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GC–MS Analysis Of Phytocomponents From The Leaf, Stem And Root Methanolic Extracts Of *Artemisia nilagirica* (C.B. Clarke) Pamp

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Abstract

Artemisia nilagirica (C.B.Clarke) Pamp, is an essential medicinal plant belonging to the Asteraceae family. The purpose of this study was to identify the bioactive chemicals in *Artemisia nilagirica* methanolic extract from its leaf, stem and root using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Phyto-chemicals are naturally occurring compounds found in plants that have biological activities such as antioxidant, antibacterial, antifungal and anti-cancer properties. They help prevent diseases by scavenging and chelating free radicals. GC-MS detected thirty-three bioactive compounds in the leaf methanolic extract, while eighteen and ten bio active compounds were detected in the stem and root methanolic extracts, respectively. 3-O-Methyl-d-glucose (64.3%), β- longipinene (25.22%), (E)-β-Famesene (22.27%) were the major bioactive molecules identified in *A. nilagirica*. Significant compounds with both pharmacological and biological properties were also found, such as Caryophyllene, Cis-α-Bisabolene,1,5-Naphthyridin-4-ol, Humulene, Germacrene D, 3-O-Methyl-d glucose, Phytol. Therefore, the isolation and purification of compounds with therapeutic potential from *A. nilagirica* could be used to combat several diseases in plants and humans.

Keywords: Artemisia nilagirica, GC-MS analysis, Phyto-chemicals, Pharmacology, Methanolic extract.

Introduction

The knowledge and usage of phyto-chemicals as medicine can be traced back to very ancient times and can be found in traditional medical systems. Bioactive substances have been shown to suppress microorganisms since 1926, and there has been evidence of a dramatic rise in the occurrence of resistance to bacteria, fungi, viruses, protozoa, and other pathogens since then as well [1]. Artemisia, a prominent genera within the Asteraceae family, encompasses an extensive variety of phyto-chemicals. These phyto-chemicals have several pharmacological effects. Consequently, a great deal of attention was attracted to the use of these medicinal herbs. *Artemisia nilagirica*, often known as Indian wormwood, is a medicinal plant that has been used for over 10 decades to cure many ailments and symptoms, including malaria, diabetes, inflammation, stress, depression, and microbiological disorders. *A. nilagirica* is an aromatic herb that includes many

volatile oils and biologically important compounds [2]. *A. nilagirica* is a well-known member of its genus. It grows in many places in India, including Kerala and the Western Ghats [3]. It is commonly referred to as "Nagdona" and grows up to 150 cm tall on nitrogen-rich soil in Indian Himalayan districts [4]. *A. nilagirica* was reported to possess anti-fungal [5], anti-malarial [6], anti-cancer [7], anti-inflammatory [8], antioxidant [9], anti-asthmatic [2] and anti-cancer properties [3]. These evidences demonstrate that the entire plant has a high medicinal value. The current study was to identify several biologically active compounds in *A. nilagirica's* leaf, stem, and root methanolic extract.

Material and Methods

Collection and Preparation of Plant Material

Artemisia nilagirica (Clarke) Pamp leaves, stems, and roots were collected in June and July from the Environmental Protection Training and Research Institute in Gachibowli, Hyderabad. The gathered samples were thoroughly washed with running water, dried in the shade and then homogenized. After dissolving 5g of these plant components in 50 ml of methanolic solvent, they were extracted by subjecting them to incubation for 48 hours in an orbital shaker operatingat120 rpm and a temperature of 22°C.

Gas Chromatography and Mass Spectroscopic (GC-MS) Analysis

GC-MS were used in the process of determining the quantitative and qualitative identities of organic compounds present in the sample provided. The possible bioactive components in the extracts of leaves, stems, and roots of *A. nilagirica* were evaluated using GC-MS (Agilent 8890GC system). Helium was used as the gaseous carrier, with a flow rate of 1 ml/min and an inject or volume of 1µl at a temperature of 75°C. The oven was set to a temperature of 350°C. The total GC-MS running time was 32.5 minutes for the leaf methanolic extracts of *A. nilagirica* and 34.5 minutes for the stem and root methanolic extracts, respectively. The process of identifying bioactive chemical substances is based on their retention time, peak area, molecular weight and molecular formula. We identified the phyto-chemicals by comparing their generated MS spectrum patterns to the MS spectra at the National Institute of Standards and Technology (NIST) MS Database library.

Results and Discussion

GC–MS profiling of methanol extracts of *A. nilagirica* leaves, stems and roots showed the presence of different possible organic molecules with distinct biological activities. Phyto-constituents in extracts were detected using multiple parameters, including retention time (RT) and the molecular formula of compounds. Using GC-MS, we found the 33 phyto-constituents in leaf extract, 18 compounds in stem extract, and 10 compounds in root extract (Tables 1, 2, and3).

GC-MS Analysis of Leaf Methanolic Extract

GC-MS revealed the presence of 33 bioactive components in *A. nilagirica* Leaf methanolic extract (Table 1, Fig.1). The bioactive compounds identified through GC-MS analysis were 1,2-15,16-Diepoxyhexadecane (RT-7.51), which was reported to have anti-inflammatory, antioxidant and anti-tumor activities in *Tulipa edulis*, *Artemisia annua* and *Sida cordata* [10-12]. 17-Octadecynoic acid (RT-7.51) which was similarly documented in *Cucurbita maxima* and was recognized for its nematicide properties [13,14]. cis-5,8,11,14,17-Eicosapentaenoic acid (RT-12.94) possess an anti-thrombotic agent [15]. The compound Caryophyllene identified in both leaf and stem extracts possesses antibacterial, analgesic, antioxidant, anti-inflammatory activities [16,17]. 1,5-Naphthyridin-4-ol (14.04) exhibits anti-malarial activity [18]. 8-hydroxyquinoline-5-carbaldehyde (14.04) exhibits anti-microbial and anti-fungal activity [19]. Cis-α-Bisabolene reported in both leaf and stem extracts possess anti-fungal and anti-bacterial activity [20]. Humulene (RT-14.45) has anti-inflammatory activity and anti-tumor activity in *Cordia verbenacea* [21,22]. The bioactive compound Germacrene D, identified in both leaf and stem, has anti-microbial activity [23]. Interestingly, it was discovered that the structure of amorpha-4,11-diene and Germacrene D were closely related. Amorpha-4,11-diene, which possesses an amorphane structure, serves as a direct precursor of Artemisinin [24]. 3-O-Methyl-d-glucose, a bioactive compound found in both leaf and stem extracts, was reported to have anti-cancer properties [25]. 2-Octyl cyclopropene-1-heptanol (RT-23.08) also found in *Gloriosa superba* exhibited anti-bacterial activity [26]. 3-Decanynoic Acid (RT-23.08) is a bioactive with numerous biological features,

including acidifier, arachidonic acid inhibitor, and inhibit uric acid production [27]. Hexadecanoic acid, methylester (RT-25.53) exhibits anti-fungal activity [28]. Pentadecanoic acid, 14-methyl-methyl ester, and Undec-10-ynoic acid, undecyl ester, are two additional substances identified as having antioxidant properties [29]. Phytol (RT-30.11) also displays a number of biological characteristics like antinociceptive effect, antioxidant effect, anti-inflammatory and anti-allergic effects [30,31]. Undec-10-ynoic acid, undecyl ester (RT-30.11)has been discovered to have antioxidant action in the bark of Tampoi (*Baccaurea macrocarpa*) [32]. Undec-10-ynoic acid, nonyl ester (RT-30.11) was reported to have antibacterial and anticancer properties in *NeoLamarckia cadamba* [33]. A few substances pharmacological properties have not yet identified and reported (Table1).

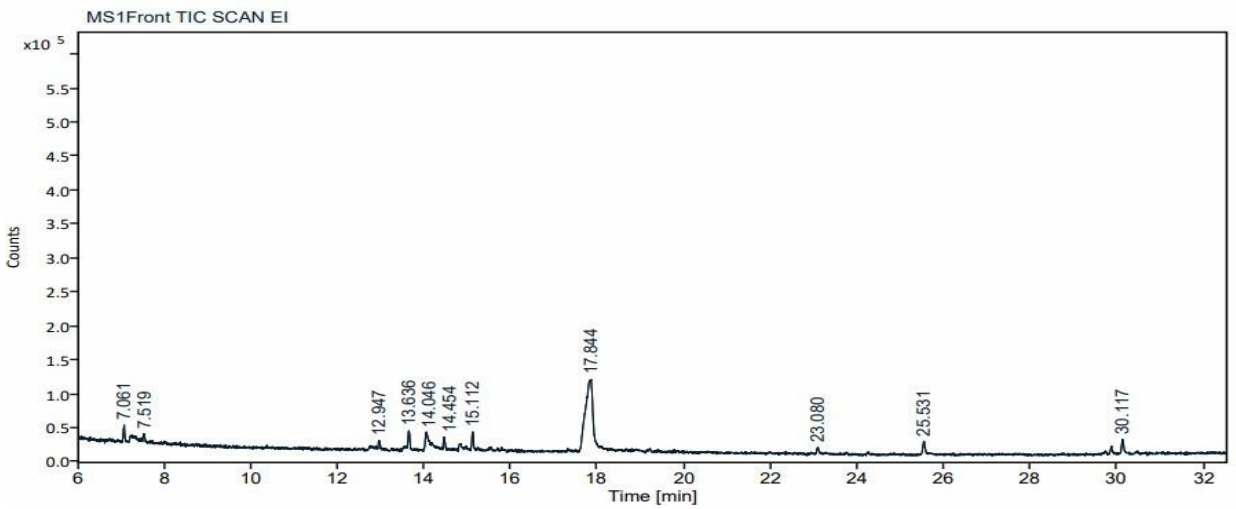

Fig.1 GC-MS chromatogram of *Artemisia nilagirica* leaf methanolic extract.

Table1: Phyto-chemical components identified in *Artemisia nilagirica* leaf methanolic extracts using GCMS analysis and their reported biological activities.

S.No	CAS	RT	Name of compound	Molecular Wt. (g/mol)	Molecular Formula	Area %	Recorded Biological activity
1	61465-24-5	7.061	(1S-(1Alpha,2alpha,4beta))-1-isopropenyl-4-methyl-1,2-cyclohexanediol	170.25	$C_{10}H_{18}O_2$	3.05	No activity
2	77954-13-3	7.061	Benzofuran, octahydro-6-methyl-3-methylene-	152.23	$C_{10}H_{16}O$	3.05	No activity
3		7.061	1,2-15,16-Diepoxyhexadecane	254.41	$C_{16}H_{30}O_2$	3.05	Antitumor, Antioxidant, Anti-inflammatory
4		7.519	1,2-15,16-Diepoxyhexadecane	254.41	$C_{16}H_{30}O_2$	2.21	Antitumor, Antioxidant, Anti-inflammatory
5	63521-76-6	7.519	Tridecanedial	212.33	$C_{13}H_{24}O_2$	2.21	No activity
6	34450-18-5	7.519	17-Octadecynoic acid	280.4	$C_{18}H_{32}O_2$	2.21	Nematicide, Pathogenesis of periapical Abscesses
7	10417-94-4	12.947	cis-5,8,11,14,17-Eicosapentaenoic acid	316.5	$C_{21}H_{32}O_2$	2.09	Anti-thrombotic agent
8	21698-41-9	12.947	1,4-Dimethyl-7-(prop-1-en-2-yl) decahydroazulen-4-ol	222.3	$C_{15}H_{26}O$	2.09	No activity
9	4677-90-1	12.947	Cyclopropa [d]naphthalen-2 (4aH)-one, 1,1a,5,6,7,8-hexahydro-4a,8,8-trimethyl-	204.3	$C_{14}H_{20}O$	2.09	No activity
10	87-44-5	13.636	Caryophyllene	204.3	$C_{15}H_{24}$	4.01	Anti-bacterial, Analgesic, Antioxidant, Anti inflammatory
11	13877-93-5	13.636	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-	204.3	$C_{15}H_{24}$	4.01	No activity

12	41432-70-6	13.636	β-Longipinene	204.3	$C_{15}H_{24}$	4.01	No activity
13	5423-54-1	14.046	1,5-Naphthyridin-4-ol	146.1	$C_8H_6N_2O$	6.89	Anti malarial activity
14	2598-30-3	14.046	8-Hydroxyquinoline-5-carbaldehyde	173.17	$C_{10}H_7NO_2$	6.89	Antimicrobial and Anti fungal activity
15	186028-84-2	14.046	3',4'- Methylenedioxy-Ntert butyl cathinone	285.80	$C_{14}H_{19}NO_3.HCl$	6.89	No activity
16	29837-07-8	14.454	Cis-α-Bisabolene	204.35	$C_{15}H_{24}$	2.54	Anti-fungal, Anti-bacterial activity
17	24048-44-0	14.454	Spiro [4.5] dec-7-ene,1,8-dimethyl-4-(1-methylethenyl)-,[1S-(1α,4β,5α)]-	204.35	$C_{15}H_{24}$	2.54	No activity
18	6753-98-6	14.454	Humulene	204.35	$C_{15}H_{24}$	2.54	Anti-inflammatory, Anti-tumor activity
19	1374-15-5	15.11	1H-Cyclopenta [1,3] cyclopropa [1,2] benzene, octahydro-7-methyl-3-methylene	204.35	$C_{15}H_{24}$	4.15	No activity
20	18252-44-3	15.11	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02,7] decane	220.35	$C_{15}H_{24}O$	4.15	No activity
21	23986-74-5	15.11	Germacrene D	204.35	$C_{15}H_{24}$	4.15	Anti-microbial activity
22		17.84	3-O-Methyl-d-glucose	194.18	$C_7H_{14}O_6$	64.3	Anti- tumor activity
23	26295-70-5	17.84	β-d-Mannofuranoside, methyl	194.18	$C_7H_{14}O_6$	64.3	No activity
24		17.84	3-Methylmannoside	194.18	$C_7H_{14}O_6$	64.3	No activity
25	54467-85-5	23.08	2-Octylcyclopropene-1-heptanol	266.5	$C_{18}H_{34}O$	2.43	Anti bacterial activity
26	141033-65-0	23.08	Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-, [2α(R*),3α]-(.+-.)-	182.26	$C_{11}H_{18}O_2$	2.43	No activity
27	27007-78-9	23.08	3-Decanynoicacid	168	$C_{10}H_{16}O_2$	2.43	Acidifier, Arachidonic Acid Inhibitor, Inhibit Production of uric acid
28	112-39-0	25.53	Hexa decanoic acid, methyl ester	270.45	$C_{17}H_{34}O_2$	4.60	Anti-fungal activity
29	5129-60-2	25.53	Penta decanoic acid, 14-methyl-,methylester	270.45	$C_{17}H_{34}O_2$	4.60	Anti-oxidant
30		25.53	Methyl 3-Methyl penta decanoate	270.45	$C_{17}H_{34}O_2$	4.60	No activity
31	150-86-7	30.11	Phytol	296.53	$C_{20}H_{40}O$	3.68	Anti nociceptive effect Antioxidant effect Anti-inflammatory and anti-allergic effects
32		30.11	Undec-10-ynoic acid, undecyl ester	336.5	$C_{22}H_{40}O_2$	3.68	Anti-oxidant activity
33		30.11	Undec-10-ynoic acid, nonyl ester	308.4	$C_{20}H_{36}O_2$	3.68	Anti-bacterial, Anti-cancer

GC–MS Analysis of Stem Methanolic Extract

GC-MS revealed the presence of 18 distinct bioactive components in *A. nilagirica* stem methanolic extract (Table 2, Fig.2). The initial chemical that came out was found to be D-Mannopyranose (RT-4.0), which prevents urinary tract infections [34]. The other substances identified were DL-Arabinose (RT-6.77), which was previously discovered in *Chenopodium album* and is well-known for its antifungal properties [35]. 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (RT-7.08) is a biologically active chemical having a few biological properties like antioxidant effect, antiproliferative, proapoptotic effects [36]. Isosorbide Dinitrate (RT-9.31) also exhibit a number of biological properties like antianginal agent, anti-ulcerative, anti-oxidative, and anti-inflammatory effects [37]. The bioactive chemical Muramic acid (RT-10.14) can be used as bacterial markers in airborne particles and settled dust [38]. It was recognized that the substance Caryophyllene (RT-13.8) had anti-fungal, anti-cancer, anti-bacterial and analgesic properties in *Eugenia caryophyllata* [39,40,16]. cis-α-Bisabolene (RT-14.61) was well known for its anti-fungal and anti-bacterial properties reported in the Rhizosphere of Acacia [41]. The bioactive molecule, Germacrene D with a retention time of 15.27 was found to have anti-microbial activity. 3-O-Methyl-d-glucose (RT-19.11) was the main substance (with a peak area of 34.6%) found in the stem extract and it is well-known for its anticancer

effect in murine L1210 leukemia. n-Hexadecanoic acid, which has a retention time of 26.6 minutes, has been utilized as an anti-inflammatory, antioxidant, nematicide, pesticide, and anti-bacterial substance [42-44]. The chemical eluted at (RT-30.3) was recognized as Phytol, which has antinociceptive, antioxidant, anti-inflammatory and antiallergic effects. Sucrose (with a peak area of 10.4%) was the second significant chemical found at retention time 14.15, which has numerous biological properties such as antiarthritic, antifungal, antibacterial, analgesia for neonatal circumcision, and activates crucial metabolites and the accumulation of free sugars [45]. The chemical Linoelaidic acid (RT-30.7) was well known for its apoptosis activity [46]. The bioactive compound eluted at 30.8 minutes was identified as 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, which is known to have a various biological actions, such as anti-cancer, cardiovascular protection, protecting the nervous system, preventing osteoporosis, reducing inflammation, blood clot reduction, anti-obesity, and anti-metabolic syndrome and possessing antioxidant characteristics [47](Table2).

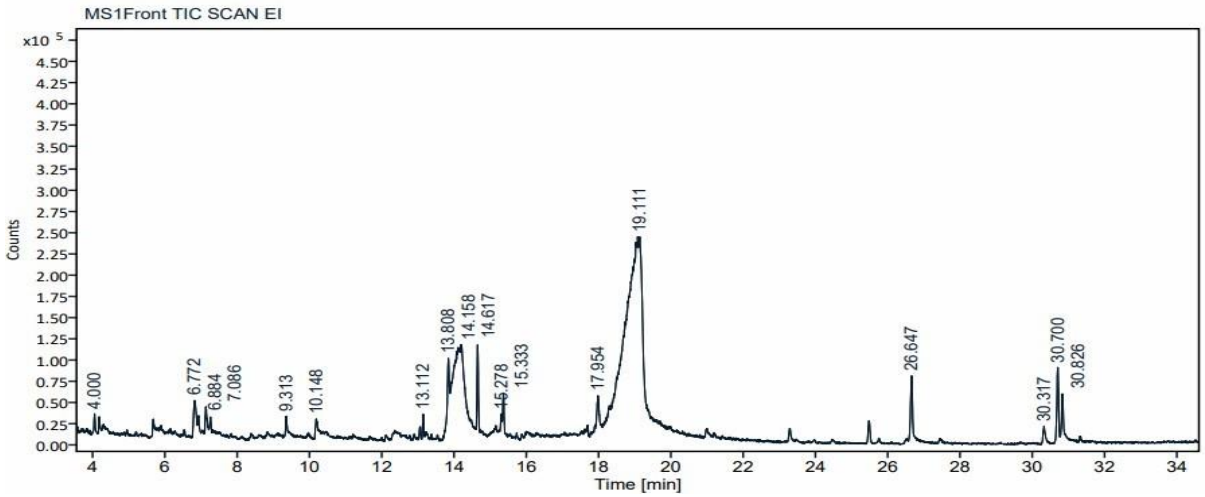

Fig.2 GC-MS chromatogram of *Artemisia nilagirica* stem methanolic extract.

Table 2 Phyto-chemical components identified in *Artemisia nilagirica* stem methanolic extract using GC-MS analysis and their reported biological activities.

S.No	CAS	RT	Name of compound	Molecular Wt. (g/mol)	Molecular Formula	Area%	Recorded Biological activity
1	530-26-7	4.0	D-Manno pyranose	180.1	$C_6H_{12}O$	1.03	Prevent urinary tract infections
2	20235-19-2	6.77	DL-Arabinose	150.1	$C_5H_{10}O_5$	4.66	Anti-fungal activity
3	23235-99-6	6.88	2-Deoxy-2-fluoro-1,6-anhydro-β-d-glucopyranose	164.1	$C_6H_9FO_4$	1.41	No activity
4	28564-83-2	7.08	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.12	$C_6H_8O_4$	2.40	antioxidant effect anti proliferative, proapoptotic effects.
5	87-33-2	9.31	Isosorbide Dinitrate	236.14	$C_6H_8N_2O_8$	1.14	Anti anginal agent Anti-ulcerative, anti-oxidative, anti-inflammatory effects
6	1114-41-6	10.14	Muramic acid	251.23	$C_9H_{17}NO_7$	2.91	Bacterial markers
7	515-13-9	13.11	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-	204.3	$C_{15}H_{24}$	1.45	No activity
8	87-44-5	13.8	Caryophyllene	204.3	$C_{15}H_{24}$	3.93	Anti-fungal, Anti-cancer activity Anti-bacterial and Analgesic Properties
9	57-50-1	14.15	Sucrose	342.3	$C_{12}H_{22}O_{11}$	10.4	Anti-arthritic, antimicrobial, antifungal, analgesic for circumcision and stimulates important metabolite and free sugars accumulation.

10	29837-07-8	14.61	cis-α-Bisabolene	204.3	$C_{15}H_{24}$	5.28	Anti-fungal, Anti-bacterial activity
11	23986-74-5	15.27	Germacrene D	204.3	$C_{15}H_{24}$	1.49	Anti-microbial activity
12	41432-70-6	15.33	β-Longipinene	204.3	$C_{15}H_{24}$	3.19	No activity
13	35810-56-1	17.9	Alpha-l-rhamnopyranose	164.1	$C_6H_{12}O_5$	4.34	No activity
14		19.11	3-O-Methyl-d-glucose	194.18	$C_7H_{14}O_6$	34.6	Anti- tumor activity
15	57-10-3	26.6	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	6.77	Cytotoxic activity, Anti-Inflammatory Property, Anti-oxidant, Anti-bacterial activity
16	150-86-7	30.3	Phytol	296.5	$C_{20}H_{40}O$	2.27	Antinociceptive effect Antioxidant effect Anti- inflammatory and antiallergic effects
17	506-21-8	30.7	Linoelaidic acid	280.4	$C_{18}H_{32}O_2$	6.75	Apoptosis activity
18	463-40-1	30.8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278.4	$C_{18}H_{30}O_2$	5.92	Cardiovascular-protective, anti-cancer, neuro-protective, anti-osteoporotic, anti-inflammatory, anti-oxidative properties, reduces blood clots. anti-obesity, anti-metabolic syndrome.

GC-MS Analysis of Root Methanolic Extract

GC-MS revealed the presence of 10 bioactive components in *A. nilagirica* root methanolic extract(Table 3, Fig.3). Within the set of ten compounds, it was seen that the molecule with the lowest retention time of 6.10 minutes was identified as 1-Undecene,4-methyl-. This compound exhibited a peak area of 3.1%. Conversely, the compound with the longest retention time of 30.69 minutes was identified as Linoelaidic acid, which displayed a peak area of 5.35%. The bioactive molecule, (E)-β-Famesene (RT-14.6) was one of the main substances (with a peak area of 22.2%) found in the root extract, and it is well-known for its aphid repellant activity in *Ageratum conyzoides* [48]. n-Hexadecanoic acid (RT-26.6) is a bioactive substance that exhibits a variety of biological characteristics, including cytotoxic activity, anti-inflammatory property, antioxidant activity, and anti-bacterial activity. Linoelaidic acid (RT-30.6), a chemical found in both stem and fruit extracts, has an apoptosis action. Among the bioactive chemicals reported in the root methanolic extract of *A. nilagirica*, the biological action of few chemicals had not been determined at this point (Table3).

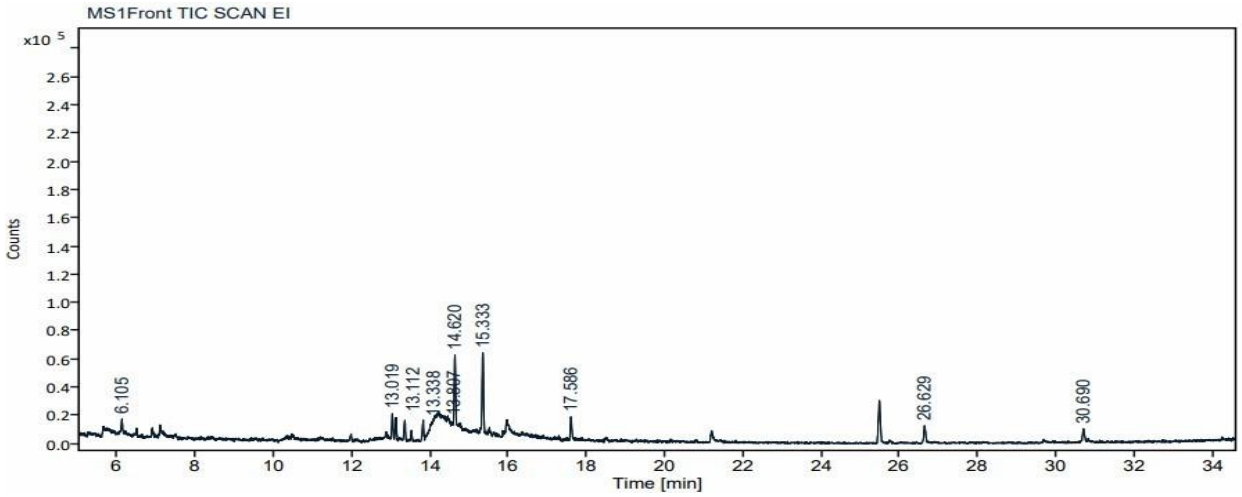

Fig.3 GC-MS chromatogram of *Artemisia nilagirica* root methanolic extract.

Table 3 Phytochemical components identified in *Artemisia nilagirica* Root methanolic extracts using GCMS analysis and their reported biological activities.

S.No	CAS	RT	Name of compound	Molecular Wt. (g/mol)	Molecular Formula	Area %	Recorded Biological activity
1	74630-39-0	6.10	1-Undecene, 4-methyl-	168.3	$C_{12}H_{24}$	3.1	No activity

2	65372-78-3	13.01	(1R,3aS,5aS,8aR)-1,3a,4,5a-Tetramethyl-1,2,3,3a,5a,6,7,8-octahydrocyclopenta[c]pentalene	204.3	$C_{15}H_{24}$	6.9	No activity
3	110823-68-2	13.11	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	204.3	$C_{15}H_{24}$	6.9	No activity
4	473-14-3	13.3	Naphthalene, 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-	204.3	$C_{15}H_{24}$	6.5	No activity
5	118-65-0	13.8	Bicyclo [7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	204.3	$C_{15}H_{24}$	6.2	No activity
6	18794-84-8	14.6	(E)-β-Famesene	204.3	$C_{15}H_{24}$	22.2	aphid repellant
7	41432-70-6	15.3	β-Longipinene	204.3	$C_{15}H_{24}$	25.2	No activity
8	20777-39-3	17.5	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate, (R)-	196.2	$C_{12}H_{20}O_2$	10.4	No activity
9	57-10-3	26.6	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	6.8	Cytotoxic activity, Anti-Inflammatory Property, Anti-oxidant, Anti-bacterial activity
10	506-21-8	30.6	Linoelaidic acid	280.4	$C_{18}H_{32}O2$	5.35	Apoptosis activity

Conclusion

The study species *A. nilagirica* possesses a variety of therapeutic qualities, which might be attributed to the presence of all the chemicals reported. The therapeutic activities of the other chemicals found in the leaf, stem, and root of *A. nilagirica* have not yet been reported. The findings of the current study indicate that *A. nilagirica* is a dependable source of bioactive compounds, including fatty acid esters, alcohols, hydrocarbons, alkanes, amines, terpenes, and sugars, used for treating infectious diseases and preventing toxicity. Since GC-MS is the initial step in determining the nature of active principles, more research in this species is advised to produce novel medications.

5. ACKNOWLEDGEMENT

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