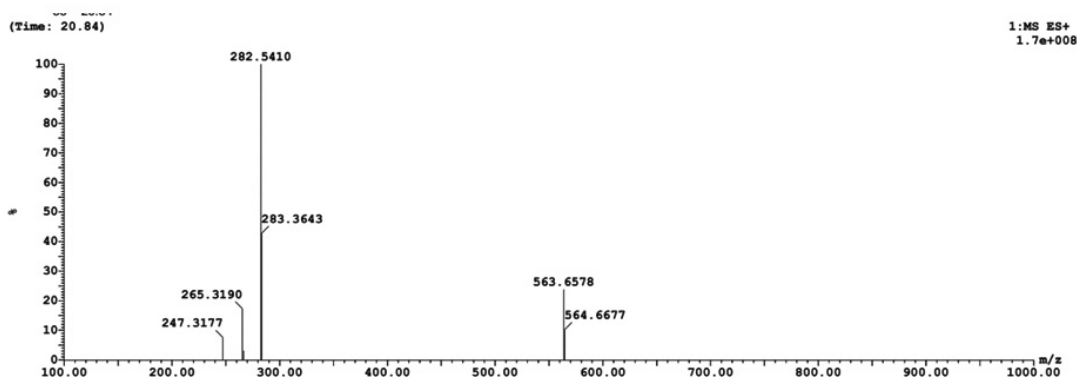


Fig. 8. mass<sup>1</sup> spectrum of Artemisinin dimer

**Table 2.** Peaks assignments for compounds detected in the chloroform fraction of the extract of *Xanthium strumarium* fruits were made using the precursor ions fragmentation pattern and their daughter ions in positive ionization mode

Peak no.	Identified compound	Precursor ion [M+H]	MS\MS m/z ESI-	Rt. min	Ref.
33	atractylenolide II	233	215,187,175,	11.78	26,27
19	4-epi-Xanthanol	249	229,205,189	7.53	26,28
25	2-acetyl-atractyligenin	363	345,328,303,297	9.43	29
53	Artemisinin dimer	564	282,265,247,97	20.54	30,31,32
57	dihydroartemisinin	285	267,249,144,88	23.05	33
33	ent-kaurene	273	233,215,187	11.78	34
6	Carboxyatractyligenin	365	318,235,128	6.61	35,29
58	Pungiolide C	512	493,449,343	24.28	36,37,38
51	Dihydroxyxanthatin	281	263,245,128	18.98	37,38

Hexane fraction contains some major unidentified peaks ( peak 5,15,23,31 ) which may reflect new compounds that need to be isolated and characterized. So this type of research allows

researchers to focus on compounds that are not being identified yet instead of separating already-known compounds.

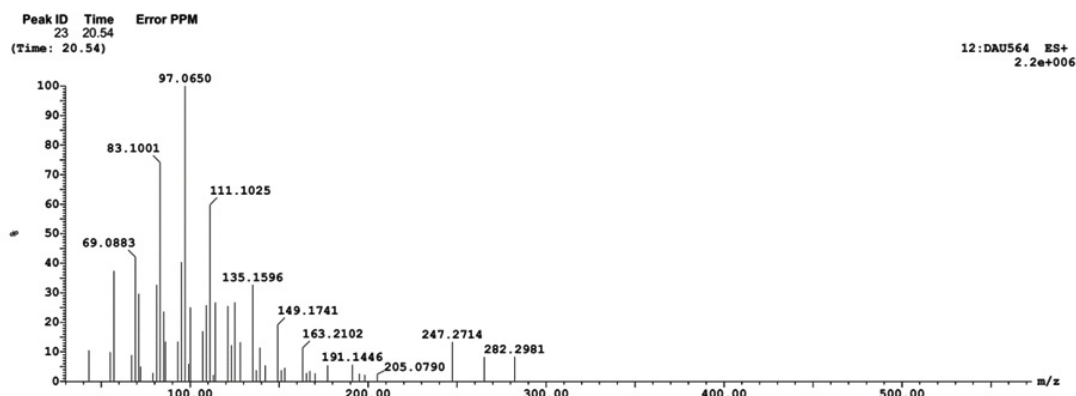


Fig. 9. Mass<sup>2</sup> spectrum of Artemisinin dimer

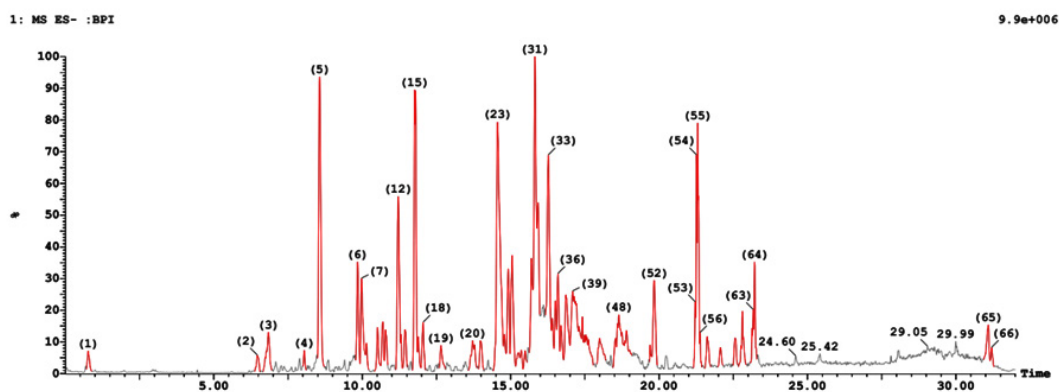


Fig. 10. (TIC) Total ions chromatogram of hexane fraction of *X. strumarium* fruit extract.

**Table 3.** The recognition of the compounds presents in hexane fraction of the extract obtained from *Xanthium strumarium* fruits was done by analyzing the fragmentation pattern of precursor ions and their diagnostic daughter ions. This analysis was performed in negative ion mode to determine the peak assignments accurately

Peak no.	Identified compound	Precursor ion [M-H]	MS/MS m/z ESI-	Rt.min	Ref.
26	beta-Sitosterol	414	396,297,273,133	15.5	18,2
55	â-amyrin	425	407,393,218,191	21.30	26,18
61	Hexadecanoic acid	255	239, 200, 127, 97	22.76	27
50	Kaur-16-ene	271	255,239,187	18.9	28
46	stigmasterol	411	367, 271, 255,	18	29
15	Unknown sesquiterpene	249	192,191,148,57	11.77	

## CONCLUSION

This study highlights the significance of using UPLC-ESI MS/MS for the provisional identification of unidentified chemical compounds. although this type of identification is not sufficient it gives important information before isolation of the required compounds, it also plays a role in confirmation of known compounds.

The toxicity of the fruit is due to atractyloside derivatives identified in N-butanol and chloroform fractions with 4-desulfated carboxyatractyloside being the major one in butanol fraction and 2-acetyl-atractyligenin in chloroform fraction. with no identified toxic compounds in hexane fraction. There are many of unidentified compounds in hexane fraction that needed to be identified.

## ACKNOWLEDGEMENT

We need to thank Central laboratory staff of Tikrit university for their valuable support.

### Conflict of Interests

The writers state that they do not have any competing interests to declare.

### Funding Sources

The work is self-funded.

## REFERENCES

- Sastry, S. K., Mandal, B., Sano, T. & Hammond, J. *Xanthium strumarium* (Cocklebur). in *Encyclopedia of Plant Viruses and Viroids* 2809–2811 (Springer, 2019).
- Fan, W. *et al.* Traditional uses, botany, phytochemistry, pharmacology, pharmacokinetics and toxicology of *Xanthium strumarium* L.: A review. *Molecules* 24, 359 (2019).
- Vladymyrova, I. V., Georgiyants, V. A., Tishakova, T. S. & Shumova, H. S. Investigation of the effect of *xanthium strumarium* l. extract on the level of thyroid-stimulating hormones and mass coefficient of rat thyroid gland. *Îàó-íúé æóðíàè «Áâñðìèè ÍÁÍ ÐË»* 15–22 (2019).
- Han, T. *et al.* Authentication and quantitative analysis on the chemical profile of *Xanthium* fruit (Cang-Er-Zi) by high-performance liquid chromatography-diode-array detection tandem mass spectrometry method. *Anal Chim Acta* 634, 272–278 (2009).
- Juan-xiu, L. I. U. *et al.* Differences of Chemical Compositions in *Xanthii Fructus* before and after Stir-Frying by UPLC-Triple TOF MS/MS. *Journal of Chinese Mass Spectrometry Society* 38, 157 (2017).
- Han, T. *et al.* Bioactivity-guided fractionation for anti-inflammatory and analgesic properties and constituents of *Xanthium strumarium* L. *Phytomedicine* 14, 825–829 (2007).
- Al-Saleem, M. S. M. *et al.* Metabolic Profiling and In Vitro Assessment of the Biological Activities of the Ethyl Acetate Extract of *Penicillium chrysogenum* “Endozoic of *Cliona* sp. Marine Sponge” from the Red Sea (Egypt). *Mar Drugs* 20, 326 (2022).
- van Kiem, P., Hoang, N. H., Thu, V. K., Tai, B. H. & Nhiem, N. X. Diterpene glycosides and phenolic compounds from the fruits of *Xanthium strumarium*. *Vietnam Journal of Chemistry* 58, 648–653 (2020).
- Botha, C. J., Lessing, D., Rösemann, M., van Wilpe, E. & Williams, J. H. Analytical confirmation of *Xanthium strumarium* poisoning in cattle. *Journal of Veterinary Diagnostic Investigation* 26, 640–645 (2014).
- Brucoli, F., Borrello, M. T., Stapleton, P., Parkinson, G. N. & Gibbons, S. Structural characterization and antimicrobial evaluation of atractyloside, atractyligenin, and 15-didehydroatractyligenin methyl ester. *J Nat Prod* 75, 1070–1075 (2012).
- Yang, L. *et al.* RRLC-MS/MS method for the quantitation of atractyloside in *Fructus Xanthii* (*Xanthium sibiricum*). *Analytical Methods* 5, 2093–2097 (2013).
- Varga, E., Domokos, E., Kelemen, H., Fulop, I. & Kursinszki, L. HPLC-ESI-MS/MS Profiling of Phenolic Acids, Flavonoids And Sesquiterpene Lactones from *Xanthium spinosum*. *Rev. Chim* 71, 558–564.
- Jiang, H. *et al.* The fruits of *Xanthium sibiricum* Patr: A review on phytochemistry, pharmacological activities, and toxicity. *World Journal of Traditional Chinese Medicine* vol. 6 408–422 Preprint at [https://doi.org/10.4103/wjtc.wjtc\\_49\\_20](https://doi.org/10.4103/wjtc.wjtc_49_20) (2020).
- Tsukui, A. *et al.* Direct-infusion electrospray ionization-mass spectrometry analysis reveals atractyligenin derivatives as potential markers for green coffee postharvest discrimination. *LWT* 103, 205–211 (2019).
- Cheng, Y., Fu, J., Chen, L., Li, L. L. & Qu, J. Diterpenoid glycosides and monoterpenoid glycosides from the fruits of *Xanthium chinense*. *J Asian Nat Prod Res* 21, 207–216 (2019).
- Lang, R., Beusch, A. & Dirndorfer, S. Metabolites of dietary atractyligenin glucoside in coffee drinkers’ urine. *Food Chem* 405, 135026 (2023).



17. Poljuha, D. *et al.* LC–DAD–MS phenolic characterisation of six invasive plant species in Croatia and determination of their antimicrobial and cytotoxic activity. *Plants* 11, 596 (2022).
18. Amina\_Sultana\_Chemistry\_2015\_FUUAST\_Karachi\_22.06.2016.
19. Han, T., Li, H., Zhang, Q., Zheng, H. & Qin, L. New thiazinediones and other components from *Xanthium strumarium*. *Chem Nat Compd* 42, 567–570 (2006).
20. Kamboj, A. & Saluja, A. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). *International Journal of Green Pharmacy* vol. 4 129–139 Preprint at <https://doi.org/10.4103/0973-8258.69154> (2010).
21. Wollenweber, E., Dörr, M., Fritz, H. & Valant-Vetschera, K. M. Exudate flavonoids in several Asteroideae and Cichorioideae (Asteraceae). *Zeitschrift für Naturforschung C* 52, 137–143 (1997).
22. Zhang, Y. *et al.* Qualitative and quantitative determination of *Atractylodes* rhizome using ultra-performance liquid chromatography coupled with linear ion trap–Orbitrap mass spectrometry with data-dependent processing. *Biomedical Chromatography* 33, (2019).
23. Pandey, D. P. & Rather, M. A. Isolation and Identification of Phytochemicals from *Xanthium strumarium*. *Int J Chemtech Res* 4, 266–271 (2012).
24. Varga, E., Domokos, E., Kelemen, H., Fulop, I. & Kursinszki, L. HPLC-ESI-MS/MS Profiling of Phenolic Acids, Flavonoids And Sesquiterpene Lactones from *Xanthium spinosum*. *Rev. Chim* 71, (2020).
25. Favre, G. *et al.* New acylated flavonols identified in *Vitis vinifera* grapes and wines. *Food Research International* 112, 98–107 (2018).
26. Opiyo, S. A. Triterpenes and Sterols from *Ocimum suave*. (2022).
27. Kaur, M., Kamboj, A., Rathour, A. & Saluja, A. K. Isolation and Characterization of Constituents from the Leaves of *Xanthium strumarium* and their Evaluation for Antioxidant and Antimicrobial Potential. *Nat Prod Chem Res* 3, 2 (2015).
28. Silva, M. L. *et al.* Selective cytotoxicity of entkaurene diterpenoids isolated from *Baccharis lateralis* and *Baccharis retusa* (Asteraceae). *Arch Pharm (Weinheim)* 355, 2200083 (2022).
29. Kamboj, A., Pooja, A. & Saluja, A. K. Isolation and Characterization of Bioactive Compounds from the Petroleum Ether Extracts of Leaves of *Xanthium Strumarium* Linn. *BioMedRx* 1, 235–238 (2013).