Journal of King Saud University – Science 33 (2021) 101461

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Chemical fingerprinting of three *Anemone* species and an adulteration study to detect cross mixing of medicinal plants by HPLC-HR-ESI-MS/MS method

Muzna Syed [a], Muhammad Noman Khan [a], Adeeba Khadim [a], Hamna Shadab [a], Anjum Perveen [b], Hesham R. El-Seedi [c,d,*], Syed Ghulam Musharraf [a,e,f,*]

[a] *H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*
[b] *Department of Botany, University of Karachi, Karachi 75270, Pakistan*
[c] *Pharmacognosy Group, Department of Pharmaceutical Biosciences, BMC, Uppsala University, SE-751 23 Uppsala, Sweden*
[d] *International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang 212013, China*
[e] *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*
[f] *T.C.M. Hospital of Southwest Medical University, Luzhou, Sichuan, China*

ARTICLE INFO

Article history:
Received 7 July 2020
Revised 21 March 2021
Accepted 27 April 2021
Available online 4 May 2021

Keywords:
Anemone obtusiloba
Anemone falconeri
Anemone tetrasepala
Ziziphus jujube
LC-ESI-MS/MS

ABSTRACT

The adulteration of plant raw materials used for the preparation of herbal drugs with foreign plant material is one of the important issues in the quality control of herbal products. Chemical fingerprinting is a well-known approach for the characterization of secondary metabolites associated with the plant species and can be used for quality control of plant material. The current study centred on the development of chemical fingerprinting of three medicinal plants of genus *Anemone* including *A. obtusiloba*, *A. falconeri* and *A tetrasepala* through identification of their metabolites using LC-ESI-QTOF-MS/MS analysis. Thirty compounds were identified by using high-resolution positive and negative electrospray- ionization (ESI) modes and MS/MS analysis. The identified compounds belong to diterpenoids, alkaloids, phenols, flavonoids and other classes and their distribution among the analysed species was studied using different statistical tools. Moreover, an LC-HR-ESI-MS/MS method was developed to detect the cross mixing of *A. obtusiloba* with *Ziziphus jujuba*. Seven chromatographically differentiative peaks confined to *A. obtusiloba* were selected to detect its contamination in adulterated samples. The method was able to detect as low as 20% mixing of *A. obtusiloba* in *Z. jujuba*. This study can play a significant role to manage the quality control of herbal medicines and to identify lead natural products of these plants.

1. Introduction

Plants have been a rich source of therapeutics since early times. These herbal medicines have once again attracted interest of people and are widely consumed by a large portion of population not only in developing countries but also in the developed ones. The main reasons behind re-emerging popularity of herbal medicines are lack of new and effective drugs to counter emerging diseases such as drug-resistant infections, and the increased cost and side effects of pharmaceuticals. Herbal products are not always as safe to use as they are considered. These medicines are usually prepared from crude extract of a plant or a mixture of plants as polyherbal formulation, in both cases the product is a combination of thousands of chemical compounds. Due to the

* Corresponding authors at: Pharmacognosy Group, Department of Pharmaceutical Biosciences, BMC, Uppsala University, SE-751 23 Uppsala, Sweden (Hesham R. El-Seedi). H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan (Syed Ghulam Musharraf).
E-mail addresses: hesham.el-seedi@farmbio.uu.se (H.R. El-Seedi), musharraf@iccs.edu (S.G. Musharraf).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101461
1018-3647/

presence of such enormous number of compounds, the quality control of these medicines is quite difficult. Despite the traditional back-ground and present significance of herbal medicines, many countries have still not recognised these medicines due to their authenticity, safety and lack of quality control (Liang et al., 2004).

The raw herbal material acquired by herbal processing units is mostly in its dried form, which is almost impossible to identify physically, so there is an escalating risk of adulteration of other plant material. The adulteration can be intentional or unintentional mixing of other plant having different medicinal properties than the desired one or simply no medicinal values (Zhang et al., 2012). The presence of such undesired plant material not only affects the efficacy of these medicines but can also cause serious harm or mistreatment of diseases. World Health Organisation (WHO) also highlighted the presence of adulterants as the possible risk associated to traditional herbal drugs and insisted for its prevention (Qi, 2013; Organization, 2000).

To overcome the problem, reliable quality control protocols should be followed during production of these formulations. Extensive chemical profiles serve as the fingerprint for any plant material and thus can be used for quality control purpose. For the generation of these chemical profiles, chromatographic separation along with mass spectrometric detection is a reliable and high-throughput technique. High-resolution LC-ESI-MS/MS approach is one of the most useful tools for the detection and identification of metabolites without their prior isolation and purification (Schymanski et al., 2014).

Genus *Anemone* of family Ranunculaceae is known for its medicinal species. This genus is comprised of more than 150 plant species. Among them, more than 50 species have been used for the treatment of various diseases (Hao et al., 2017). In the Chinese medicine, 38 species of *Anemone* are reported to be used for the treatment of malaria, parasitic diseases, tinea, ulcers, sore, arthritis, and traumatic injuries (Xiao et al., 1989). These species are mostly widespread in mountainous regions of Pakistan, Tibet, Nepal and Burma. The genus *Anemone* is rich in low molecular weight compounds such as triterpenoids, saponins, steroids, fats and oils, saccharides, alkaloids, coumarins, flavonoids, lactones, lignans, phenolic compounds, and other compounds (Zou et al., 2004; Lu et al., 2001; Cao et al., 2004). Many compounds isolated from various *Anemone* species show interesting biological activities such as antimalarial, anti-convulsant, anti-histamine, anthelmintic, anti-inflammatory, antimicrobial, anticancer and antioxidant activities (HAO et al., 2015; Da et al., 2015; Lee et al., 2008).

Anemone obtusiloba grows as weed with many other crops. This plant is reported to be used in Unani and other medicine systems for its numerous biological activities such as antirheumatic, antispasmodic, antimicrobial, antifungal, antiviral, antipyretic, sedative, anti-inflammatory, nervine and diuretic effects (Quattrocchi, 2016). *Anemone falconeri* is used for fever, cold, pain and gastritis (Kaul, 1997) while *Anemone tetrasepala* is used in Tibetan medicines to treat stomach worms, bronchitis, gonorrhoea, cold tumour and as sedative (Hao et al., 2017).

In the present study, chemical profiles of three medicinal plant species of genus *Anemone* were developed using high-resolution LC-ESI-QTOF-MS/MS analysis. On the other hand, adulteration studies were performed to develop a method to detect cross mixing/adulteration of *A. obtusiloba* with *Ziziphus jujuba*. *Z. jujuba* is used in many herbal and polyherbal formulations for the cure of different bronchial and chest problems (Mirakilova et al., 2016).

2. Experimental

2.1. Sample collection

Plant material of *A. obtusiloba* (AO) and *A. falconeri* (AF) were collected from Neelum Valley, Azad Kashmir, *A. tetrasepala* (AT) from Hazara valley, while *Z. jujuba* (ZJ) was collected from Swat valley, Pakistan. Botanical authentication and identification of all the samples were done by taxonomist Mr. Shabbir Aijaz and a voucher specimen was deposited for each plant species at Herbarium, University of Karachi, Pakistan. The voucher numbers for *A. obtusiloba*, *A. falconeri* and *A. tetrasepala* were GH-95587, GH-95588 and GH-95589, respectively.

2.2. Chemicals and reagents

Chemicals used for LC-ESI-MS analysis were acquired from different sources. Formic acid was used as an additive for mobile phase and purchased from Daejung (Daejung Chemicals & Metals Co. Ltd., Korea). Analytical grade methanol for mobile phase and sample preparation was acquired from Merck (Merck KGaA, Germany). Type I water for chromatography was purified using Ultrapure Water Purification assembly (Barnstead™ GenPure™, USA). All sample solutions were filtered through 0.22 μm PTFE membrane before analysis.

2.3. Preparation of plant samples for LC-MS analysis

Shadow-dried whole plant materials were ground into powder form and one gram of each was accurately weighed and extracted with 10 mL methanol through sonication for 20 min at room temperature. These samples were then centrifuged at 6000 rpm for 30 min and the supernatant was filtered using 0.22 μm PTFE syringe-driven filter. 50 μL of each sample was diluted to 1000 μL with HPLC grade methanol for LC-MS analysis.

For adulteration, *A. obtusiloba* was mixed with another medicinal plant *Ziziphus jujuba* in different percentages with total weight of 10 g. In total, nine samples were homogenized with 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, and 60% by weight of *A. obtusiloba*. One gram of each sample was accurately weighed and extracted with 10 mL methanol. The remaining sample preparation method was same for LC-MS analysis.

2.4. LC-MS/MS analysis

HPLC-MS/MS analysis for natural product identification was performed on Bruker maXis HR-QTOF mass spectrometer (Bremen, Germany) coupled to Dionex UltiMate 3000 series HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a binary RS pump, column thermostat and auto-sampler. Sample chromatography was performed on Macherey-Nagel Nucleodur C18 Gravity column (3.0 × 100 mm, 1.8 μm) which was kept at 40 °C. 4 μL sample was injected while mobile phase consisted of A (0.1% formic acid in water) and B (0.1% formic acid in methanol) with a constant flow rate of 0.7 mL/min. Chromatography was optimized using HPLC-DAD prior to LC-MS analysis. The mobile phase gradient was started at 10% B, increased to 90% B in 5.5 min, maintained for 1.5 min, and then returned to 10% B in 1 min. Total run time was 10 min including the equilibration (1mineach) at the start and end of the gradient. Mass spectra were recorded using electrospray ionization, employing the Bruker CaptiveSpray ion source. MS and MS/MS spectra were recorded separately both in positive and negative ionization modes to confirm

that all types of compounds can be ionized, detected, and identified. Ion source parameters used are: capillary voltage for positive mode kept at 4500 V while −3500 V for negative mode, end plate offset at 500 V, nebulizer gas pressure at 45.0 psi, drying gas at 12.0 L/min flow rate and 270 °C temperature. All spectra were recorded in the mass range from 100 to 2000 *m*/*z* while the scan speed was set at 5 Hz for MS while 12 Hz for MS/MS spectra to make sure that maximum possible data could be recorded in a single HPLC-MS/MS run.

As many natural products tends to be abundant and may suppress the low abundant natural products, therefore it was necessary to make sure that compounds present in low abundance are not left without MS/MS. To resolve this issue, active exclusion feature of the instrument was used which removes repeated precursor ions from consideration after the set number of spectral averages have been achieved. To ensure mass accuracy, sodium formate (10 mM in 1:1 water:2-propanol) was used at the start of each LC-MS/MS analysis. MS and MS/MS files were presented using two efficient tools: Bruker Compass Data Analysis (ver. 4.4 SR1, 64-bit) and Bruker Compass Target Analysis (ver. 1.3). The spectral background subtraction algorithm was built using Data Analysis to remove noise from obtained data.

2.5. Identification of compounds and chemometric analysis

The profiling of *Anemone* species was performed through an untargeted metabolomics workflow. A custom-made database of reported phytochemicals from these species is developed after an extensive search using the Dictionary of Natural Products (DNP ver. 26.2) and other literature sources. On the other hand, different available ESI-MS/MS libraries were incorporated in NIST MS search. These libraries include, NIST MS/MS library, Mass bank of North America and Mass Bank of Europe. The compounds were identified by comparing high resolution masses, isotopic pattern and MS/MS fragmentation with libraries and databases. The tolerance levels were set at 5 ppm for exact masses and 50 mSigma value for isotopic pattern. Chemometric studies were done to visualise the distribution of identified compounds by generating heatmap clusters using software Perseus (ver: 1.6.2.1).

3. Results and discussion

3.1. LC-ESI-MS/MS analysis and identification of compounds

The overall workflow which is used in this study is summarized in Fig. 1. The base peak chromatograms (BPCs) of all the plant extracts were quite clear with well separated peaks. Fig. 2 shows the BPCs in positive and negative ion modes of all analysed species along with the numbers of identified compounds marked on each peak. Before data analysis, each mass spectrum was recalibrated by internal calibration using high-precision calibration (HPC) mode. A total of thirty compounds were identified in three *anemone* species. Twenty-seven compounds were identified using positive ionization mode of MS, twelve were identified in negative ionization mode while nine were appeared common in both modes. The identified compounds belong to triterpenoid, steroid, alkaloid, coumarin, carboxylic acid and flavonoid classes of compounds. Among the identified compounds, twenty-six were present in *A. obtusiloba*, twenty-five were present in *A. tetrasepala* while sixteen were present in *A. falconeri*. During the MS and MS/MS study it was noted that the most compounds were observed as protonated and deprotonated molecules under the positive and negative ionization modes, respectively (Table 1). Supplementary Fig. 1 compares the Base Peak Chromatograms from three species in positive and negative modes of ionization.

3.2. Heatmap cluster analysis

Perseus (ver: 1.6.2.1) was used to generate heatmap clusters based on log2(X) transformed peak areas of all the identified compounds in *Anemone* spp. in both positive and negative modes of ionization. Difference in metabolite distribution can be visualized in cluster analysis of all three species. Compounds, hydroxycoumarin hexoside, docosenamide, aminobenzoic acid, hydroxycoumarin, triallyloxy triazine, quercetin hexoside, triphenylphosphine oxide, chlorogenic acid, gycerol stearate, caffeic acid, adenosine, tryptophan, hydroxy methoxy cinnamic acid and palmitic acid were found common in samples of all three species. Hydroxycoumarin hexoside and glycerol stearate were the two most abundant compounds with high peak area in all the samples. Species *A. obtusiloba* and *A. tetrasepala* are physically similar but have shown different chemical profiles. Compounds 15, 23, 28 and 30 were only present in *A. obtusiloba* while compounds 03, 20, 25 and 29 were specifically found in *A. tetrasepala*. These differentiative compounds can be used as their distinguishing character. Heatmap clusters of identified compounds through positive and negative modes of ionization is given in Figs. 3 and 4, respectively.

3.3. Adulteration studies by using LC-ESI-QTOF-MS/MS

Adulteration study was conducted by taking two medicinal plants, *A. obtusiloba* and *Z. jujuba*. These two plants found proximately together and well distributed in Northern regions of Pak-

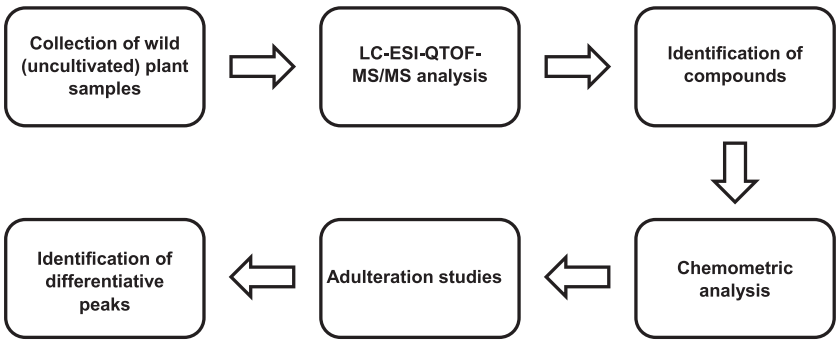

Fig. 1. The overall workflow used in the study.

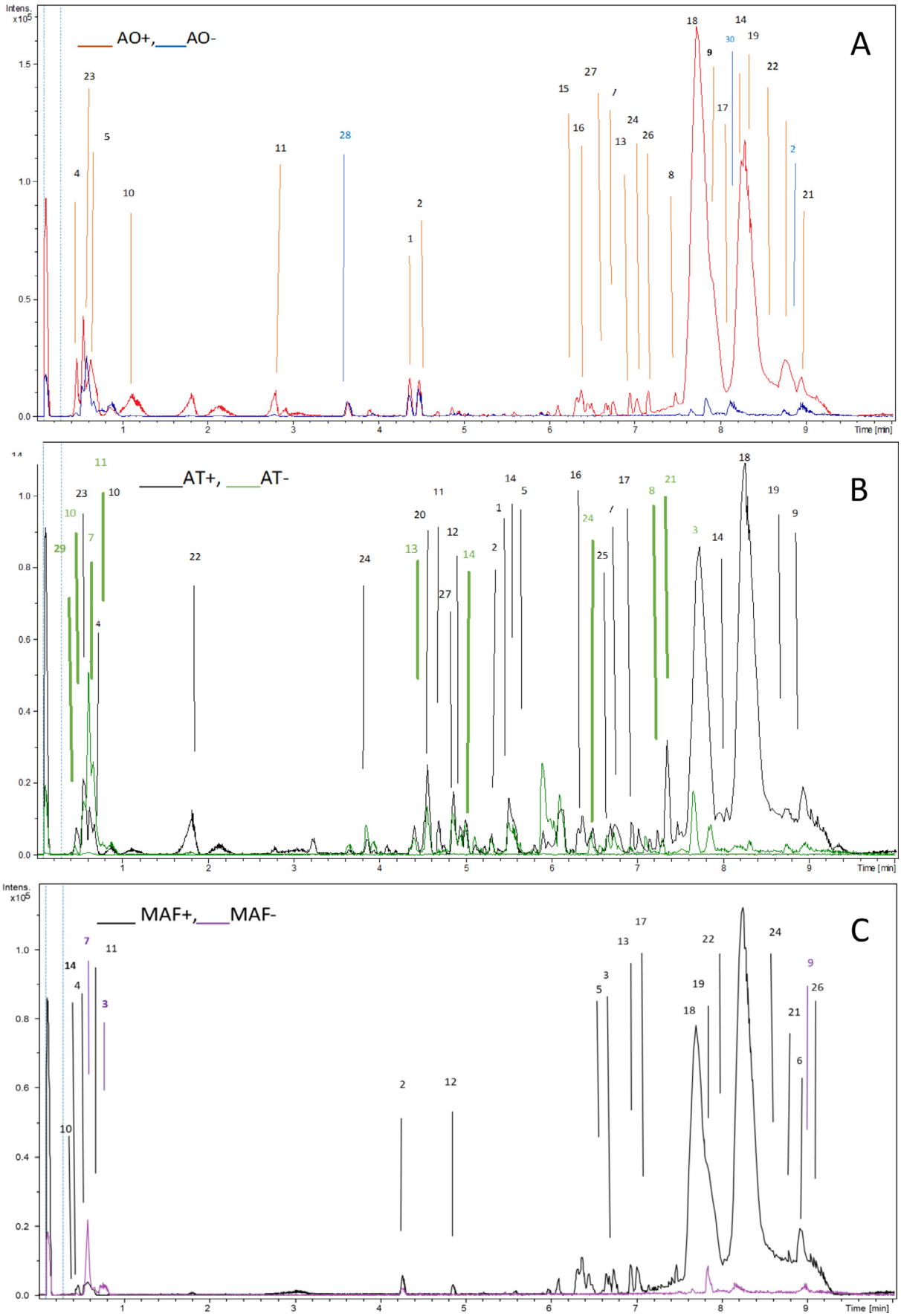

Fig. 2. Base peak chromatograms (BPC) of AO (A), AT (B) and MAF (C) in positive and negative modes of ionization with peaks numbered with identified compounds.

Table 1
Compounds identified from *Anemone* spp. using positive and negative modes of ionization.

S. No.	Compound Name	Formula	Exact Mass	Ion type	RT min	Observed *m/z*	Calculated *m/z*	Error ppm	Fragments *m/z*(% rel. abund.)
1	Cyanidin dihexoside [a,b]	$C_{27}H_{30}O_{16}$	610.1533	$[M+H]^+$	4.3	611.1608	611.1607	−0.16	449.1079(4.9)
2	Kaempferol hexoside[a,b]	$C_{21}H_{20}O_{11}$	448.1005	$[M+H]^+$	4.2	449.1079	449.1078	−0.22	287.0551(21.3), 288.0586 (20.1), 289.0611(18.1)
3	Mannobiose [b]	$C_{12}H_{22}O_{11}$	342.1162	$[M+H]^+$	0.6	343.1315	343.1311	1.16	341.1100(9.7)
				$[M-H]^-$	0.6	341.1091	341.1090	−1.10	179.0500(8.8)
4	Aminobenzoic acid [a, b,c]	$C_7H_7N_1O_2$	137.0480	$[M+H]^+$	0.6	138.0550	138.0551	−0.72	120.0041(32.7)
5	Hydroxycoumarin [a,b]	$C_9H_6O_3$	162.0316	$[M+H]^+$	3.3	163.0391	163.0390	−0.61	163.0462(84)
6	Palmitic acid [a,b,c]	$C_{16}H_{32}O_2$	256.2402	$[M+H]^+$	8.0	257.2477	257.2475	−0.77	211.2426(17.2), 239.2375(9.1).
				$[M-H]^-$	9.0	255.3000	255.3003	−1.10	210.2426(20.2)
7	Caffeic acid [a,b,c]	$C_9H_8O_4$	180.0422	$[M+H]^+$	5.1	181.0501	181.0503	1.10	145.0278(31.9), 163.0381(12.7)
				$[M-H]^-$	0.6	179.0345	179.0348	1.67	134.0447(23.8)
8	Dihydroxyolean-enoic acid [a,b]	$C_{30}H_{48}O_4$	472.3552	$[M+H]^+$	7.3	473.3625	473.3630	−1.00	495.3445(37.1)
				$[M-H]^-$	4.5	471.3459	471.3455	0.80	355.6095(37.3), 370.2920(46)
9	Vinpocetine [a,b]	$C_{22}H_{26}N_2O_2$	350.1994	$[M+H]^+$	9.1	351.2066	351.2067	0.28	294.1363(63.2)
10	Adenosine [a,b,c]	$C_{10}H_{13}N_5O_4$	267.0967	$[M+H]^+$	0.6	268.1041	268.1040	−0.37	136.0620(45.7)
11	Tryptophan [a,b,c]	$C_{11}H_{12}N_2O_2$	204.0898	$[M+H]^+$	2.8	205.0971	205.0972	0.48	146.0599(12.7), 159.0917(15.6), 170.0603(32.6), 188.0706y.
				$[M-H]^-$	0.5	203.0819	203.0819	0.00	Not found
12	Chlorogenic acid [a,b,c]	$C_{16}H_{18}O_9$	354.0950	$[M+H]^+$	3.6	355.1025	355.1024	0.28	163.0300(87.6), 337.0892(11.4)
13	Hydroxymethoxy cinnamic acid [a,b,c]	$C_{10}H_{10}O_4$	194.0579	$[M+H]^+$	3.8	195.0651	195.0652	0.51	177.0549(58.5)
				$[M-H]^-$	4.2	193.0506	193.0508	−1.03	134.0360(26.2),178.0260(15.0)
14	Quercetin hexoside [a, b,c]	$C_{21}H_{20}O_{12}$	464.0955	$[M+H]^+$	4.2	465.1027	465.1028	0.21	85.0285(41.2), 97.0801(20.2), 303.0489(19.2)
				$[M-H]^-$	3.1	463.0882	463.0882	0.00	300.0285(21.2), 301.0353(8.6)
15	Peonidin hexoside cation [a]	$C_{22}H_{23}O_{11}$	463.1240	$[M+H]^+$	5.3	463.1236	463.1235	−0.21	286.0496(7.6), 301.0718(19.0).
16	Triphenylphosphine oxide [a,b,c]	$C_{18}H_{15}OP$	278.0860	$[M+H]^+$	6.6	279.0934	279.0933	−0.35	201.0462(31.2)
17	Triallyloxy triazine [a, b,c]	$C_{12}H_{15}N_3O_3$	249.1113	$[M+H]^+$	6.5	250.1187	250.1186	−0.39	Not found
18	Glycerol stearate [a,b,c]	$C_{21}H_{42}O_4$	358.3083	$[M+H]^+$	7.7	359.3156	359.3156	0.00	267.2679(25.5), 285.2793(11.1), 341.3048(14.6).
19	Docosenamide [a,b,c]	$C_{22}H_{43}NO$	337.3344	$[M+H]^+$	8.7	338.3417	338.3417	0.00	149.1332(23.7), 156.1384(12.6), 163.1487(7.5), 177.1630(6.6), 226.2138(2.8), 268.2635(10.2), 321.3162(0.9).
20	Hydroxymethoxy coumarin [b]	$C_{10}H_8O_4$	192.0422	$[M+H]^+$	4.5	193.0495	193.0496	0.50	176.0473(14.3)
21	Hydroxycoumarin hexoside [a,b,c]	$C_{15}H_{16}O_9$	340.0794	$[M+H]^+$	0.6	341.0867	341.0868	0.29	179.0348(17.0), 341.1804(12.7).
				$[M-H]^-$	2.0	339.0716	339.0716	0.00	133.0304(34.0), 177.0194(18.8)
22	Candenatenin D [a,b,c]	$C_{16}H_{20}O_3$	260.1410	$[M+H]^+$	8.3	261.1484	261.1485	0.50	Not found
23	Acetylneuraminic acid [a]	$C_{11}H_{19}NO_9$	309.1059	$[M+H]^+$	0.6	310.1133	310.1133	0.00	274.0922(10.4), 292.1030(17.5).
24	Geniposidic acid [a,b,c]	$C_{16}H_{22}O_{10}$	374.1212	$[M+H]^+$	3.8	375.1263	375.1263	0.00	217.0476(20.9), 185.0424(7.1).
				$[M-H]^-$	3.0	373.1178	373.1176	0.50	Not found
25	Methylprostaglandin [b]	$C_{21}H_{36}O_5$	368.2557	$[M+H]^+$	6.7	369.2635	369.2635	5.00	367.2503(10.4).
26	Octadecatrienoic acid [a,c]	$C_{18}H_{30}O_2$	278.2250	$[M+H]^+$	7.9	279.2319	279.2319	0.00	261.2218(52.5).
27	Anemonin [a,b]	$C_{10}H_8O_4$	192.0422	$[M+H]^+$	4.1	193.0495	193.0495	0.00	Not found
28	Heptadecanoic acid [a]	$C_{17}H_{34}O_2$	270.2560	$[M-H]^-$	3.9	269.2493	269.2491	0.70	251.2378(21.4), 271.2540(18.2)
29	Cuminoid E [b]	$C_{24}H_{26}O_{10}$	474.1520	$[M-H]^-$	0.7	473.145	473.1453	0.60	373.1651(40.3), 355.1545(19.6)
30	Stearic acid [a]	$C_{18}H_{36}O_2$	284.2720	$[M-H]^-$	8.0	283.2654	283.2654	0.00	267.2688(27.0), 285.2794(13.4).

a = identified in *Anemone obtusiloba, b* = identified in *Anemone tetrasepala, c* = identified in *Anemone falconeri.*

istan, so have a huge chance of mixing together. Profiles of *A. obtusiloba* and *Z. jujuba* were generated using LC-ESI-QTOF MS/MS analysis and chromatograms were compared in Supplementary Fig. 2. Seven peaks A, B, C, D, E, F, H and G having retention times 0.7, 1.1, 1.3, 1.9, 4.3, 8.1 and 8.5 min, respectively, were taken as distinguishing character of *A. obtusiloba* (Fig. 5). These peaks were absent in profile of *Z. jujuba* and were thus used to detect the presence of *A. obtusiloba* as adulterant. One of the peaks was identified as compound, cyanidin dihexoside (E), while rest were unidentified compounds. Samples with different percentages (w/w) of *A. obtusiloba* mixed in *Z. jujuba* were analysed and peak areas of selected marker peaks were checked (Fig. 6). All the samples showed significantly intense peaks up to limit of 20% mixing while in samples with less ratio of mixing, peak intensities were not much clear. It was deduced that the developed method was able to detect as low as 20% adulteration of *A. obtusiloba* in *Z. jujuba*. A detailed chemical fingerprinting of *Z. jujuba* has already been reported (Khan et al., 2020).

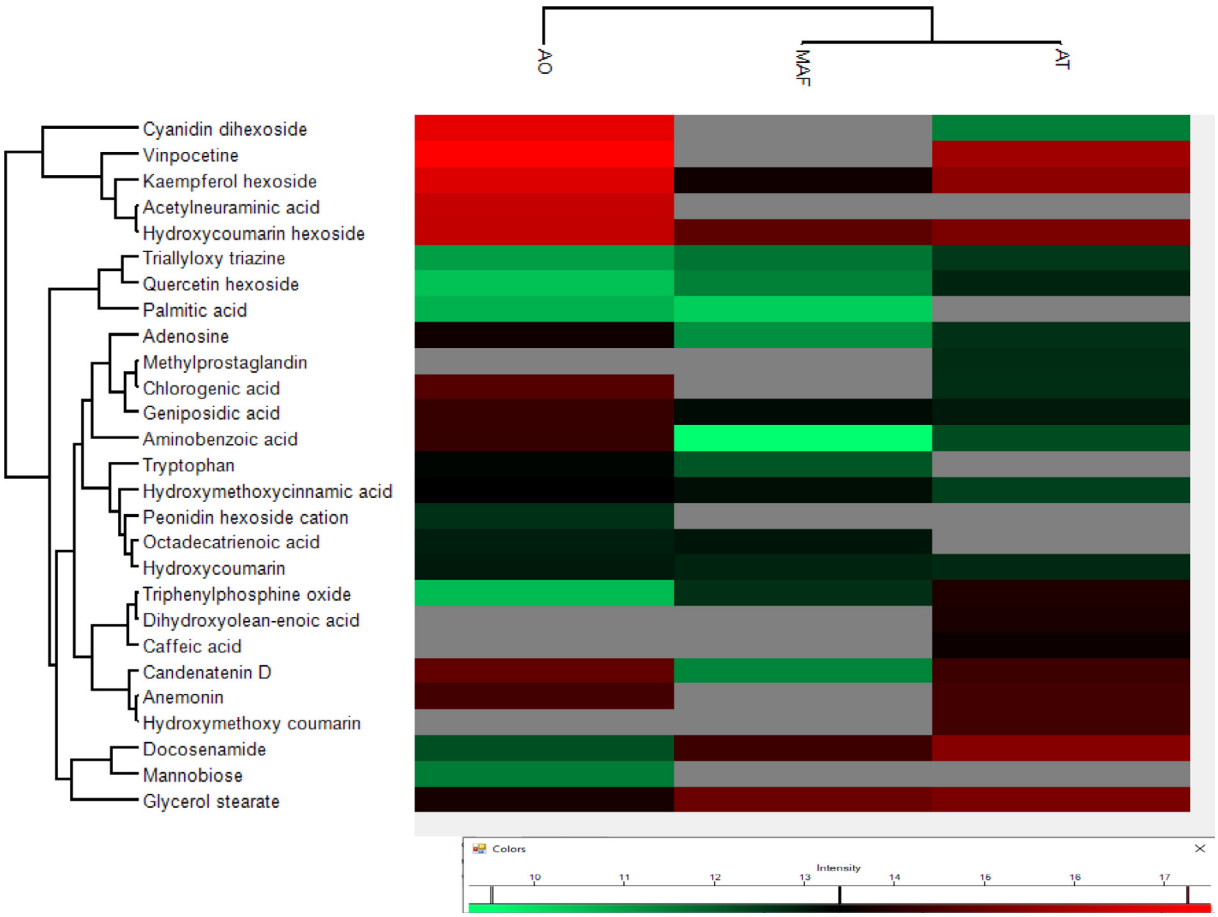

Fig. 3. Heat map cluster analysis of identified compounds in positive mode.

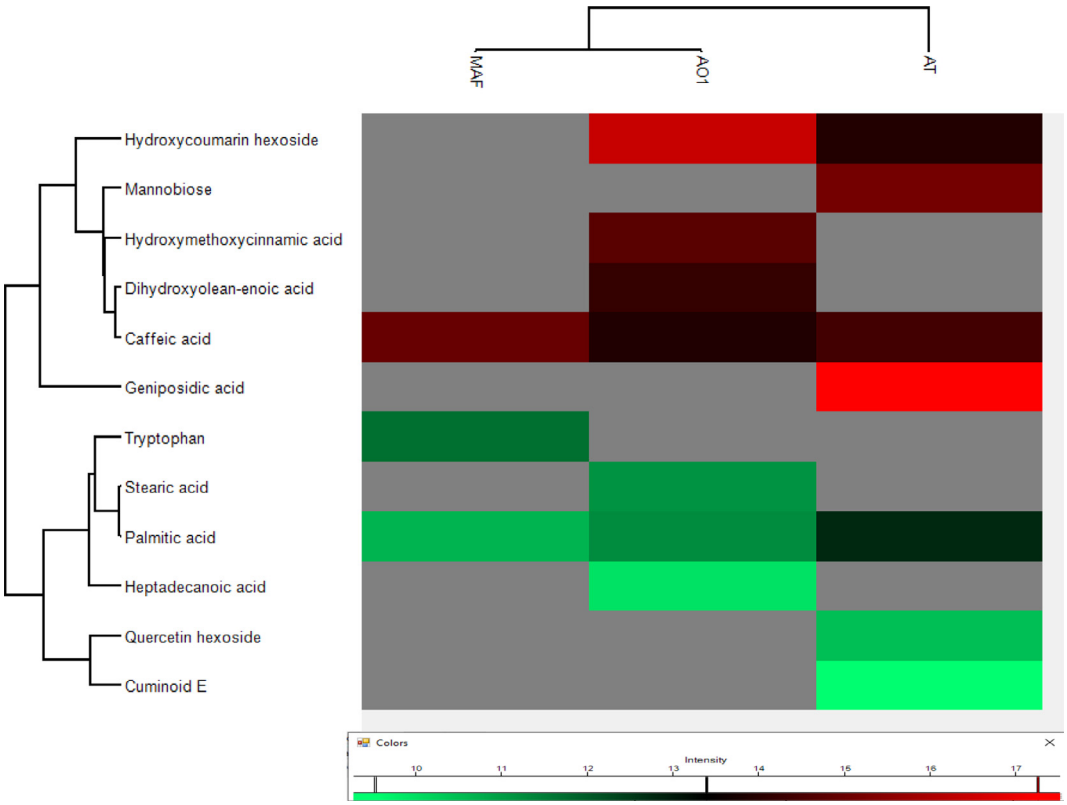

Fig. 4. Heat map cluster analysis of identified compounds in negative mode.

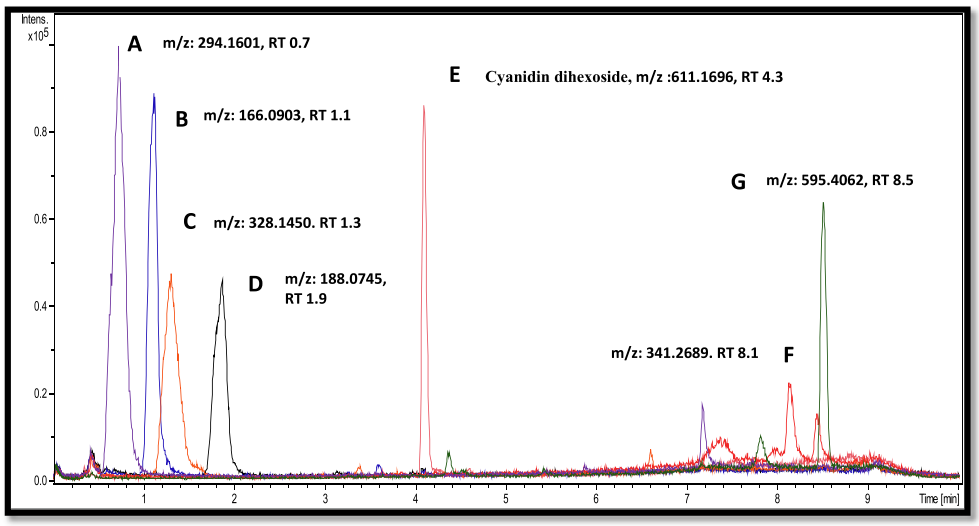

Fig. 5. Extracted ion chromatogram (EIC) of selected peaks from *Anemone obtusiloba.*

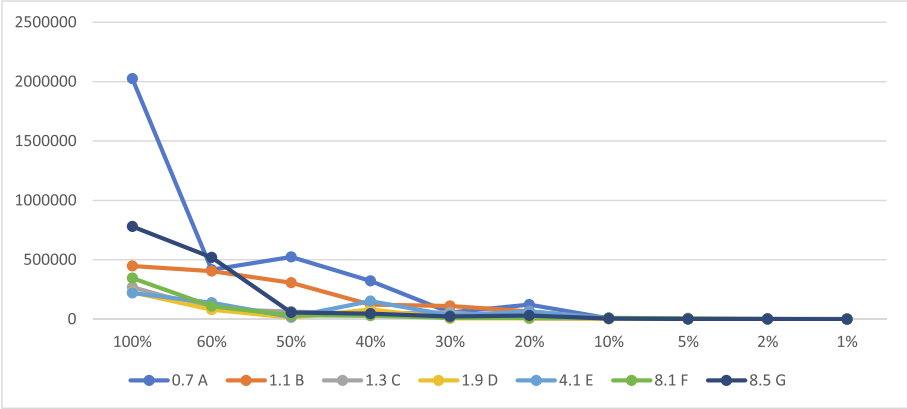

Fig. 6. Areas of differentiative peaks with different ratios of *A. obtusiloba* mixed in *Z. jujuba.*

4. Conclusion

The present work is focused on the development of a method for the identification of natural products in three *Anemone* species. A total of thirty compounds belonging to various classes of natural products were identified using LC-ESI-MS/MS approach. A sensitive and simple method was designed to identify the adulteration of *A. obtusiloba* (grows as weed) with *Ziziphus jujuba.* The method was able to detect as low as 20% mixing of *A. obtusiloba* in medicinal plant using seven distinguishing peaks.

5. Future prospects

This work will help to develop quality control procedures for prevention of accidental adulteration of *A. obtusiloba in Ziziphus jujuba.* The developed chemical profiles of three species will also be useful in natural product study, drug discovery, plant taxonomy and targeted isolation of bioactive metabolites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the support of Organization for the Prohibition of Chemical Weapons (OPCW) for research funding under project number L/ICA/ICB/210500/17. The authors also like to acknowledge Mr. Arsalan Tahir and Mr. Junaid Ul Haq for their technical assistance in UHPLC-MS/MS analysis and Mr. Shabir Ijaz for plants collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2021.101461.

References

LIANG, Y., XIE, P., CHAN, K., 2004. Quality control of herbal medicines. J. Chromatogr. B 812 (1-2), 53–70.
Zhang, J., Wider, B., Shang, H., Li, X., Ernst, E., 2012. Quality of herbal medicines: challenges and solutions. Complementary therapies in medicine. 20 (1-2), 100–106.
Qi, Z., 2013. Who traditional medicine strategy 2014–2023. World Health Organization, Geneva.
Organization, W.H., 2000. General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization.

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules *via* high resolution mass spectrometry: communicating confidence. ACS Publications 48 (4), 2097–2098.

Mirakilova, D.B., Rakhimov, D.A., Azizov, U.M., 2016. Chemical Compositions of Fruit and Dry Aqueous Extract of *Ziziphus jujuba*. Chem. Nat. Compd. 52 (3), 472–474.

Hao, D.-C., Gu, X., Xiao, P., 2017. *Anemone* medicinal plants: ethnopharmacology, phytochemistry and biology. Acta pharmaceutica sinica B. 7 (2), 146–158.

Xiao, P., Wang, L., Giu, G., 1989. Statistical analysis of ethnopharmacologic data based on Chinese medicinal plants using a computer (II) Hamamelidae and Caryophyllidae. Zhong xi yi jie he za zhi= Chinese journal of modern developments in traditional medicine. 9 (7).

Zou, Z.-j., Liu, H.-x., Yang, J.-s., 2004. Chemical constituents and pharmacological activities of genus *Anemone*. CHINESE PHARMACEUTICAL JOURNAL-BEIJING-. 39 (7), 493–495.

Lu, J., Sun, Q., Sugahara, K., Sagara, Y., Kodama, H., 2001. Effect of six compounds isolated from rhizome of *Anemone raddeana* on the superoxide generation in human neutrophil. Biochem. Biophys. Res. Commun. 280 (3), 918–922.

Cao, P., Wu, F., Ding, L., 2004. Advances in the studies on the chemical constituents and biologic activities for *Anemone* species. Natural Product Research and Development. 16 (6).

HAO, D.-C., XIAO, P.-G., MA, H.-Y., PENG, Y., HE, C.-N., 2015. Mining chemodiversity from biodiversity: pharmacophylogeny of medicinal plants of Ranunculaceae. Chinese journal of natural medicines. 13 (7), 507–520.

Da, H., Gu, X.J., Xiao, P.G., 2015. Medicinal plants: Chemistry, biology and omics. Woodhead Publishing.

Lee, T.H., Huang, N.K., Lai, T.C., Yang, A.T.Y., Wang, G.J., 2008. Anemonin, from *Clematis crassifolia*, potent and selective inducible nitric oxide synthase inhibitor. J. Ethnopharmacol. 116 (3), 518–527.

Quattrocchi U. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology (5 Volume Set): CRC Press; 2016.

Kaul, M.K., 1997. Medicinal Plants of Kashmir and Ladakh: Temperate and Cold Arid Himalaya. Indus Publishing Company.

Khan, M.N., Ul Haq, F., Rahman, S., Ali, A., Musharraf, S.G., 2020. Metabolite distribution and correlation studies of *Ziziphus jujuba* and *Ziziphus nummularia* using LC-ESI-MS/MS. J. Pharm. Biomed. Anal. 178, 112918. https://doi.org/10.1016/j.jpba.2019.112918.