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Research Article

Gas Chromatography Mass Analysis (GCMS) of fruits and leaves extract of *Ziziphus nummularia* to find out the functional group of the active components by the Maceration Process and Hot continuous extraction method by Soxhlet apparatus

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Abstract

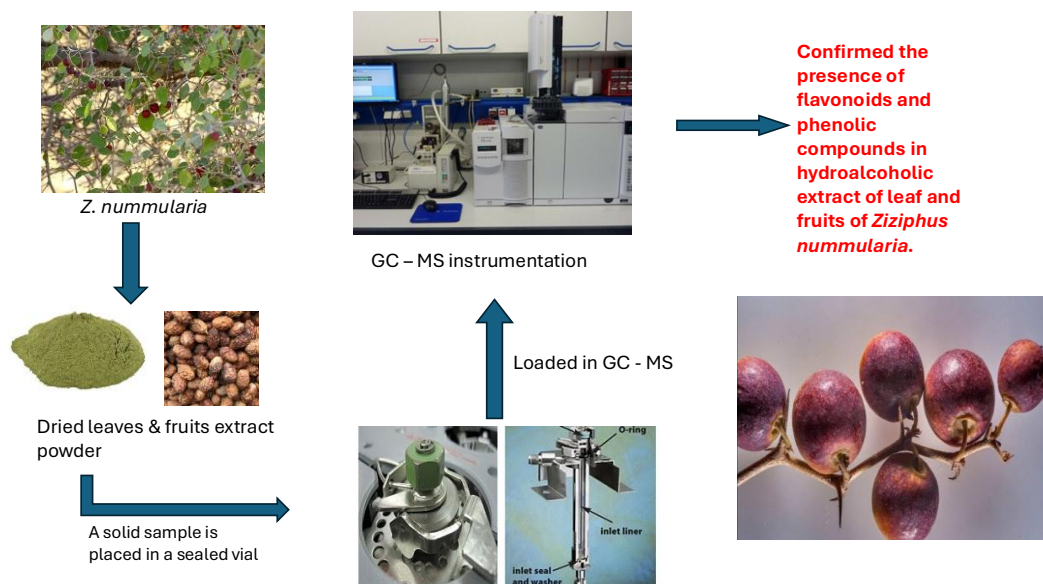
Ziziphus nummularia (Burm. f.) Wight and Arn. (Rhamnaceae), commonly known as "Jharberi, and as Bairi or Karkanrha in Pakistan, is a branched thorny shrub with a height of 1 - 2 m found in Pakistan, India, and Iran. This plant is known for its palatable and vitamin C-rich fruits. *Ziziphus nummularia* leaves and fruits are used for mental retardation, preventing frequent attacks of colds and influenza, treating diarrhoea, dysentery and colic, indigestion, inflammation of gums and tonic. The unripe fruits of the plant are prescribed in the management of vomiting, burning sensations and as tonic, while dried fruits are useful as an anticancer, sedative, stomach ache and in treatment of anaemia, bronchitis, burns, chronic fatigue, diarrhoea, hysteria, loss of appetite and pharyngitis.

The objective of the present research investigations to confirm various active components (antioxidants) present in leaves and fruit extract of *Ziziphus nummularia* so this plant further used as an antidiabetic by reducing free radicals.

The mass analysis performed for identification of phytoconstituents present in leaf and fruit extracts of *Z. nummularia*. Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. GC - MS analysis of leaves extract of *Ziziphus nummularia* prepared by maceration and soxhlet method revealed the existence of 9 and 15 compounds respectively. GC - MS analysis of fruits extract of *Ziziphus nummularia* prepared by maceration and soxhlet method revealed the presence of 7 and 10 phytocompounds.

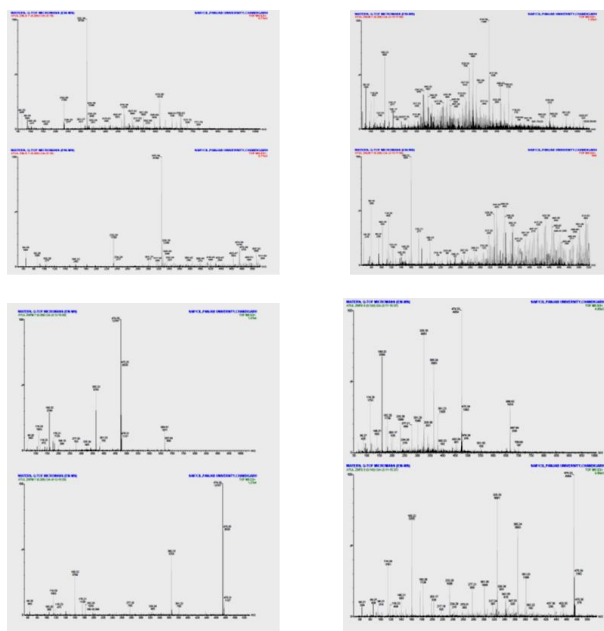
Keywords: Gas Chromatography, Mass Analysis, Extraction, *Ziziphus nummularia*, antidiabetic

Graphical Abstract





GCMS graphs leaf and fruit extract by maceration and soxhlation



Introduction

The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India, out of which 150 species are used commercially on a large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world ¹. A list of medicinal plants with proven antidiabetic and related beneficial effects and of herbal drugs used in the treatment of diabetes is compiled ^{2,3}. These include *Allium sativum*, *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenumgraecum* and *Withania somnifera* ³. To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore.

Methods

Characterization of *Ziziphus nummularia*

Ziziphus nummularia, also called Jharberi, is species of *Ziziphus* native to the western India and south-eastern Pakistan and south Iran ⁴. *Ziziphus nummularia* leaves and fruits are used for mental retardation, preventing frequent attacks of colds and influenza, treating diarrhoea, dysentery and colic, indigestion, inflammation of gums and tonic ⁵. The unripe fruits of the plant are prescribed in the management of vomiting, burning sensations and as tonic, while dried fruits are useful as an anticancer, sedative, stomach ache and in treatment of anaemia, bronchitis, burns, chronic fatigue, diarrhoea, hysteria, loss of appetite and pharyngitis ⁶. It is also used for wound healing, ulcers, irritability, fever and as nerve tonic.

Chemical constituents are present in leaves and fruits of *Ziziphus nummularia* are Glutamine synthetase, nitrate reductase and glutamine dehydrogenase, Ascorbic acid (vitamine C), nummularine-T, nummularine-M and N, Nummularine E, nummularine S, frangulofoline,

nummularine R, sterols and/or triterpenes, alkaloids, flavonoids, tannins and saponins ⁷, nummularine-P (I), mauritine-D, jubanine B, nummularine O (I), Jubanine-A, -B, and mauritine-C, N-desmethyl-jubanine-B (I) ⁸, nummularogenin, (25S)-3 β -hydroxy-5 β -spirostane-2,12-dione (I), nummularine B, nummularine M (I), nummularine N (II), Zizynummin, sitosterol, stigmasterol, betulinic acid, oleanolic acid, ceanothic acid, β -D-glucosides of sitosterol and stigmasterol, n-octacosanol and quercetin-3-O-galactoside, Peptidal alkaloids nummularine-G (I), -H (II), and -K (III), steroidal sapogenin, manogenin, and the flavanonol, taxifolin, Nummularine D and F, frangulofoline, amphibine A, intergerrenine, and mauritine F, mucronine D, amphibine H, nummularine A,B,C, vitamin A, Na, K, Ca, Mg, Fe, Al, Cu, nummularine-T, nummularogenin, (25S)-3- α -hydroxy-5- α -spirostane-2, 12-dione echinocystic acid-3-o- β -D-galactopyranoside ⁹.

Mass spectrometry and Gas Chromatography

Mass spectrometry is fast becoming an indispensable field for analysing biomolecules. The only analytical techniques that provided similar information were electrophoretic, chromatographic or ultracentrifugation methods. The results were not absolute as they were based on characteristics other than the molecular weight. Thus, the only possibility of determining the exact molecular weight of a macromolecule was to calculate it based on its chemical structure.

The development of desorption ionization methods based on the emission of pre-existing ions such as plasma desorption (PD), fast atom bombardment (FAB) or laser desorption (LD), allowed the application of mass spectrometry for analysing complex biomolecule.

Result and Discussion

GC – MS is the best technique to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, and esters etc. Peak area, retention time, and molecular formula were used for the

confirmation of phytochemical compounds. The active principles with their retention time (RT), peak area in percentage, molecular weight, molecular formula, and compound names present in the leaves and fruits extract prepared by maceration and soxhlet method are given in Tables 1.1 to 1.4.

GC – MS analysis of leaves extract of *Ziziphus nummularia* prepared by maceration and soxhlet method revealed the existence of 9 and 15 compounds respectively. GC – MS analysis of fruits extract of *Ziziphus nummularia* prepared by maceration and soxhlet method revealed the presence of 7 and 10 phytochemicals respectively. Table 1.5 to 1.8 shows the phytochemical compounds and their biological activities present in the leaves and fruits extract of *Ziziphus nummularia*.

The chromatogram of leaves extract prepared by maceration fig (2.1, 2.2) shows 9 prominent peaks as Pentane, 3-methyl with retention time 1.56 and peak area of 0.599%, 4- Decylaniline with retention time 6.36 and peak area of 1.705%, di- n- Undecylamine with retention time 17.36 and peak area of 6.709%, Succinic acid 8 chloro ethyl penta decyl ester with retention time 4.90 and peak area 1.270%, Benzamide, N-N, diundecyl 3 bromo with 3.305 retention time and 0.880% peak area, 1- Valine, n heptafluoro butyl-hepta decyl ester with retention time 3.004 and peak area 0.808%, Iron, tetra- μ 3-carbonyltetra- π -cyclopentadienyltetra-, tetrahedro-; Cyclopentadienylcarbonyl tetramer with retention time

of 1.759 and peak area 0.639%, Syroscingopine with retention time 2.004 and peak area 0.738%, Tridecaethylene glycol monomethyl ether with retention time 2.01 and peak area 0.752% (Table 1.1, 1.2).

The chromatogram of leaves extract by soxhlet method (Fig.2.3,2.4) shows 15 prominent peaks as 1, 3-cyclopentadiene, 1- methyl with retention time 3.052 and peak area 0.508%, Hexane 3 ethyl with retention time 2.001 and peak area 0.406%, Decane 1- fluoro with retention time 8.045 and peak area 0.909%, Bronopol with retention time 1.026 and peak area 0.201%, 1,7 - octa decyonic acid 4,4- di methyl oxazoline diox derivatives with retention time 5.074 and peak area 0.478%, Cis - 10- nonadecyonic acid with retention time 2.141 and peak area of 0.483%, Propionyl tetradecyl ester with retention time 2.145 and peak area of 0.329%, Tetrahexylammonium bromide with retention time 3.152 and peak area of 0.381%, Silane with retention time 3.147 and peak area of 0.354%, Decanic acid with retention time 3.158 and peak area of 0.363%, Isoleucine with retention time 5.223 and peak area of 0.756%, Pentamethyl pi cyclopenta dienyl with retention time 15.221 and peak area of 1.348, Ascorbic acid with retention time 1.866 and peak area of 0.305%, Cyclononasiloxane with retention time 1.874 and peak area of 0.526%, Tridecaethylene glycol with retention time 2.568 and peak area of 0.513% (Table 1.3, 1.4).

Table 1: GC-MS analysis of leaves extract of *Z. nummularia* by maceration:

s.no	Retention time	% Area	Molecular weight	Molecular formula	Compound name
1	1.563	0.599	86.26	C ₆ H ₁₄	Pentane, 3 - methyl
2	6.368	1.705	233.28	C ₁₆ H ₂₇ N	4- Decylaniline
3	17.366	6.709	325.34	C ₂₇ H ₄₇ N	di- n- Undecylamine
4	4.906	1.270	474.34	C ₇ H ₁₂ O ₄	Succinic acid, 8 chloro ethyl penta decyl ester
5	3.305	0.880	507.52	C ₇ H ₆ BrNO	Benzamide, N-N, diundecyl 3 bromo
6	3.004	0.808	551.55	C ₅ H ₁₀ NO ₂	1- Valine, n heptafluoro butyl-hepta decyl ester
7	1.759	0.639	595.60	C ₂₄ H ₂₀ Fe ₄ O ₄	Iron, tetra- μ 3-carbonyltetra- π -cyclopentadienyltetra-, tetrahedro-; Cyclopentadienyliron carbonyl tetramer
8	2.004	0.738	666.61	C ₃₅ H ₄₂ N ₂ O ₁₁	Syroscingopine
9	2.019	0.752	700.63	C ₁₀ H ₂₂ O ₄	Tridecaethylene glycol monomethyl ether,

Table 2: GC-MS analysis of fruits extract of *Z. nummularia* by maceration:

S.no	Retention time	% area	molecular weight	Molecular formulae	compound name
1	3.512	0.943	64.20	B ₅ H ₉	pentaborane
2	11.669	1.953	114.24	C ₈ H ₁₈	hexane 3 ethyl
3	5.328	0.472	118.23	C ₈ D ₁₀	1, 4 -Di (methyl- d3) benzene d4
4	17.996	3.764	160.22	C ₇ H ₂₀ N ₄	1, 3-propanediamine, N, N' - bis(2 aminoethyl)
5	8.023	1.129	176.21	C ₁₃ H ₂₀	cyclohexane, 1, 3 diisopropenyl(6- methyl)
6	19.001	5.79	365.23	C ₁₉ H ₃₁ N ₃ O ₄	9z, 11E - TetraDecadiene 1- yl Acetate- adduct with 4 methyl-1, 2, 4
7	10.277	1.87	666.61	C ₃₅ H ₄₂ N ₂ O ₁₁	Syroscingopine

Table 3: GC-MS analysis of leaves extract of *Z. nummularia* by soxhlation:

s.no	Retention time	% area	Molecular weight	Molecular formula	Compound name
1	3.052	0.508	80.16	C ₉ H ₁₄	1,3- cyclopentadiene, 1- methyl
2	2.001	0.406	114.24	C ₈ H ₁₈	Hexane 3 ethyl
3	8.045	0.909	160.22	C ₁₀ H ₂₁ F	Decane 1- fluoro
4	1.026	0.201	198.17	C ₃ H ₆ BrNO ₄	Bronopol
5	5.074	0.478	333.32	C ₂₂ H ₄₁ NO	1,7 – octa decyonic acid 4,4- di methyl oxazoline dmo derivatives
6	2.141	0.483	349.32	C ₁₅ H ₂₈ O ₂	Cis – 10- nonadeceonic acid
7	2.145	0.329	417.39	C ₁₇ H ₃₂ O ₂	Propionyl tetradecyl ester
8	3.152	0.381	433.38	[CH ₃ (CH ₂) ₅] ₄ N(Br)	Tetrahexylammonium bromide
9	3.147	0.354	445.39	SiH ₄	Silane
10	3.158	0.363	513.53	C ₁₀ H ₂₀ O ₂	Decanic acid
11	5.223	0.756	529.53	C ₆ H ₁₃ NO ₂	Isoleucine
12	15.221	1.348	616.58	C ₁₀ H ₁₀ V ⁻⁶	Pentamethyl pi cyclopenta dienyl
13	1.866	0.305	632.59	C ₆ H ₈ O ₆	Ascorbic acid
14	1.874	0.526	666.63	C ₁₈ H ₅₄ O ₉ Si ₉	Cyclononasiloxane
15	2.568	0.513	700.63	C ₆ H ₁₄ O ₄	Tridecacylene glycol

Table 4: GC-MS analysis of fruits of *Z. nummularia* by soxhlation:

Sno.	Retention time	% area	Molecular weight	Molecular formula	Compound name
1	1.003	0.426	86.27	C ₆ H ₁₄	Butane 2,3 – dimethyl
2	2.303	1.761	114.24	C ₈ H ₁₈	Hexane 3 ethyl
3	6.125	3.355	160.22	C ₇ H ₂₀ N ₄	1,3 – propanediamine, N-N'-bis (2-amino ethyl)
4	1.529	1.134	182.20	C ₁₃ H ₂₆	Heptylcyclohexane
5	1.123	1.096	233.28	C ₁₈ H ₃₆	4- decylcyclo hexane
6	5.175	1.046	301.26	C ₂₀ H ₃₁ NO	Trihexyphenidyl
7	7.063	4.001	325.35	C ₂₂ H ₄₇ N	di- n- undecylamine
8	5.956	3.065	365.24	C ₂₂ H ₄₄ O ₃	Sarcosine, N- (3-fluorobenzoyl-,undecyl ester)
9	8.004	4.954	474.33	C ₆ H ₁₀ O ₄	Adipic acid
10	2.009	1.614	666.02	C ₁₈ H ₅₄ O ₉ Si ₉	Octadecamethyl siloxane

Table 5: Biological activity of phytochemicals of leaves extract through maceration

s.no	Compound name	Biological activity
1	Pentane, 3 – methyl	Antidiabetic, antifungal, antitumor
2	4- Decylaniline	No activity reported
3	di- n- Undecylamine	Antimicrobial, antibacterial
4	Succinic acid, 8 chloro ethyl penta decyl ester	Antimicrobial, antioxidant, ovicidal
5	Benzamide, N-N, diundecyl 3 bromo	No activity reported
6	1- Valine, n heptafluoro butyl-hepta decyl ester	Antimicrobial
7	Iron, tetra-μ3-carbonyltetra-π-cyclopentadienyltetra-, tetrahedro-; Cyclopentadienyliron carbonyl tetramer	Antitumor
8	Syrosingopine	Antidiabetic, anticancer
9	Tridecaethylene glycol monomethyl ether, trifluoro acetate	No activity reported

Table 6: Biological activity of fruits extract through maceration:

Sr. no	Compound name	Biological activity
1	pentaborane	No activity reported
2	hexane 3 ethyl	Antimicrobial
3	1, 4 -Di (methyl- d3) benzene d4	No activity
4	1, 3-propanediamine, N, N'- bis(2 aminoethyl)	Antitumor
5	cyclohexane, 1, 3 diisopropenyl(6- methyl)	Antibacterial
6	9z, 11E - TetraDecadiene 1- yl Acetate- adduct with 4 methyl-1, 2, 4	Antibacterial
7	Syrosingopine	Antidiabetic, anti-cancer

Table 7: Biological activity of leaves extract through soxhlation:

s.no	Compound name	Activity
1	1,3- cyclopentadiene, 1- methyl	Antibacterial
2	Hexane 3 ethyl	Antimicrobial
3	Decane 1- fluoro	Antioxidant, antimicrobial
4	bronopol	Antibacterial, antimicrobial
5	1,7 - octa decyonic acid 4,4- di methyl oxazoline dmox derivatives	No activity reported
6	Cis - 10- nonadeceonic acid	Antimicrobial, anticancer
7	Propionyl tetradecyl ester	Antimicrobial, antibacterial
8	Tetrahexylammonium bromide	No activity reported
9	Silane	Antimicrobial
10	Decanic acid	Anti-inflammatory
11	Isoleucine	Bactericidal
12	Pentamethyl pi cyclopenta dienyl	Anticancer
13	Ascorbic acid	Antioxidant, antidiabetic
14	Cyclononasiloxane	Antioxidant, antidiabetic
15	Tridecaethylene glycol	Antibacterial, antimicrobial

Table 8: Biological activity of fruit extract through soxhlation:

Sr no	Compounds name	Activity
1	Butane 2,3 - dimethyl	Anti-inflammatory
2	Hexane 3 ethyl	Antioxidant, antimicrobial, antibacterial
3	1,3 - propanediamine, N-N'-bis (2-amino ethyl)	Antibacterial, antifungal
4	Heptylcyclohexane	Antimicrobial, antifibrotic
5	4- decylcyclo hexane	Antimicrobial, antibacterial
6	Trihexyphenidyl	No activity reported
7	di- n- undecylamine	Antimicrobial
8	Sarcosine, N- (3-fluorobenzoyl-,undecyl ester)	Antimicrobial, antibacterial
9	Adipic acid	Antimicrobial
10	Octadecamethyl siloxane	Antimicrobial

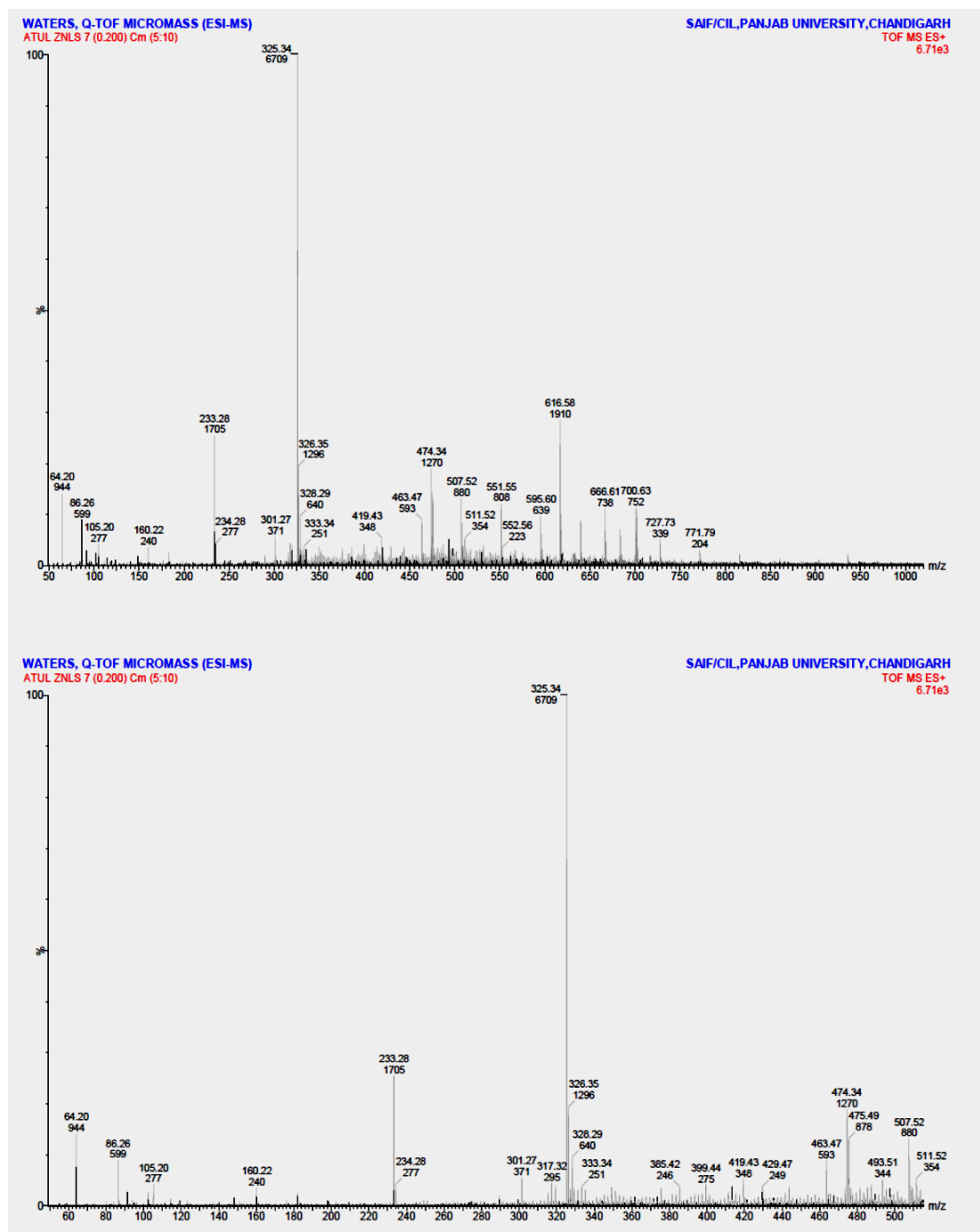


Figure 1: Chromatogram of MASS analysis of leaves extract using Maceration method

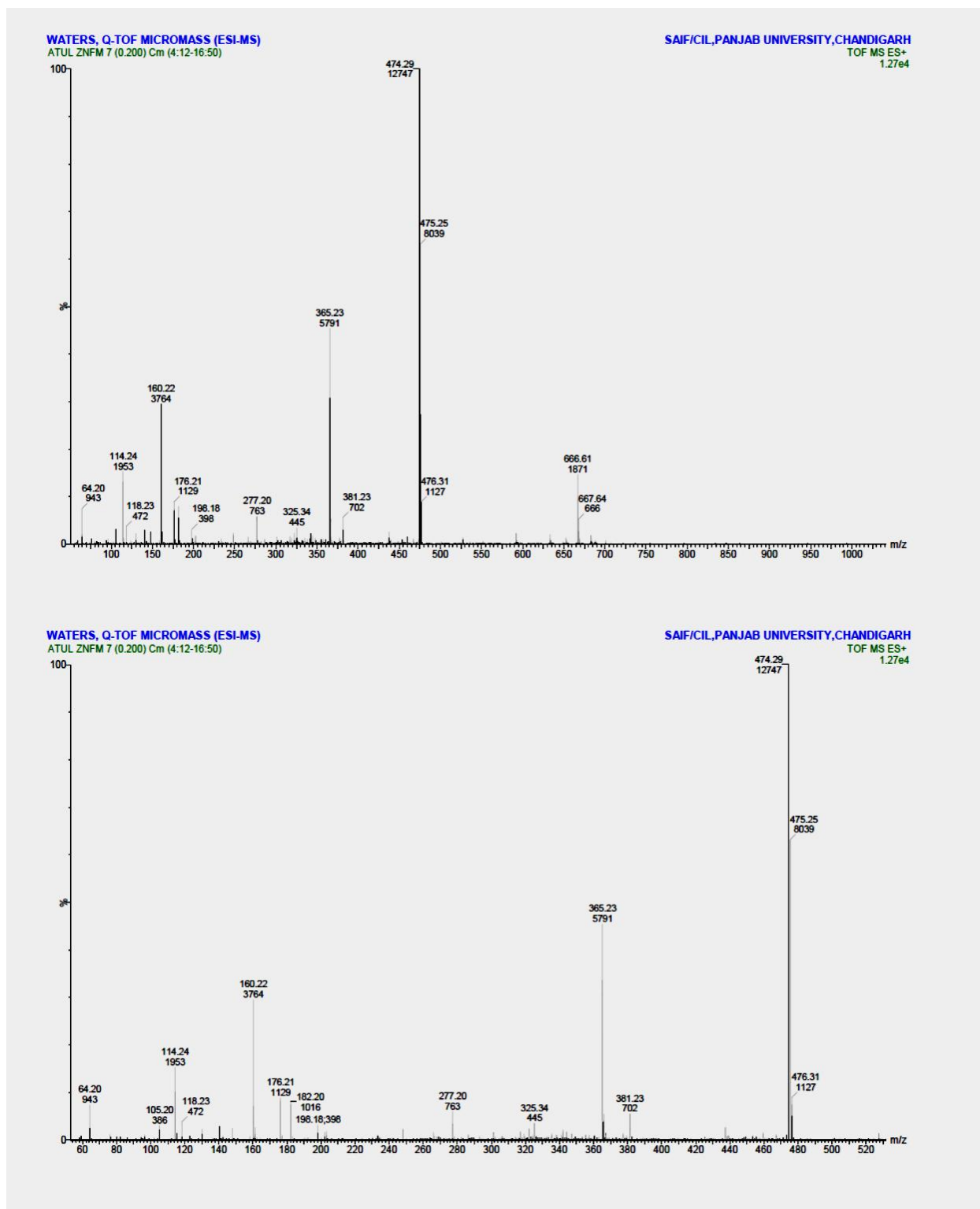


Figure 2: Chromatogram of MASS analysis of fruits extract using Maceration method

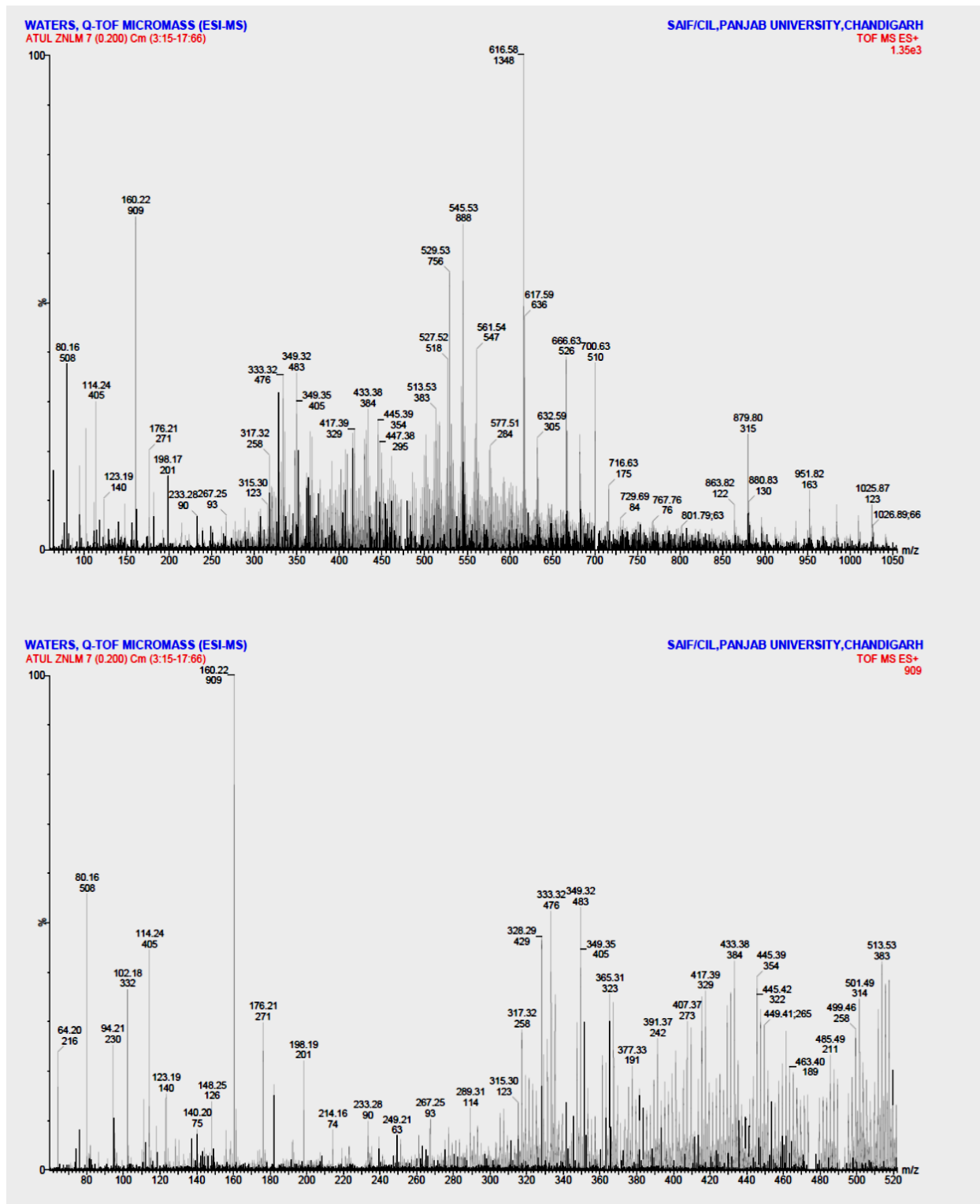


Figure 3: Chromatogram of MASS analysis of leaves extract using Soxhlet method

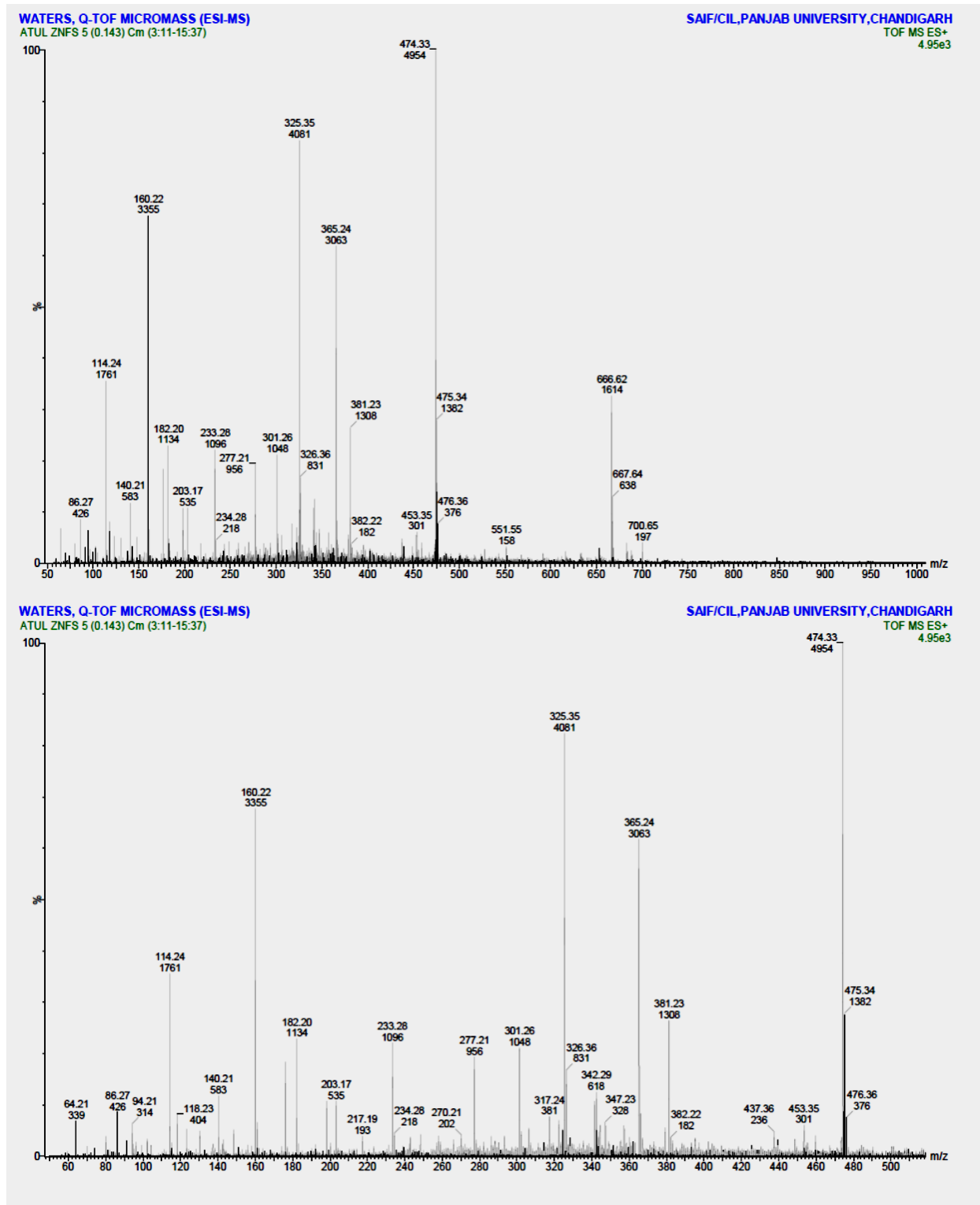


Figure 4: Chromatogram of MASS analysis of fruits extract using Soxhlet method

Conclusion

The major peaks along with many small peaks indicates the presence of major components. The small peaks may be attributed to the compound present in small quantities as well as disintegrated major compounds. The peaks related to low retention times are mainly low polar plant compounds. Metabolite profiling in plant species was done by GC – MS method for last few years but only a limited numbers laboratory has done GC – MS. The identified compound occupied many biological

properties. GC–MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical value of that plant. Thus, this type of GC–MS analysis is the first step towards understanding the nature of medicinal properties in the medicinal plant, and this type of study will be helpful for further detailed study.

Out of 37 isolated compounds of the present study Pentane 3 methyl, Syroingopine, Ascorbic acid, Cyclononasiloxane are major compounds of leaves and fruits of *Ziziphus nummularia* which are observed to have

antidiabetic activity. Ascorbic acid, succinic acid, 8 chloro ethyl Penta decyl ester, Decane-1-fluro, Cyclononasiloxane and hexane 3 ethyl are identified as antioxidants. Potential antidiabetic, antioxidant, antitumor activities were found in these compounds. The compounds identified by the initial qualitative analysis and GC – MS analysis have many biological properties and can be used in various purposes to treat many diseases. Most of the compounds identified have their unique characteristics to treat multiple diseases. Further studies are needed to reveal its importance in a specific field for treating diabetes.

In the field of plant senses, a natural product chemistry metabolomics has been developed to measure all metabolites both qualitatively and quantitatively, which can provide a clear picture of living organism under certain conditions. The plant metabolon is very complex as the metabolites present in a plant are all different regarding their polarity chemical behaviour, stability, and concentration. This makes the analysis of metabolites in one single experiment extremely difficult. GC – MS analysis of hydroalcoholic extracts of leaves and fruits of *Z. nummularia* reveals the presence of medicinally valued bioactive components like alkaloids, flavonoids, phenolic compounds, tannins, Saponines, steroids, cardiac glycosides. As the medicinal value of similar components in other plant extracts has already been proven, it is no wonder *Z. nummularia* may also be equally effective.

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Authors' contributions: The author conceived and designed the study, performed the experimental work, analysed the data, and prepared the manuscript.

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