



Phytochemicals and antioxidant activity of *Sisymbrium irio* L. seeds

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Sisymbrium irio (Family: *Cruciferae*) is used in Unani medicine. The qualitative and quantitative analysis showed the presence of different types of bioactive secondary metabolites for like phenols, flavonoids, saponins, terpenoids, except reducing sugar and cardiac glycosides. The antioxidant activity was checked by DPPH and ABTS free radical trapping methods by using standards such as ascorbic acid and trolox. The methanol extract of the Indian variety of the seeds of *Sisymbrium irio* showed a very high antioxidant activity. The major pharmacological bioactive chemical compounds identified in different solvent extracts of *Sisymbrium irio* were characterized as: 2, 4-Di-tert-butylphenol, n-Hexadecanoic acid, 3', 5'-Dimethoxyacetophenone, γ -Tocopherol, Cholesterol, 1-Nonadecene, γ -Sitosterol, Ergot-5-en-ol, (3- Beta, 24R)-3- Methylcrotononitrile, 1, 2 - Cyclopentadi-one, 1, 3 - Cyclohexanedione, 4-Isothiocyanato-1-Butene, Isosorbide, Sinapic acid methyl ester, 1, E-8, Z-10-Pentadecatriene and 7-Tetradecenal, (Z) and GC-MS fatty acid composition of the cold pressed seeds oil of plant showed the presence of major fatty acids such as: Linolenic acid (36.29%), Linoleic acid (17.99%), Oleic acid (12.58%), cis-11-Eicosanoic acid (9.2%), Erucic acid (9.19%) and Palmitic acid (6.66%). The elemental analysis of the seeds showed the presence of magnesium, calcium, strontium, titanium, chromium, manganese, copper, zinc, boron, aluminium, silicon, barium, iron, phosphorus, sodium, potassium and lead.

Keywords: Antioxidant activity, Elemental analysis, GC-MS analysis, Phytochemicals, *Sisymbrium irio* L. seeds

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The medicinal plants are the foundation of effective sources of bioactive compounds used as phytomedicines & the chemical compounds obtained from these therapeutic plants played vital role in the discovery of novel drugs for the treatment of the various categories of human, and animal diseases¹. They contain various types of secondary metabolites (phytochemicals) and these phytochemicals perform many physiological activities in humans and animals. The therapeutic plant *Sisymbrium irio* L belongs to the family *Cruciferae* and is called Khubakalan (Urdu), Asalio, Khubkalan; Khakasi, Khakshi (Persian), khubkhala (Hindi), Khubah (Arabic) and London Rocket/Rocket Mustard (Common names) and is found in various parts of the world². In 1980, Vohora *et al.*³, investigated the seeds of *Sisymbrium irio* L. Indian origin for antipyretic, analgesic and antimicrobial activity. In 1991, Khan *et al.*, isolated isorhamnetin, quercetin, β -sitosterol and β -sitosterol-3 β -D-glucoside from aerial parts of the *Sisymbrium irio* L. collected from the campus of Jamia Hamdard, New Delhi, India⁴. The phytochemical and biological studies (LD₅₀, antioxidant activity) of the aerial part of Saudi Arabia species (Najed Region) of *Sisymbrium irio* L.

contained flavonoids⁵, β -sitosterol, stigmasterol and β -sitosterol glucoside⁶. A research study on the investigation of Baghdad-Iraq (Al-Jadrea) species of *Sisymbrium irio* L. showed the presence of nicotine in the aerial part of the plant⁷. Another study conducted on the aerial part of the *Sisymbrium irio* L. collected from the Irbid area (north of Jordan) showed the oil comprised of four fatty alcohols (2.49%), five aromatic compounds (3.53%), six aliphatic hydrocarbons (6.29%), fifteen terpenes derivative (terpenoids) (8.19%), eleven sulfur and eleven nitrogen containing compounds (36.41%), two esters and seven acids (38.80%) and three additional compounds (1.17%)⁸. Rauf and Ahmed⁹ reported fatty acid distribution pattern in triacylglycerol (isolated from oil) of *S. irio*. Another research study conducted by Nengroo and Rauf stated the fatty acid composition of petroleum extract of *Sisymbrium irio* L. seed were: Palmitic acid C16: 0 (22.3%), Stearic acid C18:0 (6.1%), Oleic acid C18:1 (30.7%), Linoleic acid C18:2 (30.3%), Arachidic acid C20:0 (2.1%), Gondoic acid C20:1 (0.6%), Behenic acid C22:0 (0.5%), Erucic acid C22:1 (0.3%), Lignoceric acid C24:0 (0.3%), total unsaturated fatty acids (61.9%) and total saturated fatty acids (31.3%)¹⁰. The seeds of the Indian variety of *Sisymbrium irio* L. contained isorhamnetin¹¹ and is used

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as expectorant, febrifuge, rubefacient and are used in asthma, and in the preparation of poultices¹². In 1998, Guil *et al.*¹³, from Spain investigated the leaves of wild edible *Sisymbrium irio* L. (Hedge Mustard) for nutritional purposes because of the various amounts of nutrients (protein 3.43%, carbohydrates 1.43%, lipid 2.14%, oxalic acid/Calcium ratio 1.1) and fatty acids ω3 (46.57%) & ω6 (13.02%) together with erucic acid C22:1ω9 (2.52%). Ethanolic solvent extracts of fresh plant of *Sisymbrium irio* L. seeds collected at flowering stage from Peshawar University Campus, Pakistan reported the cytotoxic, phytotoxic and insecticidal activities¹⁴. Other studies conducted on Indian variety of ethanolic extract of *Sisymbrium irio* L., seeds on *in vitro* rat mast cells has a significant broncho protective role in mast cell degranulation induced by the compound 48/80 and active anaphylaxis¹⁵. In 2017, Gamal *et al.*, from Egypt reported the n-hexane extract of *Sisymbrium irio* L. leaves collected from Bahariya Oasis, inhibited the growth of microbial strains such as *Staphylococcus epidermidis* and *Klebsiella pneumoniae*, whereas the n-hexane extract of *Sisymbrium irio* L. seed demonstrated greater inhibitory effect against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The ethyl acetate fraction of the leaves of *Sisymbrium irio* L. was active against the gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and the aqueous extract of *Sisymbrium irio* L. was active to against all tested pathogenic microbial such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Acinetobacter baumannii*, *Enterobacter cloacae* and *Candida albicans* and the purified compound showed dose dependent cytotoxic activity against Vero cell line¹⁶. The various polarity-based extracts of the seeds and leaves of *Sisymbrium irio* L. collected from Islamabad and Rawalpindi showed inhibition against the bacterial and fungal strains¹⁷. A study conducted on aerial part of *Sisymbrium irio* L. collected from Al-Jadriya area of Baghdad-Iraq declared the methanol and ethyl acetate extract of the plant owns hepatoprotective activity against CCl₄ induced hepatotoxicity in rats¹⁸. Research conducted on the acute-toxicity, anti-inflammatory and bronchial smooth muscles investigation of Indian variety of *Sisymbrium irio* L. (seeds) is safe up to the doses of 1000 mg/kg and caused no mortality and normal behaviour on the tested animal¹⁹. In view of the significance of the plant, the present investigation was conducted to carry out the chemical examination, antioxidant and elemental analysis of seeds of Indian variety of *Sisymbrium irio* L. used in Unani medicine collected from Delhi.

Experimental Methods

Assortment and Documentation of the plant material

Seeds of *Sisymbrium irio* L. were obtained from Khari Baoli, Delhi, Indian Drugs House. Botanical specimens of the plant were identified by Dr. Mokhtar Alam, Central Council for Research in Ayurvedic Sciences, Ministry of Ayush, Government of India, New Delhi and the voucher specimen/file number 6238 was deposited at the Ministry of Ayush, Government of India, New Delhi.

Preparation of extracts

The seed oil of *Sisymbrium irio* L. was extracted by cold press machine, then 100 g of the powdered seeds was extracted sequentially with the solvents of increasing polarity viz; n-Hexane, Chloroform, Ethylacetate and Methanol for 48 h on a rotary shaker. The extracts obtained after extraction were filtered using Whatman filter paper and concentrated on a rotary vacuum evaporator.

Qualitative phytochemical tests

The qualitative phytoconstituents tests of *Sisymbrium irio* L. extracts were done using standard procedures²⁰⁻²².

Estimation of the total phenolic contents

The phenolic contents were estimated following Folin-Ciocalteu index protocol²³.

Estimation of the total flavonoid contents

The flavonoid contents were calculated according to some modifications of Khalil *et al.*²⁴.

DPPH free radical trapping activity

DPPH free radical trapping action of the solvent extracts was carried with some modification²⁵.

ABTS-scavenging analyses

The positive control such as trolox used for the analyses ABTS-antioxidant activity of the extracts²⁶.

Gas Chromatography-Mass Spectrometry analysis

Sisymbrium irio L. seeds extracts of n-hexane, chloroform, ethyl acetate and methanol were injected in GC-MS (Shimadzu GCMS-QP2010 Plus) for obtaining the results. The samples were introduced in split mode at 300°C. The oven was heated from 40°C (1 min) to 300°C (28 min). The column flow rate was 1.21 mL/min with Helium being the carrier gas. Metal quadrupole mass filter with pre-rod with a range of m/z 1.5–1000 was used in scan mode. Electron Ionization (EI) was used as the ionization mode. The bioactive phytochemicals present in the extracts were identified by means of their retention time and comparison of their mass spectra with the National Institute of Standard and Technology (NIST) library data and literature.

Table 1 — Phytochemical investigation of *Sisymbrium irio* seeds extracts

Phytochemicals	Test	n-Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Flavonoids	Alkaline test	+	+	+	++
Phenols	Ferric Chloride test	+	+	+	++
Terpenoids	Liebermann-Burchard test	-	+	+	+
Reducing sugar	Benedict's test	-	-	-	-
Saponins	Foam test	-	-	+	-
Cardiac glycosides	Salkowski test	-	-	-	-

+ = the presence of phytochemicals - = the absence of phytochemicals

Preparation of fatty acid methyl esters

In order to make fatty acids present in the seeds oil volatile, derivatization was done prior to GC-MS analysis. Derivatized fatty acid methyl esters were analyzed using a Shimadzu GC-2010.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

The seeds of *Sisymbrium irio* L. were milled, homogenized and digested by microwave. The elemental analysis of the sample was done by ICP-MS (Perkin Elmer Sciex Elan II DRC).

Results and Discussion

Phytochemical investigation of *Sisymbrium irio* L. seeds extracts

The qualitative phytochemical analysis showed the presence of flavonoids and phenols in all extracts, Terpenoids were found in chloroform, ethyl acetate and methanol extracts, except n-hexane extract. Saponins were only identified in the ethyl acetate extract. Reducing sugar and cardiac glycosides were not identified in any of the extracts (Table 1). The phytochemical compounds in the plant parts were recognized to be biologically active compounds and they had been accountable for diverse activity, for example, antimicrobial, antioxidant, antifungal and anticancer^{27,28}. The terpenoids show antiviral, antibacterial, antimalarial, anti-inflammatory and anticancer activities²⁹. The phenolic compounds are known for anti-microbial activity³⁰, antiviral, antimutagenic, antioxidant, antiinflammatory, anticarcinogenic activities³¹⁻³³.

Quantitative estimation of the total phenolic contents

Phenolic compounds widely distributed in plant parts are beneficial to human health due to their antioxidant activity^{34,35}. Quantitative estimation of the total phenolic contents varied from n-hexane extract to methanol extract of *Sisymbrium irio* L. seeds ranging from 7.8 ± 0.278 to 55.3 ± 0.319 $\mu\text{g GAE}/\text{mg}$ of dry extract (Fig. 1). Methanol extract contained the highest percentage of total phenolic components, followed by ethyl acetate, chloroform and n-hexane extracts.

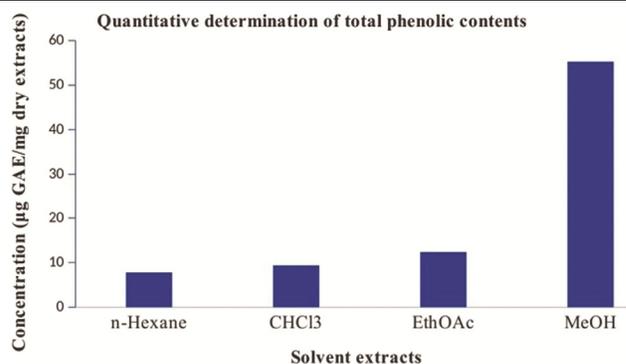


Fig. 1 — Total phenolic contents in different extracts of *Sisymbrium irio* seeds

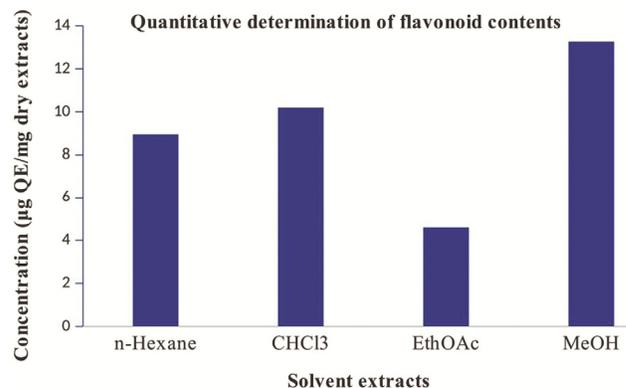


Fig. 2 — Total flavonoid content in the seeds extracts of *Sisymbrium irio*

Quantitative estimation of the total flavonoid contents

Total flavonoids content varied from 8.958 ± 0.420 to 13.266 ± 0.015 $\mu\text{g QE}/\text{mg}$ of dry extract (Fig. 2). Methanol extract contained the highest number of flavonoid contents, followed by chloroform and n-hexane and ethyl acetate extracts. Unlike phenolic contents, the ethyl acetate extract contained less amount of flavonoids as compared to other extracts.

DPPH radical scavenging activity

We analysed the antioxidant activity test of extracts via DPPH (Diphenylpicrylhydrazyl) and ABTS (2, 2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) test. Diphenylpicrylhydrazyl is a steady free radical

compound & has an absorbance in its oxidized form to 515-520 nm^{36,37}. Diphenylpicrylhydrazyl is moderately fast & productive technique to assess free radical. The colour changes, from purple to yellow demonstrates a decline in absorbance of DPPH radical. This is the confirmation of the interaction of the antioxidant found in sample with the free

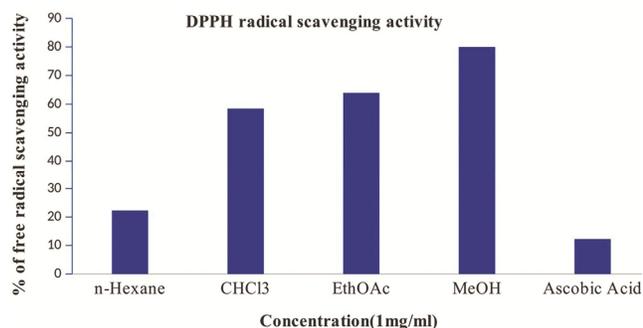


Fig. 3 — % of free radical scavenging assay of seeds extracts of *Sisymbrium irio*

radicals³⁸. DPPH free radical scavenging activities of seed extracts of Diphenylpicrylhydrazyl L extended from 22.3% to 79.94%. The methanol extract showed the highest percentage of free radical scavenging activity as shown (Fig. 3) at the concentration of 1 mg/mL.

ABTS radical scavenging assay

The solvent extracts of *Sisymbrium irio* L. exhibited ABTS free radical scavenging activities ranging from 43% to 94.3%. Out of all the extracts, methanol extract as shown in (Fig. 4) contain the highest percentage of free radical scavenging activity.

Gas Chromatography-Mass Spectrometry analysis of *Sisymbrium irio* L. seeds extracts

The main constituents of the seeds extracts of *Sisymbrium irio* L. identified by GC-MS as shown in (Table 2) was found to be 2, 4-Di-tert-butylphenol, 3', 5'-Dimethoxyacetophenone, n-Hexadecanoic acid, 7,10,13-Hexadecatrienal, 9, 12-Octadecadienoic acid

Table 2 — GC-MS analysis of bioactive compounds from seeds extracts of *Sisymbrium irio*

Name of compounds	R. time	Area%	n-Hexane extract		Present/Absent	Chloroform extract	Ethyl acetate extract	Methanol extract
			Mol. formula	M.wt		Present/Absent	Present/Absent	Present/Absent
2,4-Di-tert-butylphenol	24.408	0.60	C ₁₄ H ₂₂ O	206	+	+	-	-
3',5'-Dimethoxyacetophenone	25.668	0.55	C ₁₀ H ₁₂ O ₃	180	+	+	+	+
n-Hexadecanoic acid	34.563	5.46	C ₁₆ H ₃₂ O ₂	256	+	+	+	-
9,12-Octadecadienoic acid (Z, Z)-	38.060	37.57	C ₁₉ H ₃₄ O ₂	294	+	-	-	-
9,12-Octadecadienoic acid (Z, Z)-	38.090	5.02	C ₁₉ H ₂₆ O ₂	280	+	-	-	-
cis, cis, cis-7,10,13-Hexadecatrienal	38.158	24.08	C ₁₆ H ₂₆ O	234	+	-	-	-
γ-Tocopherol	51.491	3.26	C ₂₈ H ₄₈ O ₂	416	+	-	+	-
Cholesterol	52.383	1.91	C ₂₇ H ₄₆ O	386	+	+	+	-
γ-Sitosterol	54.859	9.11	C ₂₉ H ₅₀ O	414	+	+	+	+
1-Nonadecene	30.955	-	C ₁₉ H ₃₈	266	-	-	-	-
1-Nonadecene	35.001	-	C ₁₉ H ₃₈	266	-	+	-	-
(R)-(-)-14-Methyl-8-hexadecyn-1-ol	38.199	-	C ₁₇ H ₃₂ O	252	-	+	+	-
1-Heptacosanol	38.714	-	C ₂₇ H ₅₆ O	396	-	+	-	-
9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)	46.689	-	C ₁₉ H ₃₂ O ₂	306	-	-	+	-
Ergost-5-en-3-ol, (3. Beta, 24R)-	53.771	-	C ₂₈ H ₄₈ O	400	-	+	+	-
O-Ethyl S-2-dimethylaminoethylethylphosphonothiolate	43.630	-	C ₈ H ₂₀ NO ₂ PS	225	-	-	+	+
3-Methylcrotonitrile	4.378	-	C ₅ H ₇ N	81	-	-	-	+
1,2-Cyclopentanedione	8.183	-	C ₅ H ₆ O ₂	98	-	-	-	+
4-Isothiocyanto-1-Butene	9.683	-	C ₅ H ₇ NS	113	-	-	-	+
1,3-Cyclohexanedione, 2-methyl	13.267	-	C ₇ H ₁₀ O ₂	126	-	-	-	+
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	14.696	-	C ₆ H ₈ O ₄	144	-	-	-	+
Isosorbide	19.042	-	C ₆ H ₁₀ O ₄	146	-	-	-	+
2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	28.044	-	C ₁₃ H ₂₂ O	194	-	-	-	+
Sinapic acid methyl ester	36.682	-	238	C ₁₂ H ₁₄ O ₅	-	-	-	+
1, E-8, Z-10-Pentadecatriene	37.581	-	C ₁₅ H ₂₆	206	-	-	-	+
7-Tetradecenal, (Z)	37.701	-	C ₁₄ H ₂₆ O	210	-	-	-	+

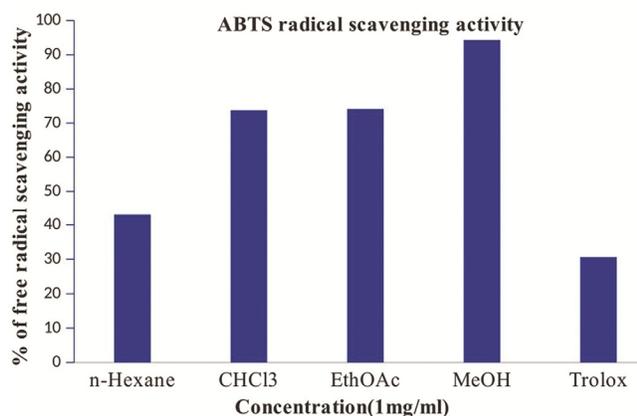


Fig. 4— % of free radical scavenging assay of seeds extracts of *Sisymbrium irio*

(Z,Z)-, γ -Tocopherol, Cholesterol, γ -Sitosterol, 1- Nonadecane, 1-Heptacosanol, 3-Methylcrotonitrile, 1,3-Cyclohexanedione, O-Ethyl S-2-dimethylaminoethylphosphonothiolate, 1, 2-Cyclopentadiene, Isosorbide, 4-Isothiocyanato-1-Butene, Sinapic acid ester, E-8, Z-10-Pentadecatriene, 9, 12, 15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z), 7-Tetradecenal and (R)-(-)-14-methyl-8-hexadecyn-1-ol. Phytochemical examination of the different extracts namely n-hexane, chloroform, ethyl acetate and methanol identified by GC-MS revealed the presence of different types of phytochemicals and these compounds may be responsible for various pharmacological activities such as hepatoprotective, antioxidant, wound healing, and antimicrobial activities.

GC-MS fatty acid profile of *Sisymbrium irio* L. cold pressed seed oil

The major fatty acid composition of *Sisymbrium irio* L. seed oil as shown in (Table 3) was found to be: Linolenic acid (36.29%), Linoleic acid (17.99%), Oleic acid (12.58%), cis-11-eicosenoic acid (9.2%), Erucic acid (9.19%), Palmitic acid (6.66%), Stearic acid (2.2%), Arachidic acid (1.75%) and cis-11, 14 Eicosenoic acid (0.95%). Linoleic acid (omega 6) is one of the fundamental fatty acids that are not created in the human body and should be given to the body from outside as such *Sisymbrium irio* L. seed oil is a good source of linoleic acid. Likewise, linoleic acid (omega 6) as a metabolic precursor of eicosanoids which forms important lipids like prostaglandins play an important role in inflammation, immunity and blood clotting.

Metal analysis of *Sisymbrium irio* L. seeds (ICP-MS)

Elemental analysis of *Sisymbrium irio* L. seeds showed the existence of various types of minerals in different concentration (Table 4). The minerals which

Table 3 — Fatty acid composition of *Sisymbrium irio* seeds oil

Peak No.	Component Name	Area (%)
1	C14 Myristic Acid	0.05
2	C15 Pentadecanoic Acid	0.03
3	C16 Palmitic Acid	6.66
4	C16: 1 Palmitoleic Acid	0.22
5	C17 Heptadecanoic Acid	0.07
6	C18 Stearic Acid	2.2
7	C18:1 Δ 9 Oleic Acid	12.58
8	C18:2 Δ 9, 12 Linoleic Acid	17.99
9	C18:3 Δ 9,12,15 Linolenic Acid	36.29
10	C20 Arachidic Acid	1.75
11	C20: 1 Cis-11 Eicosenoic Acid	9.2
12	C20: 2 Cis-11, 14 Eicosenoic Acid	0.95
13	C20:3 Cis-11, 14, 17-Eicosatrienoic Acid	0.87
14	C22 Behenic Acid	0.63
15	C22:1 Erucic Acid	9.19
16	C22:2 Cis-13, 16-Docosadienoic Acid	0.21
17	C24 Lignoceric Acid	0.28
18	C24:1 Nervonic Acid	0.81

Table 4 — Mineral content of *Sisymbrium irio* seeds

S. No.	Analyte	Sample concentration (ppm)
1	Mg	2940.1
2	Ca	5844.7
3	Sr	18.2
4	Ti	7.7
5	Cr	2.6
6	Mn	41.8
7	Co	bdl
8	Cu	5.5
9	Ni	bdl
10	Zn	40.7
11	B	1.9
12	Al	315.4
13	Si	101.6
14	Ba	3.7
15	Fe	672.8
16	Mo	bdl
17	Ag	bdl
18	P	6393.4
19	Na	54.7
20	K	8031.9
21	Pb	2.9
22	Bi	bdl
23	Cd	bdl
24	V	bdl

bdl: below detection limit

were analyzed from *Sisymbrium irio* L. seed is very useful for the health of human beings. The elements for example calcium, phosphorus and magnesium may be helpful in the buildings of our bones; potassium and sodium support in the preservation of normal blood pressure. The metal iron is the centre of haemoglobin and a part of myoglobin. During breakdown of carbohydrates, fats and proteins the

elements copper, zinc and manganese play important roles. During the metabolic processes of bone, the elements such as zinc, manganese and copper serves as cofactors for specific enzymes³⁹.

Conclusions

Phytochemical analysis of the different extracts of the Indian variety of *Sisymbrium irio* L. seeds revealed the presence of phenols, flavonoids, terpenoids, saponins, and other bioactive metabolite including 2, 4-Di-tert-butylphenol, 3', 5'-Dimethoxyacetophenone, 9, 12-Octadecadienoic acid (Z,Z)-, n-Hexanoic acid, cis, cis, cis-7, 10, 13-Hexadecatrienal, γ -Tocopherol, Cholesterol, γ -Sitosterol, 1-Heptacosanol, 1,3-Cyclohexanedione, O-ethyl-S-2-dimethylaminoethylethylphosphonothioate, 3-Methylcrotonitrile, 1,2-Cyclopentadiene, 4-Isothiocyanato-1-Butene, Isosorbide, Sinapic acid ester, 7-Tetradecenal, 9, 12, 15-Octadecatrienoic acid, ethyl ester(Z, Z, Z) and (R)-(-)-14-methyl-8-hexadecyn-1-ol. The major fatty acid profile of *Sisymbrium irio* cold pressed seed oil contained, Linolenic acid (36.29%), Linoleic acid (17.99%), Oleic acid (12.58%), cis-11-Eicosenoic acid (9.2%), Erucic acid (9.19%), Palmitic acid (6.66%), Stearic acid(2.2%), Arachidic acid (1.75%) and cis-11, 14-Eicosenoic acid (0.95%). The significant antioxidant activity of *Sisymbrium irio* L seed and its frequent use in Unani medicine prompted us to investigate this seed material of our Indian variety. There is need to validate the claims for its usage by investigating the biological activities of purified compounds and to develop a new drug candidate based on Unani system of medicines.

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Conflict of Interest

The authors express no conflict of interest

Author Contributions

T Hailu is a PhD student from the Delhi Technological University, Delhi, India has carried out all the experiments related to this manuscript. R K Gupta (co-supervisor) & A Rani (supervisor) have contributed in the manuscript writing and conceptualization of the work done.

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