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Determination of bioactive phytochemicals, antioxidant and anti-inflammatory activity of *Colchicum autumnale* L. (*Suranjanshireen*)

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In Unani System of Medicine *Suranjanshireen* (*Colchicum autumnale* L.) is primarily used for the treatment of arthritis, and it belongs to the family of Liliaceae. The current investigation was conducted to evaluate the phytochemical constituents, anti-oxidant, and anti-inflammatory activity of *C. autumnale* L. Anti-oxidant activity was done using 2, 2'-azinobis (3-ethyl benzothiazoline-6-sulfonic acid), and 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging test. The bioactive compounds of the extracts of *C. autumnale* were identified by GC-MS and UHPLC-QExactiveOrbitrap. Docking studies were carried out for anti-inflammatory activity.The subjective phytochemicals examination demonstrated the existence of phenols, flavonoids, glycosides, and terpenoids. Whereas the quantitative investigation indicated dichloromethane extract contains the maximum number of phenolic and flavonoids constituents and demonstrated the highest antioxidant activity. GC-MS, and UHPLC- QExactiveOrbitrap investigation of the extracts confirmed the existence of bioactive compounds. Docking analysis revealed that colchicoside (3 demethyl colchicine glucoside) inhibits IL-6 having binding energy -7.1 kcal/mol with an RMSD value of 0.00. Phytochemicals, antioxidant, GC-MS, UHPLC- QExactiveOrbitrap analysis and molecular docking results revealed that the compounds presented in *C. autumnale* L extracts were accountable for numerous therapeutic uses, for instance, antioxidant, and anti-inflammatory activities.

Keywords: Anti-inflammatory activity, Antioxidant, GC-MS analysis, ICP-MS analysis, UHPLC-QExactiveOrbitrap analysis,

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Introduction

Unani System of medication is one of the most established frameworks that prevails to date with its proficient medications obtained from animals, plant, and mineral. According to this system of medicine, the methods of treatment are divided into four distinct parts, in particular, Dietotherapy (Ilaj-Bil-Ghidha), Regimental treatment (*Ilaj-BilTadbeer*), Pharmacotherapy (Ilaj-Bil-Dawa), and Surgery (Ilaj-Bil-Yada). In view of pharmacotherapy, individually on its own and compound drugs are being utilized[1].

The therapeutic plants used in the *Unani* system of medicine are the foundation of effective sources of bioactive compounds, phytomedicines obtained from these therapeutic plants played a vital role in the discovery of novel drugs for the treatment of the various categories of human, and animal diseases[2]. Physicians of *Unani* medicine, make use of *Suranjan shireen* (*Colchicum autumnale* L.) for the treatment of arthritis and it's been referred to by nearly all famous *Unani* authors in their books together with *Unani* Pharmacopoeia. The medicinal properties of this plant have been notable to the Arabs. Hussain and Masihi have portrayed that the white variety (Shirin) is better than the black variety because the latter one may have toxicity[3]. *C. autumnale* belongs to the family *Liliaceae* that has a vital part in the discovery of new drugs in medicine. The plant is so-called for the land-living of Colchis by the side of the eastern part of the Black Sea. First complete description and sketches of the plant, known "Colchicon," was given in the 1st century AD via Dioscorides, father of botany in *Unani* medicine. *C. autumnale* is commonly a vital source of colchicine, which was initially extracted from bulbs and seeds. It is a class of alkaloids utilized for the cure of gout and rheumatism, inflammation, painful muscles, and patients with familial Mediterranean fever[4], cirrhosis, and Sweet's syndrome[5] asthma, liver fibrosis, behçet's disease and pericarditis with effusion[6].

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C. autumnale possesses antioxidant properties used to treat internal wounds whereas its mixture is effectively used for curing piles and gout, and has also been used for revitalizing deep sleep[7]. It is a well-known pain killer and has a great role in the removal of the pain of all types of muscular and burning muscular tissues, joints, and gastric pains, periosteum and synovial membranes of joints and has a beneficial role for the treatment of foot palm burning[8]. The plant is chosen as a medication for the treatment of joint pain in the *Unani* treatment[9]. In musculoskeletal disorders, it plays a significant role in the treatment of several ailments in the traditional system of medicine[10]. Hence, the current investigation was conducted to assess the phytocompounds and to determine bioactive metabolites, antioxidant, and anti-inflammatory activity of *C. autumnale*.

Material and Methods

Collection and identification of the plant sample

Plant material of *C. autumnale* was bought from Khari Baoli Market, Delhi, Indian Drugs House. The Botanical specimen of the *C. autumnale* was identified by the National Institute of Science Communication and Information Resources, New Delhi (SC-0171/15) and the voucher specimen was deposited at the Raw Material Herbarium and Museum, Delhi (RHMD).

Preparation of extracts

About 500 g of powdered *C. autumnale* was extracted with n-hexane, dichloromethane, and methanol for 5-8 hours in the Soxhlet apparatus. The extracts were dehydrated with 5 g of anhydrous sodium sulfate, filtered, and concentrated using the rotary evaporator under reduced pressure at 40°C. Then all concentrates were kept at 4°C until investigation.

Phytochemical analysis and antioxidant activity test

The qualitative phytoconstituents analysis of *C. autumnale* extracts were done using standard procedures[11]. The phenolic contents were estimated following the Folin-Ciocalteu index protocol[12]. The flavonoid contents were calculated according to Khalil *et al*.[13] with modifications. The stable DPPH radical was used for the determination of free radical scavenging activity of the extracts according to the modified method of Cuendet[14]. Briefly, 0.1 mL of extract was mixed with 1 mL of 0.1 mM of DPPH. Absorbance was measured at 517 nm after 30 min of incubation. Trolox was used as the positive control for the analyses of the ABTS-antioxidant activity of the extracts[15].

Gas Chromatography-Mass Spectrum analysis

C. autumnale extracts were injected in GC-MS (Shimadzu GCMS-QP2010) for obtaining the results. The samples were introduced in split mode at 260 °C. The oven temperature was planned for 50 °C (2 min) to 280 °C (16 min). The column flow rate was 1.21 mL/min and electron Ionization (EI) was used as the ionization mode. In this technique, the identification of phytocompounds can be done by comparing fragmentation pattern and retention time, along with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed as a per cent with peak area normalization[16].

UHPLC- QExactiveOrbitrapanalysis

Sample preparation and analysis by UHPLC- QExactive Orbitrap

About 2 g *C. autumnale* powder was mixed with 10 mL 1% Formic acid (FA) in water and kept for 10 min. Then, 10 mL methanol and 10 mL acetonitrile was added and vortex for one min and kept on a shaker for 40 min at 350 rpm (room temperature) and centrifuged at 5000 rpm for 5 min. Exactly 0.5 mL of supernatant was diluted by 0.5 mL acidified water. Then, 5 µL extract injects into the QE-Orbitrap focus system. In this study, the UHPLC-Q Exactive Focus orbitrap system was used to acquire raw data using Xcalibur software in full scan with a $ddMS^2$ mode, which offered simultaneously full MS (R=70,000) as well as MSMS (R=17500) spectra in a single acquisition with positive/negative polarity. The data were processed through the compound discoverer software using a non-target approach and to identify the maximum number of compounds. The analysis becomes completed on Vanquish ultra-high-performance liquid chromatography (UHPLC Thermo Scientific™), coupled with a QExactive focus (Orbitrap, Thermo Scientific, Bremen, Germany). UHPLC analysis becomes achieved with Vanquish UHPLC (Thermo Scientific™) equipped with an AccucoreaQ™ C18 (100 x 2.1 mm, 2.6 µm particle size) column maintained at 40 °C. The mobile phase consisted of phase A [water:methanol (90:10, v/v) + 0.2 % HCOOH] and phase B[methanol: water (90:10, v/v) + 0.2% HCOOH] with a constant flow rate (0.4 mL/min). A gradient program was used as follows: 0-1min, 2% B, 1-11 min, 2-100% B, 11-16-min, 100% B, 16–17-min 2% B, 17-22-min, 2% B. The full MS-$ddMS^2$ mode offered a full MS spectrum with MS/MS simultaneously in a single LC run. For ddMS2, the normalized collision

energy ramped from 10-55v. The data acquisition was performed in Xcalibur 4.1 software. The full MS spectrum provided info about the complete molecular ion (e.g., M^+, M+H+), whereas the ddMS2 discovery generated the product ion spectra with ramped collision energy. The identification and characterization of metabolites were performed by a relative comparison of formerly reported data and from online databases[17].

Inductively coupled plasma mass spectrometry (ICP-MS)analysis

All the chemicals used were of supra pure or trace metal grade. About 0.25 g of the same samples were taken in various digestion vessels. To which one mL of distilled water, 2 mL of conc. HNO_3, 1 mL of H_2O_2, and 0.2 mL conc. H_2SO_4 was added. After 30 min of pre-digestion, the vessels were closed and kept inside the digester at 483K. After the accomplishment of digestion, the samples were transferred to a volumetric flask and made up to 50 mL. NIST standards were also digested following the same procedure. The blank sample solution was prepared following a similar procedure as above without adding the sample. Instrument calibration was done for all the analyzed elements by mixing the standard solutions in the required proportions[18].

***In-silico* anti-inflammatory activity**

A molecular docking study was conducted o investigate the comparative inhibitory effect of colchicine, colchicoside (3-demethyl colchicine glucoside), deacetamido-5, 6-dihydrocolchicine, and deacetyl colchicine compounds present in the extracts of *C. autumnale* on TNF-α, IL-6, and IL-17.

Retrieving the target and ligand structures

The experimental structures of TNF-α (1TNF.pdb), IL-6 (1IL6.pdb), and IL-17 (4HR9.pdb) were downloaded from protein data bank (PDB) (htto://www.rcsb.org/pdb). The retrieved TNF-α (PDB ID: 1TNF) of resolution 2.6 Å consists of three chains (A, B, and C) with 157 amino acids sequence length. 1IL6 retrieved from PDB was having 185 amino acid consists of chain A and similarly, 4HR9 protein structure at resolution 2.48 Å consist of Chain A and B with a sequence length of 122 amino acid. 1TNF and 1IL6 protein structures downloaded were of zero mutation and the 4HR9 structure possessed 2 mutations. Structures of identified (using UHPLC) bio compounds were obtained from PubChem (https://pubchem. ncbi.nlm.nih.gov/)[19].

Docking studies

For carrying out docking studies, the lattice and docking parameter records were set up via AutoDock 4.2.1. For keeping up the electrostatics, included hydrogen atoms and afterwards converged with non-polar hydrogen. PDBQT file of ligands was generated after adding gasteiger and Kollman charges. The 3D box was designed with 126x126x126 Å and spacing 0.81 Å for TNF-α and IL-17 whereas the number of points in X, Y, Z dimension for IL-6 was set 108x98x90 Å with 0.403 spacing. Blind docking was employed to study the best possible conformation in 10 various poses per run. The best binding pose was selected by estimating minimum binding energy and root mean square deviation (RMSD) (Auto Dock Vina) via the Lamarkian algorithm[20].

Results and Discussion

Phytochemical investigation of *C. autumnale* extracts

The qualitative phytochemical analysis showed the existence of phenols, terpenoids, flavonoids, and glycosides in all the extracts, but steroids were found only in the dichloromethane and methanol extract of the *C. autumnale*. Saponins and tannins were not identified in any extracts (Table 1). The chemical compounds in the plant parts were recognized to be biologically active compounds and they had been accountable for diverse actions, for example, antioxidant, antifungal, antimicrobial, and anticancer[21]. Terpenoids are known for anti-viral, antibacterial, antimalarial, anti-inflammatory, hindrance of cholesterol combination, and anti-malignant activities[22]. The phenolic compounds have pharmacological properties, particularly antimicrobial activity[23], antiviral, mitigating & cytotoxic activity, and antimutagenic & anticarcinogenic activities[24]. The therapeutic herb which possesses good phenolic activity also has potent antioxidant properties[25]. Phenolic compounds play a role in restoring kidney and stomach problems[26]. Phenolics have antimicrobial, antioxidative, antiallergic, antimutagenic, antidiabetic,

Table 1 — Qualitative phytochemical screening results of the extracts

Phytochemicals	*Colchicum autumnale* L. extracts		
	n-Hexane	Dichloromethane	Methanol
Phenols	+	+	+
Flavonoids	+	+	+
Saponins	-	-	-
Terpenoids	+	+	+
Steroids	-	+	+
Glycosides	+	+	+
Tannins	-	-	-

+ = the existence of phytochemicals; - = the absence of phytochemicals

anti-inflammatory, and anticarcinogenic activities[27]. Flavonoids likewise have antioxidant properties as they prevent oxidative and hydrolytic chemicals, affect radical scavenging, anti-cancerous, and anti-inflammatory activity[28].

Quantitative estimation of the total phenolic contents

Phenolic compounds, widely distributed in plant parts are beneficial to human health due to their antioxidant activity[29]. Quantitative estimation of the total phenolic contents showed its level varies from n-hexane crude extract (6.448±0.008) to methanol crude extract (17.3±0.003) of *C. autumnale* extracts and represented as µg of gallic acid equivalent (GAE/mg of dry extract). The results showed that dichloromethane crude extract (26.6±0.003 µg GAE/mg of dry extract) contained the highest percentage of the total phenolic components, followed by methanol and n-hexane crude extract.

Quantitative estimation of the total flavonoid contents

Total flavonoids content varied from n-hexane to methanol extracts and is represented as µg QE/mg of dry extract. Dichloromethane extract (25.5±0.007) contained the highest amount of flavonoid, followed by methanol (6.094±0.003), and n-hexane (4.773±0.005) extracts.

Radical scavenging activity

The antioxidant activity test of extracts was analysed via DPPH **(**Diphenyl picrylhydrazyl) and ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) test. DPPH is a steady free radical compound and has an absorbance in its oxidized form at 515-520 nm[30]. DPPH analysis is a moderately fast and productive technique to assess free radicals. The colour change, from purple to yellow demonstrates a decline in the absorbance of DPPH radical. This is the confirmation of the interaction of the antioxidant found in the sample with the free radicals[31]. The level of DPPH radical scavenging action of n-hexane, dichloromethane, and methanol crude extracts of *C. autumnale* at different concentrations is given in Fig. 1. The dichloromethane extract showed the highest percentage of ABTS free radical scavenging activity. The percentage of ABTS radical scavenging activity of n-hexane, dichloromethane, and methanol extract of *C. autumnale* extracts is given in Fig. 2. Polyphenolic compounds help in lipid oxidation and possess antioxidant activity which helps in scavenging free radicals[32]. Moreover, it might show an inhibitory impact on mutagenesis and carcinogenesis in humans[33].

Gas Chromatography-Mass Spectrometric analysis

Nowadays, the investigation of the natural compounds from plants and their activity has increased.

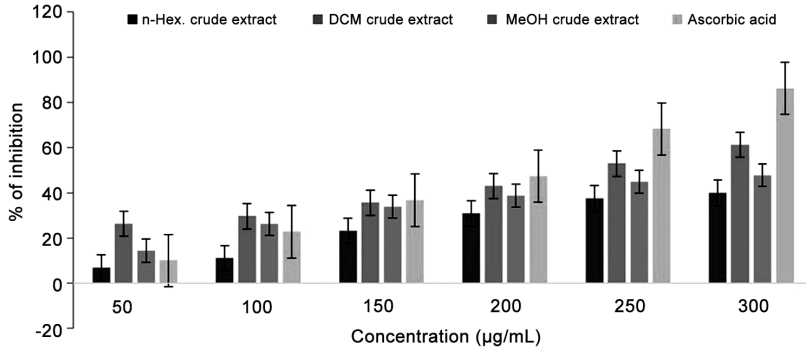

Fig. 1 — DPPH free radical scavenging activity of solvent extracts of *Colchicum autumnale* L.

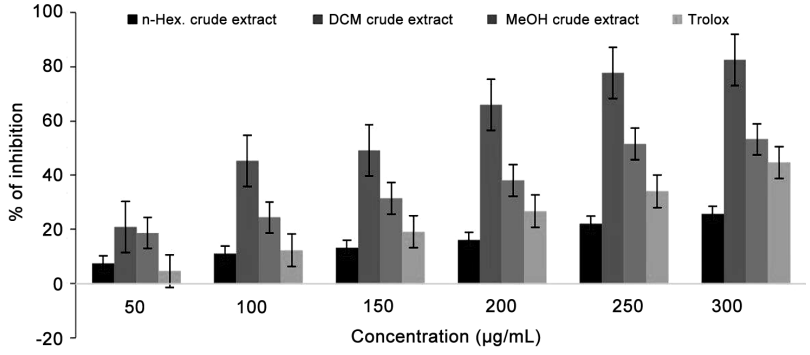

Fig. 2 — ABTS free radical scavenging activity of solvent extracts of *Colchicum autumnale* L.

The GC-MS analysis is a perfect method for the identification of volatile and semi-volatile bioactive compounds[34]. The main bioactive constituents identified from *C. autumnale* extracts are given in Table 2. Earlier studies have reported that Hexadecanoic acid methyl ester showed antioxidant, hemolytic, antiandrogenic,

Table 2 — Bioactive components identified by GC-MS in the different solvents extracts of *Colchicum autumnale* L.

Name of Compounds	R.time	Area %	Mol. formula	Mol weight g/mol	n-hexane extract	Dichloromethane extract	Methanol extract
1-Bromo Decane	12.387	20.88	$C_{10}H_{21}Br$	220	+	-	-
3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	13.810	1.82	$C_{19}H_{54}O_7Si_7$	590	+	-	-
3', 5' Dimethoxy acetophenone	15.127	1.77	$C_{10}H_{12}O_3$	180	+	-	+
3, 4-Dihydroxymandelic acid-tetratms	15.780	1.35	$C_{20}H_{40}O_5Si_4$	472	+	-	-
(-) -(4R,5S,6R)-4,5,6-tris-{[(tert-butyl) dimethyl silyl] oxy} cyclohex-2-en	17.478	0.74	$C_{24}H_{50}O_4Si_3$	486	+	-	-
Hexadecanoic acid, methyl ester	19.035	8.33	$C_{17}H_{34}O_2$	270	+	+	+
n-Hexadecanoic acid	19.428	13.10	$C_{16}H_{32}O_2$	256	+	-	+
9,12-Octadecadienoic acid, methyl ester	20.675	10.98	$C_{19}H_{34}O_2$	294	+	+	-
8,11,14-Docosatrienoic acid, methyl ester	20.732	5.01	$C_{23}H_{40}O_2$	348	+	-	+
9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	21.147	22.93	$C_{18}H_{30}O_2$	278	+	-	-
1-Pentanol, 5-cyclopropylidene	23.848	1.11	$C_8H_{14}O$	126	+	-	-
10-Undecyn-1-ol	24.453	0.92	$C_{11}H_{20}O$	168	+	-	-
9,12,15-Octadecatrienal	24.551	0.75	$C_{18}H_{30}O$	262	+	-	-
beta -Sitosterol acetate	31.919	2.23	$C_{31}H_{52}O_2$	456	+	-	-
Methanol, [5,7,9 trimethyl -4-(1-propenyl)-3 oxabicyclo [3.3.1] non-6-en-	34.480	1.69	$C_{15}H_{24}O_2$	236	+	-	-
3-Methoxy Phenol	10.750	6.33	$C_7H_8O_2$	124	-	+	+
1-Tetradecene	12.903	0.56	$C_{14}H_{28}$	196	-	+	-
2,4-bis(1,1-dimethylethyl) Phenol	14.432	2.54	$C_{14}H_{22}O$	206	-	+	-
E-14-Hexadecenal	15.435	2.20	$C_{16}H_{30}O$	238	-	+	-
1-Octadecene	17.698	1.93	$C_{18}H_{36}$	252	-	+	-
Hexadecanoic acid, ethyl ester	19.762	11.17	$C_{18}H_{36}O_2$	284	-	+	+
Methyl stearate	21.027	1.47	$C_{19}H_{38}O_2$	298	-	+	-
Bis(2-ethylhexyl) maleate	21.158	1.52	$C_{20}H_{36}O_4$	340	-	+	-
Linoleic acid ethyl ester	21.396	11.33	$C_{20}H_{36}O_2$	308	-	+	-
(E)-9-Octadecenoic acid ethyl ester	21.446	4.30	$C_{20}H_{38}O_2$	310	-	+	-
7-Tetradecenal, (Z)-	21.639	16.97	$C_{14}H_{26}O$	210	-	+	-
Methyl-18-methylnonadecanoate	22.874	0.83	$C_{21}H_{42}O_2$	326	-	+	-
Nonacos-1-ene	23.586	1.19	$C_{29}H_{58}$	406	-	+	-
Decanedioic acid, bis(2-ethylhexyl) ester	29.241	0.94	$C_{26}H_{50}O_4$	426	-	+	-
Alpha -Tocopheryl acetate	32.274	0.53	$C_{31}H_{52}O_3$	472	-	+	-
Beta -Sitosterol	34.588	1.03	$C_{29}H_{50}O$	414	-	+	+
1-Phenyl Ethanone	8.183	2.22	C_8H_8O	120	-	-	+
Methanol, alpha.,. alpha -dimethyl Benzene	8.514	1.52	$C_9H_{12}O$	136	-	-	+
4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	9.639	3.92	$C_6H_8O_4$	144	-	-	+
5-Hydroxymethylfurfural	11.068	6.88	$C_6H_6O_3$	126	-	-	+
2,4-Ditert-butylphenol	14.422	0.88	$C_{14}H_{22}O$	206	-	-	+
4-Methoxy-7-methylindan-1-one	15.416	1.32	$C_{11}H_{12}O_2$	176	-	-	+
2, 6-Dimethoxy-4-(2-propenyl)- Phenol	15.523	0.30	$C_{11}H_{14}O_3$	194	-	+	+
Pentadecanoic acid	18.425	0.47	$C_{15}H_{30}O_2$	242	-	-	+
Benzene propanoic acid, 3, 5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	19.142	1.97	$C_{18}H_{28}O_3$	292	-	-	+
Hexadecanoic acid, ethyl ester	19.712	0.52	$C_{18}H_{36}O_2$	284	-	+	+
9,12-Octadecadienoic acid (Z, Z)-, methyl ester	20.706	10.00	$C_{19}H_{34}O_2$	294	-	+	+
Octadecanoic acid, methyl ester	20.974	0.40	$C_{19}H_{38}O_2$	298	-	-	+
9,12-Octadecadienoic acid (Z, Z)-	21.298	31.42	$C_{18}H_{32}O_2$	280	-	+	+
Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	21.445	0.43	$C_{22}H_{44}O_4$	372	-	-	+

'+' shows presence and '-' shows absence

pesticide lubricant, and hypocholesterolemic activity[35]. Linoleic acid (omega-6) is one of the fundamental fatty acids, that is not created in the human body and should be provided from outside. The *C. autumnale* extract is a good source of linoleic acid. Linoleic acid (omega-6), is a metabolic precursor of eicosanoids which forms important lipids like prostaglandins that play an important role in inflammation, immunity, and blood clotting[36]. The compound β-sitosterol is a plant sterol that shows superb mitigating and cholesterol-lowering action[37]. Another research study showed thatβ-sitosterol stimulates antioxidant enzymes by activation of the estrogen receptor/ P13-kinase dependent pathway. The GSH and GSH/complete glutathione proportion recouped after treatment via β-sitosterol proposing this phytosterol could be a ROS scavenger[38]. Research has shown that 9, 12, Octadecadienoic acid (Z, Z) antiarthritic and anti-inflammatory properties[39]. Phytochemical examination by GC-MS confirmed the existence of different types of bioactive metabolites and they are accountable for innumerable therapeutic uses such as hepatoprotective, antioxidant, anti-inflammatory, and antimicrobial activities.

UHPLC-Q Exactive Orbitrap analysis

UHPLC- Q Exactive Orbitrap investigation of the extract confirmed the presence of colchicine, (R/S)-deacetyl colchicine, 3-demethyl colchicine, colchicoside (3-demethyl colchicine glucoside), and deacetamido-5, 6-dihydrocolchicine as shown in Table 3. The other research examination showed the identified compound colchicine, chiefly possesses anti-inflammatory, analgesic, and antiarthritic activity. It also possesses expectorant, deobstruent, antidote, and aphrodisiac activity[40]. It is chiefly used to relieve inflammation, pain, and shorten the duration of acute gout and certain gouty infection and to treat myeloid leukemia[41]. It increases secretions of the skin, liver, kidneys, and also the flow of bile. In ascites because of liver infection, it is a strong cure. In cerebral and hepatic clogs, it acts as a purgative with benefit. The extracted colchicine is used orally in tablet form for arthritis and familial Mediterranean fever while corms and seeds are used to treat enlarged prostate, dropsy, and gout, rheumatism, and arthritis[42].

***C.autumnale* extract**

The phytochemical analysis through UHPLC confirmed the *C. autumnale* L extract contained diverse types of bioactive compounds, and these bioactive compounds have a significant part in the therapeutic system of medicine for the treatment of disease.

Elemental analysis of *C. autumnale* L (ICP-MS)

Elemental analysis of *C. autumnale* exhibited the occurrence of diverse types of minerals at a different concentration as shown in Table 4. The minerals which were analyzed from *C. autumnale* are very useful for the health of human beings. The elements

Table 3 — The mass spectrometric data of the identified compounds via UHPLC- QExactiveOrbitrap

Sr. No.	Name of compounds	Mol. formula	Mol. weight	R. time	Adduct	Observed mass	Error (PPM)	Fragments
1.	Colchicine	$C_{22}H_{26}NO_6$	399.437	7.36	$[M+H]^+$	400.1753	-1.0374	68.7195,98.4443, 127.2563, 170.5951, 239.1045, 239.1045, 267.1000, 310.1177, 358.1649, 382.1639
2.	(R/S)-Deacetyl Colchicine	$C_{20}H_{24}NO_5$	357.4	5.07	$[M+H]^+$	358.1647	-0.5075	110.0095, 116.9872, 125.9871, 137.1074, 141.9587, 173.9847 ,213.1022, 232.9297, 248.9004, 295.0490, 313.1672, 327.122 6, 344.1855
3.	3-demethyl Colchicine	$C_{21}H_{23}NO_6$	385.410	6.50	$[M+H]^+$	386.1594	-1.0892	68.8835, 84.9604, 102.9704, 158.9273, 176.9383, 207.0796, 232.9276, 250.9386, 295.0956, 344.1490, 368.1474
4.	Colchicoside (3-demethyl colchicine glucoside)	$C_{27}H_{34}O_{11}N$	547.5	5.27	$[M+H]^+$	548.2120	-1.4305	84.9598, 114.1461, 149.0593, 235.0750, 267.1013, 295.0945, 344.1479, 368.1482, 386.1591, 405.0515
5.	Deacetamido-5, 6-Dihydrocolchicine	$C_{20}H_{21}O_5$	340	6.01	$[M+H]^+$	341.1382	-0.5779	102.9707, 116.9862, 125.9864, 134.9965, 149.0122, 158.0125 , 167.0225, 198.9399, 204.887, 214.9175, 232.9281, 264.9542

Table 4 — ICP-MS analysis of *Colchicum autumnale*L

S. No.	Elements	Sample concentration (ppm)
1	Li	bdl
2	Be	bdl
3	B	5.59
4	Na	200.49
5	Mg	638.15
6	Al	76.00
7	P	1164.61
8	K	7368.93
9	Ca	1057.87
10	V	bdl
11	Cr	0.82
12	Mn	3.60
13	Fe	67.77
14	Co	0.04
15	Ni	0.57
16	Cu	6.12
17	Zn	8.25
18	As	0.10
19	Se	0.06
20	Mo	0.07
21	Cd	0.02
22	Sn	bdl
24	Sb	bdl
25	Ba	2.15
26	Hg	bdl
27	Pb	0.34

bdl: below detection limit

for example calcium, phosphorus, and magnesium may be helpful in the buildings of our bones; potassium and sodium support the preservation of normal blood pressure. The metal iron is the centre of haemoglobin and a part of myoglobin. During the breakdown of carbohydrates, fats, and proteins, the elements copper, zinc, and manganese play important roles. During the metabolic processes of bone, elements such as zinc, manganese, and copper serve as cofactors for specific enzymes[43].

Docking studies

Various interaction conformations of both the targets and compounds were studied by AutoDock4. The *in-silico* studies of the present analysis indicated that colchicoside is a strong inhibitor of IL-6 having binding energy -7.1 kcal/mol with an RMSD value of 0.00 for both lower and upper bound (Fig. 3) compared to diclofenac having binding energy -6.1 kcal/mol. In the case of IL-17, colchicoside again showed minimum binding energy (-6.5 kcal/mol) as compared to standard drug diclofenac which showed a binding affinity of -4.7 kcal/mol. There are polar contacts between protein and ligand. A list of binding

Table 5 — Representation of binding energy and RMSD

	Affinity (kcal/mol)			RMSD* (l.b./u.b.)**		
	TNF-α	IL-6	IL-17	TNF-α	IL-6	IL-17
Colchicine	-5.9	-6.4	-6.2	0.00/0.00	0.00/0.00	0.00/0.00
Colchicoside	-6.2	-7.1	-6.5	0.00/0.00	0.00/0.00	0.00/0.00
Deacetamide -5	-5.7	-6.5	5.1	0.00/0.00	0.00/0.00	0.00/0.00
Deacetyl Colchicine	-6.1	-6.4	-5.3	0.00/0.00	0.00/0.00	0.00/0.00
Declofenac	-5.6	-6.1	-4.7	0.00/0.00	0.00/0.00	0.00/0.00

*Root mean square deviation; **l.b. = lower bound; u.b. = upper bound

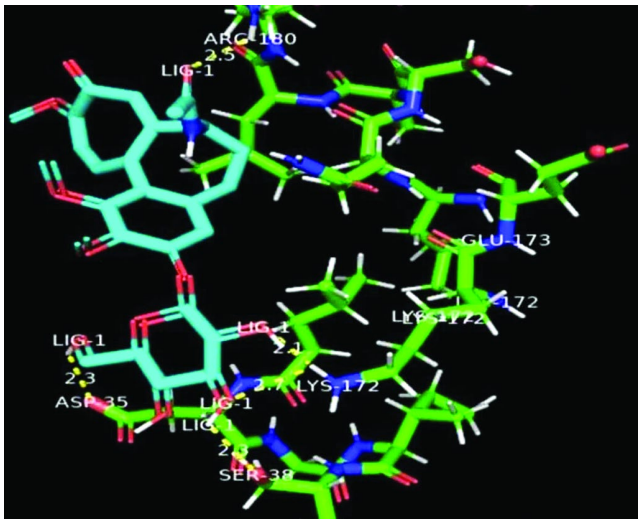

Fig. 3 — Representative image of binding of compound colchicoside (Ligand) with protein IL-6 (Receptor) showing polar contacts with distance in A and residues of binding.

energy and RMSD values have been summarized in Table 5. These results indicated that *C. autumnale* equal amount of methanolic:acetonitrile:water (1:1:1) extract had great potential to inhibit main instigator cytokines (IL-6, TNF-α, IL-17) of inflammation and hence, may be investigated for future drug design study.

Conclusion

The phytochemicals examination exhibited the existence of phenols, flavonoids, glycosides, and terpenoids in all extracts, while steroids were found in dichloromethane and methanol extracts of the plant. Saponins and tannins were absent. The dichloromethane extract of the *C. autumnale* contains the highest amount of phenolic and flavonoids constituents and also demonstrated good antioxidant activity in comparison to other extracts. The GC-MS and UHPLC-QExactive

Orbitrap analysis confirmed the existence of diverse classes of phytochemical compounds. The anti-inflammatory activity suggests that this plant can be used for relieving the symptoms of inflammation. Comparative docking studies revealed colchicoside as the most potent anti-inflammatory compound with minimum binding energy (affinity) as compared to other compounds and standard drug diclofenac. Thus, the above research results indicate that *C. autumnale* extract is a potent natural antioxidant, anti-inflammatory, and also a good source of phytomedicine with excellent therapeutic use.

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Conflict of interest

The authors declare no conflict of interest.

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