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COMPOSITIONAL ANALYSIS AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF *ARTEMISIA NILAGIRICA* LEAVES

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ABSTRACT

Essential oil composition of leaves of *Artemisia nilagirica* (Clarke) Pamp. grown in Doon valley on the food hill of the western Himalaya in the state of Uttarakhand (India) were analyzed. Altogether 29 constituents were identified on the basis of GC-FID analysis of essential oil. The major constituents identified were β-pinene (22.29%), 1,8-cineole (8.16 %), p-cymene (8.08%), β-caryophyllene (7.71%), n-hexadecane (7.39%), artemisia ketone (5.59%), (+)-camphor (5.23%), α-terpineol (4.65%), β-eudesmol (4.35%), artemisia alcohol (3.78%), germacrene D (3.07), α-thujone (2.56%), caryophyllene oxide (2.54%), and tetratriacontane (2.37%). Physicochemical values of leaves including moisture content, total ash, acid insoluble ash, water soluble ash and sulphated ash were determined and found well within permissible limits. Phytochemical screening of various extracts of leaves indicated the presence of volatile oil in hexane and benzene extracts, alkaloids, steroids and terpenoids in all extracts, tannins in diethyl ether, methanol and aqueous methanol extracts, flavonoids in chloroform, diethyl ether, methanol and aqueous methanol extracts, carbohydrate, protein, amino acids and saponins in methanol and aqueous methanol extracts with glycosides particularly in methanol and aqueous methanol extract. The leaf essential oil showed marked total phenolic content and antioxidant activity with DPPH radical scavenging assay.

KEYWORDS: *Artemisia nilagirica*, Leaves, Essential oil, GC Profiling, Antioxidant assay.

INTRODUCTION

Artemisia nilagirica (C.B.Clarke) Pamp is a wild annual hardy plant belonging to one of the largest genus *Artemisia* represented by more than 800 species under the family Asteraceae.[1] The genus *Artemisia* found throughout northern Europe, North and S. America, Africa, and Asia. Commonly known as Indian wormwood, *A. nilagirica* is found throughout the hilly regions of India, ascending to an altitudinal range of 1600-3600 m in the western Himalayas. It is also found in Mount Abu in Rajasthan, and in the Western Ghats from Konkan southwards to Kerala. The plant has traditionally been used as folk medicine since ancient times.[2] Literature reveals the application of plant parts in various forms as brain tonics, antimalarials,[3-4] expectorant, antihelmintics, antidiabetics and in treating skin diseases, wounds, ulcers, bronchitis, tuberculosis, epilepsy, and nervous diseases in traditional medicine systems.[5-10] There are also several reports concerning the antipyretic, analgesic, antimalarial, anthelmintic, anticonvulsant,[11] antioxidant, antidiabetic, antiseptic[12], hepatoprotective,[13] expectorant, astringent, antiinflammatory and cytotoxic,[14] antimicrobial,[15] antifungal[16-17] and larvicidal[18] activities. Phytochemical investigation of the plant revealed the presence of all classes of compounds with particular reference to flavonoids, steroids, terpenoids, saponins, tannins, proteins and essential oil.[14,19] The rich accumulation of essential oils and other terpenoids in the plant is responsible for its use in flavouring various kinds of foods products or liqueurs.

A number of studies have shown that the yield and composition of essential oils varies with respect to phenological stages of plants as well as seasonal, climatic and phytogepgraphic conditions of the area where they are grown.[20-25] Investigation on essential oil composition of *A. nilagirica* from different parts of India and other parts of world showed wide variability with respect to geographical origin,[12,17,18,26-28] growth stages,[20] altitudes,[29-30] seasons[31] and genetic factors.[32] In the present study, the yield and composition of essential oil of *A. nilagirica* leaves from Dehradun situated in the Doon Valley on the foothills of the Himalayas in Uttarakhad state of India were investigated. The preliminary phytochemical screening was carried out to identify the derivatives in the leaf extracts. Total phenolic content and antioxidant efficacy of essential oil extracted from leaves was also investigated.

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals, solvents and reagents used for the extraction, phytochemical screening and *in-vitro* bioassay of *Artemisia nilagirica* leaves were of

analytical grade and refer to Sdfinechem, Ranbaxy. Some of the reagents were prepared during the experiments while some of the readymade available regents were used for qualitative detection. Anhydrous sodium sulphate (Na_2SO_4) was normally used for drying the organic solvents. Analytical samples were routinely dried over P_2O_5 for 24 h in vacuum.

Plant material

Plants of *A. nilagirica* were collected from the Botanical Garden of Forest Research Institute, Dehradun ($30^0$34′41″ N and $77^0$99′79″ E), India in full vegetative stage and were identified by Botany Division of FRI, Dehradun. Leaves were separated and washed thoroughly under running tap water then shade dried at room temperature (28±2 °C) for 5–8 days. The air-dried leaves were powdered using electrical blender.

Physicochemical evaluation

Dried and powdered leaves of *A. nilagirica* was subjected to evaluation of physicochemical characteristics viz. moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash and water and alcohol soluble extractive values were determined according to the specified methods.[33-35] Each study was performed in triplicate; mean values with standard error of mean (SEM) were calculated.

Determination of extractive values

The dried and powdered leaves of plant (200g) was successively extracted with hexane, benzene, chloroform, diethyl ether, methanol and aqueous methanol (Methanol:Water–80:20) in soxhlet apparatus. Extracts so obtained were separately distilled under reduced pressure to obtained solvent free extracts. All extracts obtained were dried, weighed and their percentage yields were calculated with respect to the dry weight of leaves initially taken.

Phytochemical screening

Stock solution (1% w/v) of all the extracts including hexane, benzene, chloroform, diethyl ether, methanol and aqueous methanol extracts of *A. nilagirica* leaves were prepared and subjected to phytochemical screening to detect the presence and or absence of constituent phytochemicals.[36-38]

Test for Carbohydrates: Fehling's test: To 1 ml stock solution of various extracts, 1 ml of Fehling's solution A and B were added separately and boiled on water bath. Formation of brick red precipitate indicated the presence of sugar.

Barfoed's test: 1 ml of Barfoed's reagent was added to 2 ml stock solution of various extracts separately and boiled. Formation of reddish brown precipitate indicated the presence of carbohydrates.

Benedict's test: 5 ml of Benedict's reagent was added to each 1 ml stock solution of various extracts separately in a test tube and heated in boiling water bath for 2 minutes. Formation of red precipitate indicates the presence of sugar.

Test for Glycosides

Keller-Killiani test: 5 ml stock solution of various extracts was added to a mixture of 5 ml of water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by addition of 1 ml of concentrated sulphuric acid. Formation of a brown ring at the interface followed by formation of a violet ring below the brown ring and formation of greenish ring in the acetic acid layer just above the brown ring and gradually spread throughout layer indicated the presence of glycosides.

Test for Alkaloids

Dragendorff's test: 1 ml of Dragendorff's reagent was added to each 1 ml stock solution of various extracts separately. Formation of an orange-red precipitate indicates the presence of alkaloids.

Mayer's test: 1 ml of Mayer's reagent (Potassium Mercuric Iodide Solution) was added to each 1 ml stock solution of various extracts separately. Formation of whitish yellow or cream coloured precipitate indicated the presence of alkaloids.

Test for Flavonoids

Alkaline reagent test

Few drops of dilute ammonia were added to each 1 ml stock solution of various extracts separately, followed by addition of concentrated HCl. Formation of yellow colour indicated the presence of flavonoids.

Shinoda test

Few drops of stock solution of various extracts were added to zinc dust separately, followed by addition of concentrated HCl. Formation of red colour indicated the presence of flavonoids.

Test for Steroids

Liebermann's test: To 5 ml stock solution of various extracts 5 ml of acetic anhydride was added to each separately in a test tube, heated and cooled followed by addition of few drops of concentrated sulphuric acid along the sides of the test tube. Formation of blue colour indicates the presence of steroids.

Test for Terpenoids

Salkowski test: Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml), was carefully added to form a layer. A reddish brown coloration formed at the interface indicated presence of terpenoids.

Test for Saponin

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

Test for Tannin

Gelatin Test: To the extracts, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Protein

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicated the presence of proteins.

Test for Amino acid

Ninhydrin Test: 3 drops of 5% ninhydrin solution was added to each 3 ml stock solution of various extracts separately in a test tube and heated in boiling water bath for 10 minutes. Formation of purple/bluish colour indicates the presence of amino acids.

Test for Lignin: 2 ml stock solution of various extracts was treated with saffranine solution. Formation of pink colour indicates the presence of lignin.

Test for Volatile Oil

A drop of Sudan red III reagent was added to the thick section of various extract on a glass slide, washed with 50% alcohol after 2 mins and mounted in glycerine. Appearance of red coloured oil globule under microscope indicated the presence essential oil.

Extraction of Essential oil

The plant samples (100 g) were subjected to hydrodistillation in a Clevenger's Apparatus for 3hrs. For each batch of distillation, three replicates of 20g each of fresh plant material were dried at 60^0C for 48 hrs to determine moisture content. The extracted oil was dried over anhydrous sodium sulphate and stored in opaque plastic vials at 4^0C-8^0C for the subsequent analysis.

Analysis of Essential Oil

The isolated volatile oil was analysed by gas chromatography (GC) using gas chromatograph (Chemito) equipped with flame ionization detector (FID) and a capillary column BP-5 of 30 m length, 0.25 mm i.d. and 0.25µm film thickness. Temperature was programmed from 60-300^0C at an increment rate of 5^0C/min, held isothermally at 60^0C and 300^0C for 2 and 10 min, respectively. Injector and detector temperatures were set at 220°C and 250°C, respectively. Sample injection volume 2 µL of essential oil (diluted 5 µL oil in 2 mL dichloromethane, HPLC grade) was injected. Nitrogen was used as a carrier gas at flow rate 2 mL/min

Determination of TPC and antioxidant efficacy of oil

Total phenolic content (TPC) in the essential oil obtained from *A. nilagirica* leaves was determined using Folin-Ciocalteu colorimetric method.[39] Calculation of TPC was made with respect to the equation of the standard curve of Gallic acid and expressed as as µg/ml of gallic acid equivalents (GAE). The antioxidant potency of the essential oil was determined as DPPH radical scavenging capacity.[40] 1 ml of essential oil (at different concentrations) was mixed with 0.5 ml of 0.2 mM DPPH methanolic solution. The reaction was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm. The scavenging activity was estimated using the following equation

Scavenging effect (%) = $[(Ac - As)/Ac] \times 100$

where Ac is the absorbance of the control reaction (containing all reagents except the test sample) and As is the absorbance of the tested sample.

The experiment was done in triplicate and mean values were recorded. IC50 value was calculated as the concentration of the sample, required to scavenge 50% of DPPH free radicals.

RESULTS AND DISCUSSION

Physicochemical Evaluation: Powdered leaves of *A. nilagirica* was subjected to physicochemical evaluation for the traits like moisture content, ash values including total ash, water soluble ash, acid insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and extractive values in different organic solvents of varying polarity. The observation and results of the physicochemical studies are presented in Table 1.

Moisture content of the leaf sample was found 12.87±0.23% which is within the recommended range of 8-14% for vegetable drug materials indicating that the plant leaves can be stored for a long period of time with less probability of microbial attack. Total ash, acid insoluble ash, water soluble ash, and sulphated ash of leaf powder of *A. nilagirica* was found to be 7.83±0.32%, 0.25±0.24%, 0.35±0.16% and 0.21±0.24 respectively. The total ash is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash. From results, it is clear that the amount of water soluble ash is greater than that of acid insoluble ash. The ash content gives an idea about the inorganic content of powdered leaves under investigation and thus the quality of the drugs can be assessed. These ash values are important quantitative standards. The calculated values are within the permissible limit.

Table -1. Physicochemical values of A. *nilagirica* leaves

Parameters	Result (% w/w)
Moisture Content	12.87±0.23
Total ash	7.83±0.32
Acid insoluble ash	0.25±0.24
Water soluble ash	0.35±0.16
Sulphated ash	0.21±0.24
Alcohol soluble extractive	9.81±0.17
Water soluble extractive	6.39±0.33

On the other hand, the water soluble extractive value of the leaves was found to be 6.39±0.33% (w/w) which indicates the presence of water soluble components such as sugar, acids and inorganic compounds etc. and the alcohol soluble extractive value was found to be 9.81±0.17% (w/w) which indicates the presence of polar organic constituents like phenols alkaloids steroids glycosides flavonoids (Table 1). The results of physicochemical analyses

lie within the acceptable limit which in turn ascertains the quality as well as purity of leaf drugs. These parameters are therefore some useful quality standards included in the standardization of *A. nilagirica* leaf materials.

Extractive Value: Extractive values of *A. nilagirica* leaves determined under sequential extraction are presented in Table 2 that provided idea about extractability of chemical constituents in different organic solvents.

Plants possess different solubility behaviour in various solvents. The yields of extracts from a plant in different solvents provide information about the solubility of plant chemical constituents in different organic solvents thus suggesting the best solvent for extraction of phytochemicals.

Table 2. Extractives values under successive extraction

Solvents used	Extract Yields (%)
Hexane	2.21
Benzene	1.68
Chloroform	2.46
Diethyl ether	3.14
Methanol	12.50
Aq. Methanol	9.68

It is clear from results given in Table 2, that percentage yield of methanolic extract from leaves of *A. nilagirica* is much higher than other extracts. This indicates that methanolic extract contain much more chemical constituents other than other extracts.

Phytochemical screening

Phytochemical test were carried out on the *A. nilagirica* leaf extracts to ascertain the presence and/or absence of the natural bioactive compounds. The phytochemical screening of hexane, benzene, chloroform, diethyl ether, methanol and aqueous methanol extracts showed the presence of major derivatives and their results were summarized in Table 3.

Table 3. Phytochemical screening of *Artemisia nilagirica* leaf extracts

Phytochemicals	Hexane Extract	Benzene Extract	Chloroform Extract	Diethylether Extract	Methanol Extract	Aq. Methanol Extract
Volatile oil	+	+	–	–	–	–
Carbohydrates	–	–	–	–	+	+
Protein	–	–	–	–	+	+
Amino acids	–	–	–	–	+	+

Alkaloids	+	+	+	+	+	–
Steroids	+	+	+	+	+	–
Terpenoids	+	+	+	+	+	+
Flavonoid	–	–	+	+	+	+
Tannins	–	–	–	+	+	+
Saponins	–	–	–	–	+	+
Glycosides	–	–	–	–	+	+
Lignin	–	–	–	–	–	–

Phytochemical screening of *A. nilagirica* leaves revealed the presence of alkaloids, flavonoid, tannins, steroids, terpenoids, saponins, glycosides Protein amino acids, carbohydrates, and volatile oil in various extracts. The analysis showed the occurrence of alkaloids, steroids and terpenoids in all extracts. Tannins were present in diethyl ether, methanol and aqueous methanol extracts. Volatile oils were present in hexane and benzene extracts. Flavonoids were present in chloroform, diethyl ether, methanol and aqueous methanol extracts. Carbohydrate, protein, amino acids and saponins were present in methanol and aqueous methanol extracts with glycosides particularly present in methanol and aqueous methanol extract. Lignin was absent in all the extracts.

Furthermore, it is evident from the Table 3 that the methanol extract recorded the maximum number of chemical constituents including alkaloids, steroids, terpenoids, phenolics, flavonoids, tannins, saponins, protein, amino acids, carbohydrates and glycosides. The presence of a number of secondary metabolites indicated towards the occurrence of significant therapeutic activity. Presence of steroidal compounds is of importance in pharmaceutical application as these compounds are responsible for several biological functions in the human body. This may be the reason that it is used in facilitating the secretion of milk and also in reducing the distension of mammary glands. The presence of flavonoids, which are considered to be good free-radical scavengers, indicates that this plant may have antioxidant properties. Tannins are linked to antibacterial activity [41] and glycosides are associated in lowering blood pressure. The plant contains phenolics which are powerful antioxidants. The presence of saponins protects plant from microbial pathogens.[42] Presence of flavonoids may be responsible for anti-inflammatory activity as they act as non-steroidal anti-inflammatory agents by inhibiting the enzymes that cause the synthesis of prostaglandins.[43] Bioactive agents including flavonoids and saponins have the ability to inhibit pain perception and they can also serve as anti-inflammatory agent.[43,44] Results reveals that the all extracts has large number of phytoconstituents, which may be responsible

for many pharmacological activities; further work is required to investigate all the extracts of *A. nilagirica* for various pharmacological activities. Presence of different types of phytochemicals in *A. nilagirica*, leaves provide scientific explanation to the medicinal value of the plant.

Yield and composition of essential oil: Hydrodistillation of *A. nilagirica* leaves produced pale yellow volatile oil in the yield of 0.62% (w/w) with camphoraceous odour, pungent, bitter-sweet taste and greasy touch. The essential oil was subjected to GC-FID analysis to identify the different constituents of the oil, which resulted in the identification of 29 constituents representing 99.15 % of the oil. The GC chromatogram (Fig. 1) depicts different peaks of the essential oil constituents.

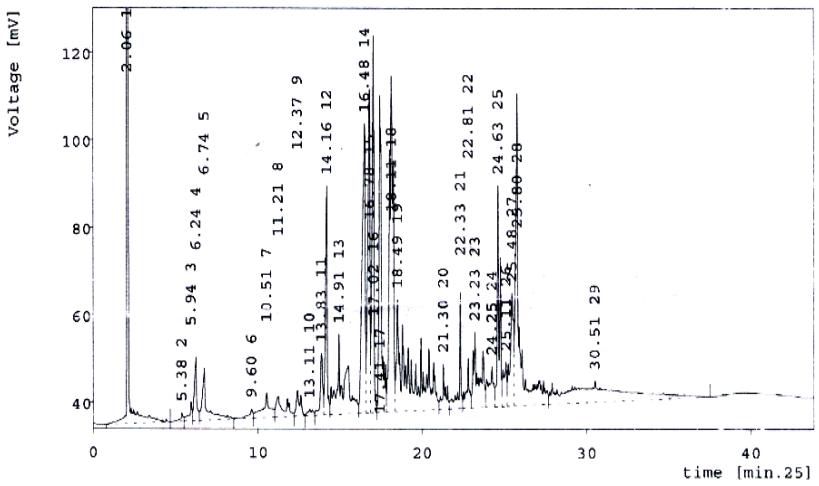

Fig. 1: GC-FID Chromatogram of *A. nilagirica* leaf essential oil

List of compounds identified in the oil are summarized in Table 4. The table shows peaks of the compounds with their corresponding retention times and their possible identities. The oil represents mainly a mixture of monoterpenes and sesquiterpenes. The major ones are β-pinene (22.29%), 1,8-cineole (8.16 %), p-cymene (8.08%), β-caryophyllene (7.71%), n-hexadecane (7.39%), artemisia ketone (5.59%), (+)-camphor (5.23%), α-terpineol (4.65%), β-eudesmol (4.35%), artemisia alcohol (3.78%), germacrene D (3.07), α-thujone (2.56%), caryophyllene oxide (2.54%), tetratriacontane (2.37%), along with that, some important constituents such as β-thujone (1.60%), amyl-alcohol (1.33%), hexatriacontane (1.31%), (+)-sabinene (1.07%) dl-limonene (0.99), γ-terpinene (0.98%), α-humulene (0.88%), pentyl butyrate (0.86%), citronellal (0.84%), isobornyl isobutyrate (0.69%), geraniol (0.64%), α-

pinene (0.35%), champhene (0.25%) γ-terpinine (0.19%) and β-myrcene (0.15%) with low percentage.

Table 4: Essential oil composition of *A. nilagirica* leaves

Peak No.	Retention Time	Peak Area (%)	Peak Height (%)	Probable Identity	Composition (%)
1	2.06	21.88	47.77	β-Pinene	22.29
2	5.38	0.15	0.13	β-Myrcene	0.15
3	5.94	0.34	0.30	α-Pinene	0.35
4	6.24	0.97	0.98	γ-Terpinene	0.98
5	6.74	1.58	0.78	β-Thujone	1.60
6	9.60	0.25	0.13	Champhene	0.25
7	10.51	1.06	0.38	(+)-Sabinene	1.07
8	11.21	0.87	0.32	α-Humulene	0.88
9	12.37	0.64	0.40	Geraniol	0.64
10	13.11	0.19	0.09	γ-Terpinine	0.19
11	13.83	0.98	0.94	dl-Limonene	0.99
12	14.16	2.52	3.53	α-Thujone	2.56
13	14.91	3.71	1.22	Artemisia alcohol	3.78
14	16.48	5.49	4.46	Artemisia ketone	5.59
15	16.78	4.28	4.98	β-Eudesmol	4.35
16	17.02	4.57	5.80	α-terpineol	4.65
17	17.41	5.14	4.88	(+)-Camphor	5.23
18	18.12	7.93	5.17	p-Cymene	8.08
19	18.49	8.01	1.72	1,8-Cineole	8.16
20	21.30	0.82	0.70	Citronellal	0.84
21	22.33	1.31	1.79	Amyl-alcohol	1.33
22	22.81	0.85	0.77	Pentyl butyrate	0.86
23	23.23	2.33	1.16	Tetratriacontane	2.37
24	24.25	1.29	0.62	Hexatriacontane	1.31
25	24.63	3.01	3.41	Germacrene D	3.07
26	25.11	0.67	0.68	Isobornyl isobutyrate	0.69
27	25.48	2.49	1.74	Caryophyllene oxide	2.54
28	25.80	7.57	4.82	β-caryophyllene	7.71
29	30.51	7.26	0.33	n-Hexadecane	7.39
SUM		**98.16**			**99.9**

In the previous studies major constituents including α-thujone, β-thujone, camphor, 1,8-cineole, borneol, linalool, artemisia alcohol, along with other mono and sesquiterpenoids were reported in *A. nilagirica* essential oil from north and south Indian origin. Padalia et al., (2014) reported that the essential oils from aerial parts of *A. nilagirica* were mainly composed of monoterpenoids (59.0%-77.3%) and sesquiterpenoids (15.7%-31.6%). The major constituents identified were artemisia ketone (38.3%-61.2%), chrysanthenone (1.5%-7.7%), germacrene D (3.1%-6.8%), β-caryophyllene (1.9%-6.8%), germacra-4,5,10-trien-1-

α-ol (1.9%-4.9%) and artemisia alcohol (1.4%-3.6%). Furthermore, Sarma *et al* (2014) reported major compounds including sabinene (3.5%), p-cymene (14.1%), 1,8, cineole (18.2%), artemesia ketone (5.7%), artemesia alcohol (3.2%), camphor (9.1%), β-eudesmol (5.8%) and δ-cadinene (3.1%) in the essential oil derived from aerial part of *A. nilagirica* from Northeastern part of India. In an earlier report, caryophyllene oxide (28.6%), borneol (35.8%) and camphor (46.9%) were reported as major constituents (Haider *et al.*, 2007). However, in the present study, β-pinene, 1,8-cineole, p-cymene, β-caryophyllene, n-hexadecane, artemisia ketone, (+)-camphor, α-terpineol, β-eudesmol, artemisia alcohol, germacrene D, α-thujone, caryophyllene oxide, tetratriacontane have been found as the major constituents along with considerable percentage of β-thujone, amyl-alcohol, hexatriacontane, (+)-sabinene and low percentage of dl-limonene, γ-terpinene, α-humulene, pentyl butyrate, citronellal, isobornyl isobutyrate, geraniol, α-pinene, champhene, γ-terpinine and β-myrcene. Compositional variation of essential oil of *A. nilagirica* leaves recorded in the present study as well in previous studies may be due to distinction in agroclimatic and phytogeographic conditions of specific regions,[45] harvesting time[46] and/or genetic factors.[32] Essential oil bearing plants growing at different geographical location are known to show qualitative and quantitative variations. The chemical composition of leaf essential oil of *A. nilagirica* grown in Doon Valley on the foothills of western Himalaya as investigated in this study may be useful with regard to use of plant leaves and its essential oil as an active pharmaceutical ingredient in drug formulations based on chemical constitution which is critical for therapeutic success.

Phenolic content and antioxidant efficacy of essential oil

The total phenolic content (TPC), expressed as mg of gallic acid equivalent (GAE)/g of dry weight was found to be 28.1±0.12 mg of GAE/g DW. Evaluation of DPPH radical scavenging activity of essential oil indicated considerable levels of antioxidant activities based on the low IC50 value recorded as 26.11 ± 1.04 µg/ml and thus suggesting the high radical scavenging activity of the oil. As per compositional analysis of *A. nilagirica* leaf essential oil is dominated by monoterpene hydrocarbons that possess a significant antioxidant capacity.[47] Presence of powerful radical scavenging compounds β-Caryophyllene and Germacrene D as major constituents in the essential oil of *A. nilagirica* leaves may be responsible for its antioxidant activity.[48] Furthermore, β-Caryophyllene reported to have high inhibitory capacity on lipid peroxidation, thus exhibiting high scavenging activities

against hydroxyl radical and superoxide anion.[49] Further, presence of α-terpineol may also be referred to elucidate the antioxidant activity of the essential oil.[50] Also, presence of phenolic compounds may also be responsible for antioxidant potential.[51] However, it is difficult to attribute the antioxidant effect of total essential oil to one or few active compounds. Essential oils capable of scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline.[52] Results of the study thus, emphasize the importance of *A. nilagirica* leaf essential oils for its potential use as natural ingredient in drug and as natural additives in food processing.

CONCLUSION

The present study reports the essential oil composition of the leaves of *A. nilagirica*, growing in Doon valley on the foothills of western Himalaya in the state of Uttarakhand (India). Altogether twenty-nine constituents were identified on the basis of GC analysis from the essential oil of the leaves of *A. nilagirica*; major ones are β-pinene (22.29%), 1,8-cineole (8.16 %), p-cymene (8.08%), β-caryophyllene (7.71%), n-hexadecane (7.39%), artemisia ketone (5.59%), (+)-camphor (5.23%), α-terpineol (4.65%), β-eudesmol (4.35%), artemisia alcohol (3.78%), germacrene D (3.07), α-thujone (2.56%), caryophyllene oxide (2.54%), tetratriacontane (2.37%). The oil showed marked antioxidant activity with DPPH radical scavenging assay.

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