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Composition, antioxidant and anti-inflammatory activities of different polarity extracts of *Artemisia nilagirica* collected from hilly areas in the Himalayan terrain of Uttarakhand

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ABSTRACT

The plant *Artemesia nilagirica*, collected from the terrain of the Himalayan region in Uttarakhand, India, was evaluated for its phytochemical composition, antioxidant and anti-inflammatory activities *in vitro*. The different polarity extracts of the plant were prepared and subjected to GC-MS analysis for their phytochemical composition. Twenty-six compounds were identified in the hexane extract of *Artemisia nilagirica* that represents 73.30% of the total area. The main compounds were tetracontane (15.21%), heneicosane (6.52%), and phytyl tetradecanoate (5.11%). The methanol extract yielded 26 compounds, accounting for 83.78% of the total compounds detected. The main compounds were palmitic acid (13.25%), alpha-linolenic acid (10.32%), oleamide (9.41%), phytol (8.58%), muco-inositol (7.27%), and neophytadiene (5.05%). The hexane and methanol extracts showed significant metal chelating activity having IC_{50} values of 2.23±0.01 and 2.41±0.02 µg/mL, respectively, with standard EDTA having an IC_{50} value of 2.22±0.01 µg/mL. The methanol extract showed a better anti-inflammatory property having an IC_{50} value of 2.97±0.03 µg/mL compared to the standard diclofenac potassium having an IC_{50} value of 3.79±0.01 µg/mL. The hexane extract showed better antioxidant activity in terms of metal chelating activity and reducing power activity than the methanol extract. The anti-inflammatory activity of both hexane and methanol extracts showed better results than the standard marketed drug diclofenac potassium. The current study reveals that *Artemisia nilagirica* plant extracts have potent antioxidant and anti-inflammatory activities. The effective biological compounds of plant extracts, such as phenols and flavonoids, can be potential alternatives to standard pharmaceuticals.

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1. Introduction

Plants have a long history of usage as traditional remedies to cure a variety of disorders. There are still many plants that need to be investigated for the existence of biologically active substances [1]. The phytochemical components of medicinal plants are responsible for their therapeutic quality. Medicinal plants can be found primarily in the eastern Himalayas, Western Ghats, and Andaman and Nicobar Islands. India is dubbed as the 'World's Botanical Garden' because it produces the most therapeutic herbs. Ayurveda and naturopathy as health-protecting and curing disease practices are popular and prevailing in India since time immemorial. Ayurveda emerged in India around 600 BC and uses medicinal plants for the treatment of various ailments [2].

Numerous plants having restorative properties exhibit extraordinary antioxidant potential as well as anti-inflammatory activities. When oxygen molecules break apart into single unpaired electron oxygen atoms, they become unstable free radicals that seek other atoms or molecules to attach to. Free radicals can negatively impact a number of significant types of biological components, including lipids, proteins, and nucleic acids, changing the normal redox state and increasing oxidative stress [3]. The antioxidants present in plants reduce oxidative stress in cells and are then helpful in the therapy of numerous human diseases, including malignancy, cardiovascular diseases, and incendiary infections [4]. The anti-inflammatory properties of plants are attributed to their potential to inhibit or down-regulate the secretion of pro-inflammatory stimuli such as cytokines, etc.

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For thousands of years, the selective use of crude plant extracts has been the earliest ritual in the ancient Indian medicinal system, 'Ayurveda,' as well as in traditional Chinese medicine. To produce high particle medications from plant components, modern methodologies have combined multidisciplinary technologies and specific chemical substances extracted and identified. Plants that produce a significant amount of polysaccharides, steroids, terpenoids, flavonoids, alkaloids, and antibiotics are useful for developing medications for a variety of diseases and disorders, including cancer therapies [5].

One of the most common groups of plants used in traditional medicine is the Asteraceae family [6]. *Artemisia nilagirica* (C.B. Clarke) Pamp is widely found in the hilly areas of India. It is known as 'Nagdona' in Hindi. It is an aromatic, herbaceous perennial plant that belongs to the Asteraceae family. It is also commonly found in Europe, Asia, northern Africa, Alaska, and North America. It grows well in the nitrogenous soils of hilly regions of India, such as the Uttarakhand Himalayan region.

The chemical composition of most members of the family is similar. All species, for example, are good sources of inulin, a natural polysaccharide with strong prebiotic characteristics [7]. *Artemisia nilagirica* has been effective against asthma, caused by exposure to common allergens present in the environment. The plant shows anticancer and antioxidant activity which may be due to the free radical quenching properties of sesquiterpene lactones and flavonoids present in the ethanolic extract of the plant. Essential oils of *Artemisia,* generally extracted from aromatic plants, contain a variety of volatile components, for example, terpenoids, phenylpropanoids, and aliphatic mixtures [8].

The purpose of the current study was to evaluate the antioxidant and anti-inflammatory properties of *Artemisia* plant in relation to its phytochemical composition from the Himalayan region. Plant extracts were prepared using two different solvents, hexane and methanol. The extracts were then subjected to a GC-MS analysis to determine their phytochemical composition. Following phytochemical analysis, the biological activities of the extracts, such as their antioxidant and anti-inflammatory properties, were evaluated using standard protocols.

Twenty-six compounds were identified in the hexane extract as well as the methanol extract of *A. nilagirica*. The results of the present study indicate that *Artemisia nilagirica* plant extracts have potent antioxidant and anti-inflammatory activities. The hexane extract showed better antioxidant activity in terms of metal chelating activity and reducing power activity than the methanol extract. The effective biological compounds of plant extracts, such as phenols and flavonoids, can be potential alternatives to traditional medications. The study reveals that the plant exhibits potent antioxidant and anti-inflammatory activities *in vitro*.

2. Experimental

2.1. Plant collection and authentication

Artemisia nilagirica was collected from the hilly region of Uttarakhand near Jeolikote at an elevation of 1320 m in September 2020. The plant sample was submitted to Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India. The identification of the plant sample was carried out by Dr. Dharmendra Singh Rawat (Assistant Professor and Plant Taxonomist), Department of Biological Sciences, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India.

2.2. Extract preparation

The whole plant of *A. nilagirica* was shade dried for four days and was coarsely powdered and successively extracted using the cold percolation method [9]. The extraction was done by using different polarity solvents based on the order of their increasing polarity. The yield obtained in the case of the methanol extract was 3.257 g from 240.57 g of the whole plant having a 1.35% yield while in the case of the hexane extract it was 2.255 g from 243.57 g of the plant having a yield of 0.92%.

2.3. Analysis and identification of compounds

To identify the phytochemical composition of plant extracts, an analytical technique, gas chromatography mass spectrometry (GC-MS), was carried out using Shimadzu GCMS-QP2010 Plus equipment with helium as the carrier gas having a pressure of 73.3 kPa and a velocity of 40.1 cm/sec. The column flow rate was maintained at 1.21 mL/min. The carrier gas saver, high pressure injection, and splitter hold were turned off, and the oven temperature was set to 60 °C RAMP@ 3 °C/min up to 210 °C (isotherm for 2 min), then 6 °C/min up to 280 ° C (isotherm for 2 min), then hold for 11 min, flame thermionic detector (FTD) [10].

The Kovats indices (KI) of the peaks in the DB-5 column were compared to the values in the literature and compared to the standard library spectra, which were constructed using pure substances and components of known essential oils. The fragmentation pattern of the mass spectra produced by GC-MS analysis was compared with those contained in the WILEY 8 library [11] and the published literature for further identification [12]. The peak areas obtained without FID response factor correction were used to calculate the relative amounts of identical components.

2.4. Antioxidant activities

2.4.1. 2,2'-Diphenylpicrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging activity of methanol and hexane extracts was determined with the help of the previously reported method [13]. The dark-colored crystalline powder DPPH is made up of stable free radical molecules. The DPPH solution in methanol was freshly prepared and stored in an amber-colored bottle in the dark to prevent the UV rays to enter inside.

In methanol, a stock solution of 0.1 mM DPPH was prepared. The free radical scavenging activity of various polarity solvent extracts, referred to as test samples, was investigated. Varied polarity extracts were prepared at different concentrations of 5, 10, 15, 20, and 25 µg/mL. Then 0.1 mL of each concentration of both extracts was mixed with 2.9 mL of 0.1 mM DPPH solution and kept in the dark for 30 min incubation. Using a UV spectrophotometer (Thermo Scientific Genesys 10S UV-VIS) the absorbance was determined at 517 nm. Ascorbic acid was utilized as the standard. The percentage of scavenging activity of the DPPH free radical was calculated with the help of the following formula [13].

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

where, $A_{control}$ is the absorbance of the DPPH radical solution and A_{sample} is the absorbance of the test sample.

The IC_{50} value was derived by plotting the percentage scavenging of the DPPH free radical versus the concentration of the test sample. The ability of a sample to inhibit a reaction is measured by the half-maximum inhibitory concentration or IC_{50}. Here, the concentration of the test sample that scavenges

50% of the DPPH free radical is called IC_{50}. Low IC_{50} means high antioxidant activity and vice versa.

2.4.2. Reducing power activity

The extracts were prepared at different concentrations of 50, 100, 150, 200, and 250 µg/mL in their respective solvents. A volume of 2.5 mL from each concentration was added to 2.5 mL of phosphate buffer (200 mM) having a pH of 6.6 and 2.5 mL of potassium ferricyanide (1% w/v). After that, the samples were incubated in a water bath for 20 minutes at 50 °C. After incubation, 2.5 mL of trichloroacetic acid (10% v/v in distilled water) was added. The mixture was then centrifuged at 50.54 g (650 rpm) for 10 minutes. Then, 5 mL of supernatant was taken and mixed with 5 mL of distilled water. The UV spectrophotometer (Thermo Scientific Genesys 10S UV-VIS) was used to measure the absorbance of the resulting solution at 700 nm after 1 mL of ferric chloride was added to the mixture. Readings were taken in triplicate. The gallic acid standard was also subjected to the same process, with measurements collected in triplicate. The same technique was used on the control, with the exception that no samples were added [14]. The percentage of reducing power activity of the extracts and the gallic acid standard was calculated with the following formula [14],

$$\text{Reducing power activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ = Absorbance of the control and A_{sample} = Absorbance of the test sample. The percentage activity graph of reducing power against concentration was plotted to calculate the IC_{50} value of the extracts and standard. The low value of IC_{50} indicates high reducing activity.

2.4.3. Metal chelating activity

The metal chelating activity is based on the chelation of ferrous ions by ferrozine. Ferrozine forms a complex with ferrous ions and forms a red color [15]. Different concentrations of 5, 10, 15, 20, and 25 µg/mL of plant extracts were prepared in their respective solvents and added to 0.05 mL of 2 mM $FeCl_2.4H_2O$. To it, 0.2 mL of 5 mM ferrozine was added and the volume was made up to 5 mL by adding methanol. The mixture was incubated for 10 min at room temperature and the absorbance was measured at 562 nm. The standard for this activity was EDTA, which was subjected to the technique described above. The following formula [15] was used to calculate the % inhibition metal chelating activity of the extracts and the EDTA standard

$$\text{\% Metal chelating effect} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (3)$$

where $A_{control}$ = Absorbance of control and A_{sample} = Absorbance of the test samples.

The graph of % chelating activity against concentration was plotted to calculate the IC_{50} value of the extracts and the standard. A low value of IC_{50} indicates high metal chelating activity.

2.5. In vitro anti-inflammatory activity

In vitro anti-inflammatory activity was investigated according to the protocol developed by Kar *et al*. [16]. Various concentrations i.e., 5, 10, 15, 20, and 25 µg/mL of methanol and hexane extracts, were made in their respective solvents. The volume of 2 mL of samples at different strengths was mixed with 2.8 mL of freshly produced phosphate buffer at pH = 6.4. The mixture was then spiked with 0.2 mL of egg albumin. As a result, the total volume was 5 mL. The mixture was incubated for 15 min at 37 °C and for 5 min at 70 °C. The absorbance was finally measured at 660 nm. The standard was chosen as diclofenac potassium. The following formula [16] was used to calculate the anti-inflammatory activity of the extracts and diclofenac potassium standard,

$$\text{Anti} - \text{inflammatory activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (4)$$

where A_{sample} = Test sample absorbance and $A_{control}$ = Control absorbance.

2.6. Statistical analysis

The mean and standard deviation of triplicates of plant extracts were calculated using Microsoft Excel. To evaluate their significance ($p < 0.05$), all results were subjected to a 5%-point Duncan test for one-way analysis (ANOVA) [17]. SPSS software was used to determine the significance and association of various extracts [17].

3. Results and discussion

3.1. GC-MS analysis of the hexane extract of the whole A. nilagirica plant

Twenty-six compounds were identified in the hexane extract of *A. nilagirica* which comprised a total of 73.30% of the area. The main compounds were tetracontane (15.21%), heneicosane (6.52%), phytyltetradecanoate (5.11%), tetratetracontane (4.96%), squalene (2.93%), alpha-linolenic acid (2.58%), and 24-norursa-3,12-diene (2.10%). The compounds present in lesser amounts were isononacosane (0.50%), phytol (0.51%), lyratyl acetate (0.55%), beta-amyrin (0.58%), cis-9-hexadecenal (0.65%) and many more. Alkanes were the class of compounds that were found most abundantly in the hexane extract of the plant, which occupied 46.99% area followed by saturated fatty acids, which occupied 7.05%, triterpenes occupying 3.51% of the area followed by unsaturated fatty acids, which occupied 2.58% area. Sterols comprised 2.55% of the class of chemical compounds. Other minor compounds comprised 10.62% area of a total of 73.30% area of chemical compound classes. Table 1 shows the chemical composition of *A. nilagirica* hexane extract and Figure 1 shows the gas chromatogram of the hexane extract of the whole plant of *A. nilagirica.*

3.2. GC-MS analysis of the methanol extract of the whole plant of A. nilagirica

Twenty-six compounds were identified in the methanol extract of *A. nilagirica,* contributing to 83.78% of the total compounds identified. The main compounds were palmitic acid (13.25%), alpha-linolenic acid (10.32%), oleamide (9.41%), phytol (8.58%), muco-inositol (7.27%), and neophytadiene (5.05%). Minor compounds include oleic acid (0.84%), 2-(dimethylamino) ethyl 3-cyclopentylpropanoate (0.78%), triacontane (0.63%), linoleoyl chloride (1.73%), octadecyl trifluoroacetate (0.57%) and urs-12-ene (0.93%). Unsaturated fatty acids were the major class of compounds comprising 14.54% area followed by saturated fatty acids comprising 13.25% area of the total composition of the methanol extract. The fatty acid amide comprised 9.41% area and oxygenated diterpenes comprised 8.58%. Esters and triterpenes were also present in large amounts. Other minor compounds had a percentage area of 41.31%. The precise information about the compounds is provided in Table 2 and Figure 2.

Table 1. Chemical composition of *A. nilagirica* hexane extract.

No	Compound	Chemical formula	Kovat's index	%Area	Class of compounds
1	1-Isopropenyl-3-propenylcyclopentane	$C_{11}H_{18}$	1095	1.09	Monocyclic monoterpenoid
2	Lyratyl acetate	$C_{12}H_{18}O_2$	1387	0.55	Ester
3	(-)-Oplopanone	$C_{15}H_{26}O_2$	1734	0.76	Sesquiterpenoid
4	Phytyl tetradecanoate	$C_{34}H_{66}O_2$	1762	5.11	Saturated fatty acid
5	*cis*-9-Hexadecenal	$C_{16}H_{30}O$	1800	0.65	Aldehyde
6	4-*tert*-Butylcalix[4]arene	$C_{44}H_{56}O_4$	1859	2.05	Macrocyclic phenols
7	24-Norursa-3,12-diene	$C_{29}H_{46}$	1897	2.10	Alkene
8	*trans-trans*-10,11-Epoxy farnesenic acid methyl ester	$C_{16}H_{26}O_3$	1921	0.70	Ester
9	Phytol	$C_{20}H_{40}O$	1949	0.51	Acyclic diterpene alcohol
10	Palmitic acid	$C_{16}H_{32}O_2$	1983	1.94	Saturated fatty acid
11	Eicosane	$C_{20}H_{42}$	2000	1.47	Alkane
12	Methyl linolenate	$C_{19}H_{32}O_2$	2067	1.24	Ester
13	alpha-Linolenic acid	$C_{18}H_{32}O_2$	2078	2.58	Polyunsaturated fatty acid
14	Heneicosane	$C_{21}H_{44}$	2109	6.52	Alkane
15	Docosane	$C_{22}H_{46}$	2200	5.73	Alkane
16	Heneicosanal	$C_{21}H_{42}O$	2307	0.97	Aldehyde
17	Pentacosane	$C_{25}H_{52}$	2500	6.26	Alkane
18	Squalene	$C_{30}H_{50}$	2663	2.93	Triterpene
19	Isononacosane	$C_{29}H_{60}$	2862	0.50	Acyclic branched alkane
20	Celidoniol deoxy	$C_{29}H_{60}$	2900	3.97	Alkane
21	Brassicasterol	$C_{29}H_{48}O$	3063	1.88	Sterol
22	Campesterol	$C_{28}H_{48}O$	3305	0.67	Sterol
23	beta-Amyrin	$C_{30}H_{50}O$	3337	0.58	Pentacyclic triterpenoid
24	Hexatriacontane	$C_{36}H_{74}$	3600	2.37	Alkane
25	Tetracontane	$C_{40}H_{82}$	4000	15.21	Alkane
26	Tetratetracontane	$C_{44}H_{90}$	4395	4.96	Alkane
	Total			73.30	

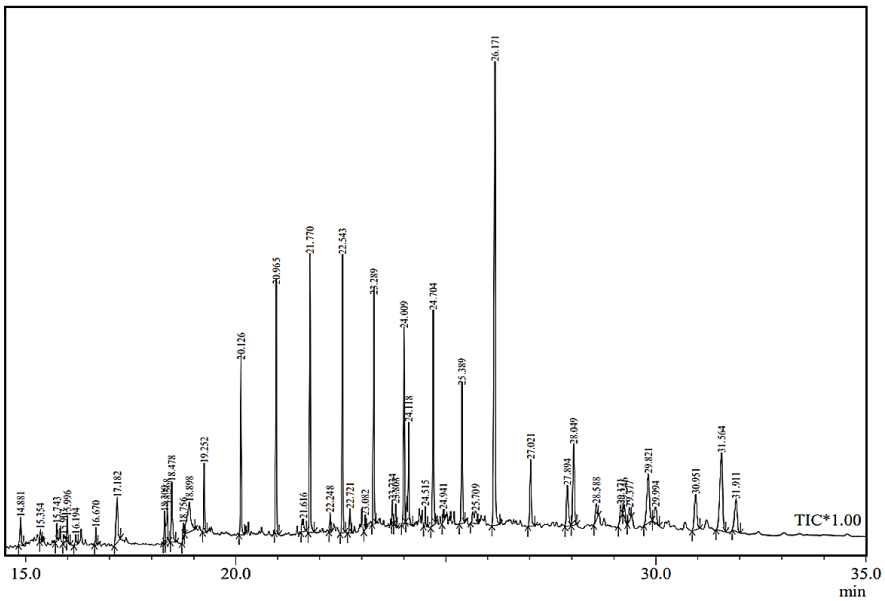

Figure 1. Gas chromatogram of the hexane extract of the whole plant of *A. nilagirica*.

3.3. In vitro antioxidant activity

Three methods were used to evaluate the antioxidant activity *in vitro.* These were free radical scavenging activity, metal chelating activity, and reducing power activity. All of these activities were evaluated by comparing them with the activity shown by the appropriate standard. Table 3 lists the mean IC_{50} values with SD of the DPPH free radical scavenging activity, the metal chelating activity, and the reducing power activity of the extracts of hexane and methanol from the whole plant of *A. nilagirica.*

3.3.1. DPPH free radical scavenging activity

By monitoring the change in violet color induced by electron transfer, the DPPH free radical activity is used to determine the antioxidant activity. At room temperature, DPPH is a stable free radical that creates a diamagnetic molecule by accepting electrons or other free radicals. Due to the existence of an odd electron, DPPH has the highest absorbance at 517 nm. As antioxidant molecules quench DPPH free radicals, lowering absorbance, the freshly generated DPPH solution fades and vanishes from deep blue to colorless [18,19]. The methanol and hexane extracts have IC_{50} values of 35.26±0.99 and 23.68±0.20 µg/mL, respectively, with standard ascorbic acid having an IC_{50} value of 2.54±0.03 µg/mL. Here, IC_{50} is the concentration of the test sample (antioxidant) that is required to inhibit DPPH by 50%. Lower IC_{50} implies higher antioxidant activity. The DPPH free radical scavenging activity of the extracts might be due to the presence of phenolic and flavonoid content in the extracts [20]. The stronger antioxidant activity of the methanol extract of *A. nilagirica* may be attributed to its higher content of α-linolenic acid occupying 10.32% which can be correlated with the previous report on the plant *Portulaca oleracea* L. [21]. Heneicosane (6.52%), which is present in good amounts in the hexane extract of *A. nilagirica,* has also been reported to have antioxidant activity in the plant *Ceropegia bulbosa* [22].

Table 2. Chemical composition of the methanol extract of *A. nilagirica*.

No	Compound	Chemical formula	Kovat's index	%Area	Class of compounds
1	Myrtanal	$C_{10}H_{16}O$	1180	0.60	Monoterpene
2	3-Cyclopentylpropionoate, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	1474	0.98	Ester
3	Linoleoyl chloride	$C_{18}H_{31}ClO$	1566	1.73	Fatty acid ester
4	Farnesal	$C_{15}H_{24}O$	1715	0.82	Sesquiterpenoid
5	Cryptomeridiol	$C_{15}H_{28}O_2$	1780	0.88	Sesquiterpenoid
6	(7*Z*,10*Z*,13*Z*)-Hexadeca-7,10,13- trienal	$C_{16}H_{26}O$	1824	0.82	Polyunsaturated fatty acid
7	Neophytadiene	$C_{20}H_{38}$	1827	5.05	Diterpene
8	Phytol	$C_{20}H_{40}O$	1949	8.58	Acyclic diterpene alcohol
9	Methyl palmitate	$C_{17}H_{34}O_2$	1950	1.42	Fatty acid methyl ester
10	Muco-Inositol	$C_7H_{14}O_6$	1974	7.27	Carbohydrate
11	Palmitic acid	$C_{16}H_{32}O_2$	1984	13.25	Saturated fatty acid
12	alpha-Linolenic acid	$C_{18}H_{32}O_2$	2078	10.32	Polyunsaturated fatty acid
13	Octadecanol	$C_{20}H_{37}F_3O_2$	2121	0.57	Saturated fatty alcohol
14	Oleic acid	$C_{18}H_{34}O_2$	2141	0.84	Unsaturated fatty acid
15	9-Hexadecenal	$C_{16}H_{30}O$	2147	2.29	Mono-unsaturated fatty aldehyde
16	Linolenic acid	$C_{19}H_{32}O_2$	2199	2.56	Polyunsaturated fatty acid
17	Oleamide	$C_{18}H_{35}NO$	2375	9.41	Fatty acid amide
18	9-Acetyl-S-octahydrophenanthrene	$C_{16}H_{20}O$	2455	4.40	Ketone
19	Calix[4]arene	$C_{28}H_{24}O_4$	2676	0.53	Macrocyclic phenols
20	beta-Monolinolein	$C_{21}H_{38}O_4$	2706	3.64	Monoglycerides
21	Triacontane	$C_{30}H_{62}$	3000	0.63	Alkane
22	6-Nitro cholesteryl acetate	$C_{29}H_{47}NO_4$	3135	2.21	Ester
23	Campesterol	$C_{28}H_{48}O$	3305	1.32	Sterol
24	Friedoolean-6-ene	$C_{30}H_{50}$	3343	0.57	Triterpene
25	alpha-Amyrin	$C_{30}H_{50}O$	3382	2.16	Pentacyclic triterpenoid
26	Urs-12-ene	$C_{30}H_{50}$	5002	0.93	Triterpene
	Total			83.78	

Table 3. Mean IC_{50} values with SD of DPPH free radical scavenging activity, metal chelating activity, and reducing power activity of the hexane and methanol extracts of the whole *A. nilagirica plant.*

Sample	Mean IC_{50} values with SD (µg/mL)		
	DPPH free radical scavenging activity	Metal chelating activity	Reducing power activity
Artemisia nilagirica methanol extract	35.26±0.99	2.41±0.02	2.03±0.02
Artemisia nilagirica hexane extract	23.68±0.20	2.23±0.01	1.86±0.01
Standard	2.54±0.03 (Ascorbic acid)	2.22±0.01 (EDTA)	4.29±0.01 (Gallic acid)

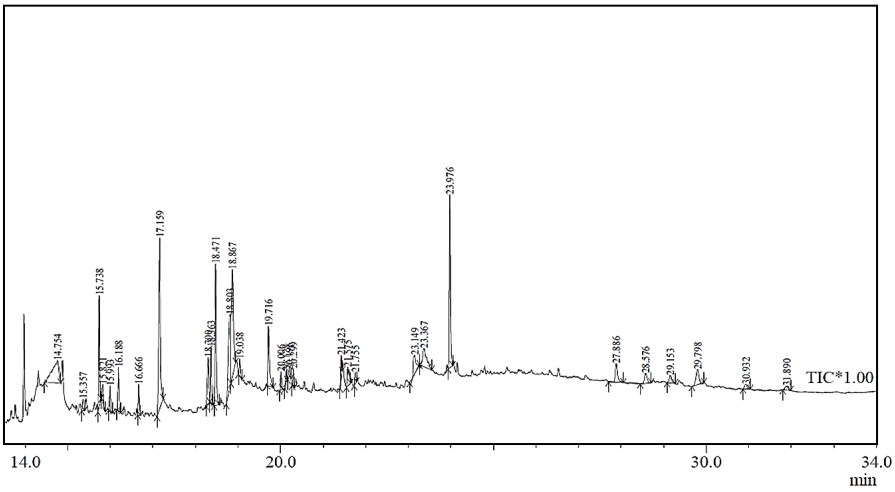

Figure 2. Gas chromatogram of the methanol extract of the whole *A. nilagirica* plant.

3.3.2. Metal chelating activity

The hexane and methanol extracts showed comparable metal chelating activity having IC_{50} values of 2.23±0.01 and 2.41±0.02 µg/mL, respectively, compared to standard EDTA that had an IC_{50} value of 2.22±0.01 µg/mL. Squalene (2.93%), which has been reported to have antioxidant properties, might have also contributed to the metal chelating activity, as it is present in plant extracts [23]. Tetracontane (15.21%) present in the hexane extract of *A. nilagirica* has been reported to exhibit antioxidant activity, as also observed in a previous study conducted on the plant *Plectranthus amboinicus* [24]. Oleamide (9.41%) present in the methanol extract of *A. nilagirica* has been reported to have antioxidant activity in endophytic fungi [25].

3.3.3. Reducing power activity of Fe^{3+}

The value of IC_{50} is the amount of antioxidant required to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by 50%. The IC_{50} values of the hexane extract, the methanol extract, and the standard (gallic acid) are 1.86±0.01, 2.03±0.02, and 4.29±0.01 µg/mL, respectively. The hexane extract showed high anti-oxidant activity in terms of reducing power activity followed by the methanol extract compared to the standard. A major compound present in the hexane extract is squalene (2.93%) which has been reported to have antioxidant properties. Therefore, the reducing power activity of the hexane extract could be due to the presence of squalene [23].

Table 4. IC_{50} with SD of the anti-inflammatory activity of standard and hexane and methanol extracts of the whole plant of *A. nilagirica*.

Samples	Mean IC_{50} values with SD (µg/mL)
Artemisia nilagirica hexane extract	3.67±0.01
Artemisia nilagirica methanol extract	2.97±0.03
Diclofenac potassium	3.79±0.01

3.4. In-vitro anti-inflammatory activity

The IC_{50} values of the plant extracts were calculated with the help of a scattered curve diagram. The IC_{50} values of the extracts of hexane and methanol were 3.67±0.01 and 2.97±0.03 µg/mL, respectively. Diclofenac potassium showed an IC_{50} value of 3.79±0.01 µg/mL. Table 4 shows the IC_{50} of the anti-inflammatory activity of plant extracts from the whole plant of *A. nilagirica*. The methanol extract showed high anti-inflammatory activity compared to the standard diclofenac potassium; however, the hexane extract showed values comparable to the standards. The anti-inflammatory effect of the methanol extract may be due to the presence of phytol (8.58%) and neophytadiene (5.05%) which are present in large amounts and have been reported to have anti-inflammatory properties [26]. Palmitic acid (13.25%) which is present in large amounts in methanol extract, is also known to have anti-inflammatory activity, as reported in fenugreek (*Trigonella foenum - graecum*) [27].

4. Conclusion

The biological characteristics of plant extracts continue to be intriguing, inspiring the creation of novel pharmaceuticals. Ethnobotany, phytochemistry, medicinal chemistry, and pharmacology must all be used together to find plant-derived compounds that can be beneficial for a variety of therapeutic purposes. The results of the present study indicate that *Artemisia nilagirica* plant extracts have strong antioxidant and anti-inflammatory activities. The plant extracts exert potent anti-inflammatory activity *in vitro*; therefore, their anti-inflammatory characteristics can be exploited further in detail to utilize them for pharmaceutical industries on a larger scale. The current study reveals that the hexane extract showed better antioxidant activity in terms of metal chelating activity and reducing power activity than the methanol extract. The anti-inflammatory activity of both the hexane and methanol extracts showed better results than the standard marketed drug diclofenac potassium. The results are still preliminary, but if different polarity extracts extracted from the plant are further investigated for their therapeutic potential, they could be used as cost-effective alternatives to synthetic drugs in the pharmaceutical industry for anti-inflammatory and antioxidant properties.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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CRediT authorship contribution statement

Conceptualization: Shiv Dubey; Methodology: Vaishali Garia; Validation: Vaishali Garia, Ananya Bahuguna; Formal analysis: Vaishali Garia, Shiv Dubey, Ananya Bahuguna; Investigation: Shiv Dubey, Om Prakash, Ravendra Kumar, Dharmendra Rawat; Resources: Shiv Dubey, Om Prakash, Ravendra Kumar, Dharmendra Rawat; Data curation: Shiv Dubey, Om Prakash, Ravendra Kumar, Dharmendra Rawat; Writing original draft: Vaishali Garia, Shiv Dubey; Writing review and editing: Vaishali Garia, Shiv Dubey; Visualization: Ravendra Kumar, Dharmendra Rawat; Supervision: Shiv Dubey, Om Prakash

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