

VALIDATION OF BIOLOGICAL ACTIVITIES AND CHARACTERIZATION OF EXTRACTS OF Anaphalis margaritacea (L.) BENTH. AND HOOK.F. FROM HILLY TERRAINS OF UTTARAKHAND HIMALAYA

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ABSTRACT

The paper focuses on the biological activities of polar and non-polar extracts made from the aerial part of the plant *Anaphalis margaritacea* that was further chemically characterized. Both extracts' constituent components were identified by their GC-MS profiles. *In vitro* antioxidant and anti-inflammatory activity of methanolic and hexane extracts was further investigated. The methanolic extract showed the identification of twenty-eight compounds that constituted 83.29% of the total methanolic extract of which 5R, 8R, 9S, 10R)-2-Formyl-3-Hydroxy-5-Isopropeny-8-8Methyl (3a10)-octahydronaptho (13.15%) was identified as the major compound. Identified compounds constituted 51.88% of the total composition of the hexane extract with hexatriacontane as one of the most prominent compounds occupying 20.43%. When compared to hexane extract, the methanolic extract is evealed lower IC₅₀ values for DPPH radical scavenging activity (75.94±0.66 µg/mL), metal chelating activity (72.90±0.096 µg/mL), and reducing power activity (79.64±5.360 µg/mL) demonstrating that it possesses strong antioxidant potential. Additionally, methanolic extract demonstrated better anti-inflammatory properties, with an IB₅₀ value of 37.581±050 g/mL. Based on these results, methanolic extract appears to be a more potent anti-inflammatory and antioxidant agent than hexane extract.

INTRODUCTION

As is well known, the medicinal plants contain significant amounts of bioactive phytochemicals or bio nutrients. The Himalayas are renowned for their richness of medicinal plants and India is one of the top exporters of raw herbal medicine worldwide (Badola and Aitken, 2003). According to ethnomedical tradition, medicinal plants are the richest in terms of bioresources. They also have therapeutic capabilities due to the presence of a wide range of complex chemical compounds with varied compositions known as plant secondary metabolites (Kandari et al., 2012). These chemical constituents viz., polyphenols, flavonoids, glycosides, alkaloids, and tannins have been prized in the pharmaceutical industry but now it is becoming more widely acknowledged that these herbs and their active components have pervasive applications in various other industries including nutrition, beverages, repellents, perfumes, cosmetics to name some. A majority of plant species, including several that have been cultivated for both culinary and medicinal purposes, are members of the Asteraceae family, which has a long history in traditional medicine. The eastern Himalayas are home to Anaphalis, the biggest genus of herbaceous plants in Asia

and a member of the Asteraceae family that possess the highest diversity of species (Nie et al., 2013). Several species of Anaphalis, which have been used traditionally, have exhibited anti-phlogistic, anti-asthmatic, and expectorant properties (Sharma et al., 2019). Anaphalis margaritacea, commonly known as the pearly everlasting belonging to the genus Anaphalis, is an herbaceous plant endemic to North America. This herb has been traditionally utilized as an expectorant, an astringent, and as a Tibetan medicine to cure cough and respiratory ailments as well as colds and rheumatism (Ren et al., 2009). This plant has been shown to exhibit other therapeutic properties, including antibacterial, antiinflammatory, antioxidant, and antifungal effects (Khemani et al., 2012). The proportional composition of the extracts derived from the plant species in two different solvents varies considerably according to altitudinal variation and different climatic and soil conditions along with the geographical locations they reside within (Sontakke et al., 2019).

Thus, the aim of the current research is to study the plant *A. margaritacea* for its known medicinal properties by preparing the extracts of the plant and then chemically characterizing the constituents of the extracts. Subsequently, the extracts are subjected to various biological activities to validate the

reported medicinal properties found within this less explored plant

MATERIALS AND METHODS

Plant Material Collection

The plant, *Anaphalis margaritacea*, was collected in September 2020 from the Kumaun district of Munsyari, Uttarakhand at an elevation of 2,200 m and was identified by submitting herbarium at G.B. Pant University of Agriculture and Technology, Pantnagar.

Preparation of extract

The extracts were prepared with the use of a soxhlet apparatus. The aerial parts of the plant were shade-dried, then finely ground, and the resulting fine powder was used to prepare extracts. Two different extracts (methanolic and hexane) were made using two separate solvents. The biological activities of these two extracts were evaluated. The percentage yield of both extracts was 1.78% (hexane extract), and 4.56% (methanolic extract) respectively.

GC-MS Analysis

The phytochemical composition of extracts (methanolic and hexane) was ascertained using analytical methods. The DB-5 column was used for the GC-MS analysis. GCMS-QP2010 Plus equipment was used with helium as carrier gas. The carrier gas server, high-pressure injection, and splitter hold were off during GC-MS. The total flow rate was16.3mL/min, the column flow rate was 1.21mL/min, with the ratio of the split of 10 and 81.9 kPa pressure with an oven temperature of 80°. The programmed temperature was 60°, RAMP @ 3°per minute at 210° (isotherm for 2 minutes) then held for 11 minutes. By comparing Kovatt indices and retention time of peaks with known reported data and the library of pure substances' spectra and comparing data with FFNSC Wiley library and NIST-MS, the compounds present in the extracts were identified.

Biological activities

Antioxidant Activities

Free Radical Scavenging Activity of 2, 2'- Diphenyl picryl hydrazyl (DPPH)

The antioxidant activity of different extracts was based on the previously described method as stated by Kabdal *et al.*, 2022. The standard used was ascorbic acid. By graphing the percentage of radical scavenging, the IC_{50} value was calculated.

Reducing power activity

The reducing power activity was determined using a previously described method (Gairola *et al.*, 2021). Gallic acid was used as standard. The reducing power activity of the extract and standard was calculated as stated by Gairola *et al.*, 2021. In order to determine the RP_{50} value, a standard graph was plotted between % reducing powers versus concentration.

Metal chelating activity

The metal chelating activity was evaluated using ferrozine with the help of the method as previously described by Gururani et *al.*, 2022. The absorbance was recorded at 562 nm and EDTA was used as the standard. The % inhibition of metal chelation in the extract and standard was calculated using the

formula afore-described (Gururani et al., 2022).

In vitro Anti-inflammatory activity

In vitro anti-inflammatory activity was determined using a method previously described by Heendeniya *et al.*, 2018. Absorbance was recorded at 660 nm. Standard was prepared using various concentrations of diclofenac ranging from 10-100 μ g/mL.

Statistical Analysis

Statistical analysis was carried out using the SPSS16.00 program to determine the mean and standard deviation of plant extract samples collected in triplicates. Their significance was analyzed by a 5% point Ducane test (one-way analysis) using ANOVA (Soni et al., 2019). To ascertain the significance and associations of different extracts, SPSS software was employed.

RESULTS AND DISCUSSION

GC-MS analysis of methanolic extract of the whole plant of *A. margaritacea*.

GC-MS analysis was used for the identification of twenty-eight compounds in methanolic extract. The compounds that were present in higher percentages are 5R,8R,9S,10R-2-Formyl-3-Hvdroxy-5-Isopropeny-8-8Methyl(3a10)-octahydronaptho (13.15%), oleic acid (11.31%), celidoniol deoxy (5.47%), 11-Octadecenoic acid methyl ester (5.46%), Glycidyl oleate (4.83%), γ-Sitosterol (3.96%), 9,12-Octadecadienoic acid methyl ester (2.82%), 13-Docosenoicacid methyl ester (3.52%),17-Oxo-6.alpha.-pentyl-4-nor-3,5-secoandrostan-3oicacidmethylester (2.84%), Hexa decanoic acid methyl ester (2.81%), n-Hexadecanoic acid (2.57%), Stigmasta-5,23-Dien-3.Beta.-ol(2.56%), Heneicosane(2.52%), 15- Hydroxypenta decanoic acid (2.34%), 9-Hexadecenoic Acid, 9-Octadecenyl Ester (1.85%), Tetracyclo [7.3.0.1E2,8.0E3,7] Tridec-10-en, 5di (1.92%), 9(11)-Dehydroergosteryl benzoate (1.90%), Methyl stearate (1.72%), Tetratetracontane (1.59%), Neo phytadiene (1.50%),phytol(1.50%),2-Pentadecanone, 6,10,14-trimethyl (1.45%), Phytyl palmitate (1.43%), Hexa triacontane (1.39%), alpha.-Tocospiro B (1.16%) while other compounds with less than 1% contribution were present in minor amounts. Table 1 provides information about the chemical compounds present in the methanolic extract of the whole plant of A. margaritacea. The compounds occupying area less than 1% are: -Amyrin (0.96%), 9,12-Octadecadienoic acid Methyl Ester (0.85%), Squalene (0.75%), Thunbergol (0.73%) and, 1-Hexadecanol (0.58%).

GC-MS analysis of hexane extract of the whole plant of A. *maragraitacea*

GC-MS was used for the identification of thirty-five compounds of total hexane extract. The compounds that were present predominantly are Hexatriacontane (20.43%), Tetracontane (14.70%), Heneicosane (2.66%), Tetratetracontane (2.05%), Eicosane (1.82%), Celidoniol Deoxy (1.83%), Pentatriacontane (1.68%), Docosane (1.32%). Table 2 provides the detailed information on the chemical composition. The compounds occupying area less than 1% are: -Sitosterol (0.96%), 5,11,17,23-Tetratert-Butylpentacyclo (0.56%), Phytyl tetradecanoate (0.37%), 2-Pentadecanone, 6,10,14-trimethyl (0.35%), 2- Pentadecanone, 6,10,14-trimethyl (0.35%), Glycidyl oleate (0.34%), 9-Octadecen-1-ol,(Z)-(0.31%), 4,8,12,16-Tetramethylheptadecan-4-olide (0.20%), Stigmasta-3,5-dien-7-one (0.21%), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.19%), Hexadecanoic acid methyl ester (0.15%), Palmitaldehyde Diallyl Acetal (0.14%), 3-Methyloctacosane (0.13%), cis-13-Octadecenoicacid methylester (0.12%), 2-Pentadecanone (0.11%), Phytol (0.09%), Hexadecanoic acid butyl ester (0.05%).

In vitro antioxidant activity

Overproduction of ROS (reactive oxygen species) is associated with aging and several chronic disorders, and it may adversely affect different cellular components, resulting in tissue damage. The imbalance between ROS generation and antioxidant defense activity is known as oxidative stress. Numerous pieces of evidence demonstrate that the antioxidant present in products derived from plants has a wide range of biomedical uses. The present study evaluated the effect of plant *Anaphlis margaritacea* on oxidative stress by three alternate assays: Fe^{+2} chelating activity, radical scavenging activity of 2, 2-diphenyl1-picrylhydrazyl (DPPH), and reducing power activity (Fe⁺³ to Fe⁺²). The antioxidant activity of the extracts was compared with standards like ascorbic acid and EDTA.

DPPH radical scavenging activity

The quenching of stable free radicals is the basis for the DPPH test. The stable, commercially available free radical DPPH

employed in this experiment has a maximum absorbance at 517 nm, and is soluble in methanol. When this stable radical absorbs an electron or hydrogen atom from a donor species, it is transformed into a stable diamagnetic molecule called diphenyl picryl hydrazine, a yellow non-radical molecule. The extent of the reaction depends on the compounds' capacity to donate hydrogen (Sandeepa et al., 2017). The IC₂₀ value is the concentration required to decrease the absorbance of DPPH by 50%. The DPPH free radical scavenging activity (IC_{10}) value of A. margaritacea in AMME was found to be $(75.94 \pm 0.66 \ \mu g/mL)$ and in AMHE was $(141.42 \pm 0.73 \ \mu g/mL)$ mL) with standard ascorbic acid showing (57.66 \pm 0.40 μ g/ mL). Methanol extract had an IC₅₀ comparable to that of standard solution while hexane extract had higher IC_{50} value. The better DPPH scavenging activity of the methanolic extract can be due to phytol, and celidoniol deoxy present in the methanolic extract which has been previously reported to show antioxidant activity (Singh et al., 2015).

The metal chelating activity of Fe⁺²

The formation of the Fe⁺² ferrozine complex was measured to determine the Fe⁺² chelating activity. When Fe (II) ions bind to ferrozine, a colored complex that shows maximum absorbance at 562 nm is produced (Akhlagi *et al.*, 2021). It is possible to calculate the chelating activity of co-existing chelators by measuring color reduction. The extract with the highest activity

Table 1: Chemical	composition of	methanolic	extract of	f the whole	plant of A.	margaritacea (AMME)
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S.No.	Compound name	Chemical forr	nula %area	Ki value	Class of compound
1	1-Hexadecanol	C ₁₆ H ₃₂	0.58	1854	Fatty alcohol
2	Neophytadiene	$C_{20}^{10}H_{38}^{12}$	1.5	1503	Sesquiterpenoids
3	2-Pentadecanone, 6,10,14-trimethyl-	C ₁₈ H ₃₆ O	1.45	1836	Ketone
4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_{7}$	2.81	1916	Fatty acid methyl esters
5	n-Hexadecanoic acid	C16H30O2	2.57	1943	Saturated Fatty acid
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C18H30	2.82	1960	Unsaturated fatty acid
7	11-Octadecenoic acid, methyl ester	C10H32O2	5.46	2069	Fatty acid
8	(2E,7R,11R)-3,7,11,15-Tetramethylhexadec -2-en-1-ol	$C_{20}^{19}H_{40}^{30}O^{2}$	1.5	2085	Diterpenoid
9	Methyl stearate	$C_{21}H_{42}O_{2}$	1.72	2093	Fatty acid methyl ester
10	Oleic acid	C, H, 40, 1	11.31	2100	Long-chain fatty acid
11	9,12-octadecadienoic acid (z,z)-, methyl ester	C, H, O,	0.85	2111	Unsaturated fatty acid
12	15-Hydroxypentadecanoic acid	C15H30	2.34	2175	Long-chain fatty acid
13	Glycidyl oleate	$C_{18}H_{34}O$	4.83	2196	Carboxylic ester and an epoxide
14	13-Docosenoic acid, methyl ester, (Z)-	C.,H.,O.	3.52	2333	Fatty acid methyl ester
15	5R,8R,9S,10R)-2-Formyl-3-Hydroxy-5-Isopropeny -8-8Methyl(3a 10)-octahydronaptho	$C_{15}^{23}H_{22}^{44}O_{2}^{2}$	13.15	2475	Sesquiterpenoids
16	Heneicosane	C, H,	2.52	2483	Alkane
17	9(11)-Dehydroergosteryl benzoate	C, H, C,	1.9	2667	Monocarboxylic acid
18	Tetracyclo[7.3.0.1e2,8.0e3,7]tridec-10-en, 5-(di	$C_{24}H_{24}$	1.92	2873	Sesquiterpenoids
19	Squalene	C_0H_0	0.75	2914	Triterpene
20	.alphaTocospiro B	$\vec{C}_{29}\vec{H}_{50}O_4$	1.16	3374	Sesterterpenoids
21	17-Oxo-6.alphapentyl-4-nor-3,5-secoandrostan -3-oic acid methyl ester	$C_{24}H_{40}O_{3}$	2.84	3531	Ester
22	Celidoniol, deoxy	$C_{44}H_{90}$	5.47	3600	Alkane
23	9-hexadecenoic acid, 9-octadecenyl ester	$C_{18}H_{36}$	1.85	4395	Fatty acid
24	Hexatriacontane	$C_{36}^{10}H_{74}^{30}$	1.39	3600	Alkane
25	³ -Sitosterol	C ₂₀ H ₅₀ O	3.96	2731	Plant steroid
26	±- Amyrin	C_H_O	0.96	2873	Pentacyclic triterpenoid
27	4-lsopropyl-1,7,11-trimethyl-2,7,11 -cvclotetradecatrien-1-ol	$C_{19}^{30}H_{32}^{30}O_{3}$	0.73	1754	Monocyclic diterpene alcohol
28	Phytyl palmitate Total	$C_{38}H_{74}O_{2}$	1.43 83.29	2045	Saturated long-chain fatty acid

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Table 2 : Chemical	composition of hexane	extract of whole	plant of A. mar	garitacea (AMHE)

	S.No.	Compound name	Chemical formula	%Area	KI value	Class of compound
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1	Neophytadiene	C _{an} H _{an}	0.1	2168	Diterpene
37,9-D1-tert-butyl-1-oxospiro(4,5)deca $C_{\mu}^{+}H_{\mu}^{-}O_{\mu}^{-}$ 0.192081Ketone-6,9-dine-2,8-dioneC_{\mu}H_{\mu}O_{\mu}^{-}0.151878Fatty acid methyl esters5Hexanoic acid, 3,5,5-trimethyl-2, 7-dimethyl/oct-1en-3-prolesterC_{\mu}H_{\mu}O_{\mu}^{-}0.122085Unsaturated acyclic monocarboxylic acids6cis-13-Octadecenoic acid, methyl esterC_{\mu}H_{\mu}O_{\mu}^{-}0.122085Diterpenoid8Palmitaldehyde, diallyl acetalC_{\mu}H_{\mu}O_{\mu}^{-}0.14Acetals99-Octadecen-1-olC_{a}H_{\mu}O_{\mu}^{-}0.312061Unsaturated fatty alcohol10Hexadec-2-en-1-olC_{a}H_{\mu}O_{\mu}^{-}0.322177Fatty acid esters10Hexadecanoic acid, butyl esterC_{a}H_{\mu}O_{\mu}^{-}0.322177Fatty acid esters10DocosaneC_{\mu}H_{\mu}O_{\mu}^{-}0.322177Fatty acid esters124.8,12,16-TetramethylheptadecC_{a}H_{\mu}O_{\mu}^{-}0.341808Carboxylic ester and an epoxide13EicosaneC_{\mu}H_{\mu}O_{\mu}^{-}0.442704Dicarboxylic acid14Glycidyl oleateC_{a}H_{\mu}O_{\mu}^{-}0.34301715Lybenzene/dicarboxylic acidC_{\mu}H_{\mu}O_{\mu}^{-}0.34301716Lybenzene/dicarboxylic acidC_{\mu}H_{\mu}O_{\mu}^{-}0.34301717Fuzzanoj,4-Dipyzahexanethyl0.153600Alkane18Hexacosane, 1-iodo-C_{\mu}H_{\mu}O_{\mu}^{-}0.343000 </td <td>2</td> <td>2-Pentadecanone, 6.10.14-trimethyl-</td> <td>C.H.O</td> <td>0.35</td> <td>1754</td> <td>Sesquiterpene</td>	2	2-Pentadecanone, 6.10.14-trimethyl-	C.H.O	0.35	1754	Sesquiterpene
6.9-diene <td>3</td> <td>7.9-Di-tert-butyl-1-oxaspiro(4.5)deca</td> <td>C H O</td> <td>0.19</td> <td>2081</td> <td>Ketone</td>	3	7.9-Di-tert-butyl-1-oxaspiro(4.5)deca	C H O	0.19	2081	Ketone
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	-	-6.9-diene-2.8-dione	17 24 3			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4	Methyl palmitate	СНО	0.15	1878	Fatty acid methyl esters
31011011011011011011011011011011011011011017cinethyloct1-ten-3-ynyl ester $C_{g}H_{g}O_{g}$ 0.122085Unsaturated acyclic monocarboxylic acids7hexadec-2-en-1-ol $C_{g}H_{g}O_{g}$ 0.092045Diterpenoid8Palmitaldehyde, diallyl acetal $C_{g}H_{g}O_{g}$ 0.312061Unsaturated fatty alcohol10Hexadecanoic acid, butyl ester $C_{g}H_{g}O_{g}$ 0.052177Fatty acid esters11Docosane $C_{g}H_{g}O_{g}$ 0.22258Diterpene lactones124,8,12,16-Tetramethylheptadec $C_{g}H_{g}O_{g}$ 0.22258Diterpene lactones13Eicosane $C_{g}H_{g}O_{g}$ 0.341808Carboxylic ester and an epoxide14Glycidyl oleate $C_{g}H_{g}O_{g}$ 0.341808Carboxylic ester and an epoxide15Heneicosane $C_{g}H_{g}O_{g}$ 0.442704Dicarboxylic ester161, Jbenzenedicarboxylic acid $C_{g}H_{g}O_{g}$ 0.34301717Celidoniol, deoxy $C_{g}H_{g}O_{g}$ 0.34301718Furzane[3,4-b]pyrazine, 5-2,3 $C_{g}H_{g}O_{g}O_{g}$ 0.34301719Furzane[3,4-b]pyrazine, 5-4,3 $C_{g}H_{g}O_{g}O_{g}$ 0.433017202,6,10,15,19,23-Hexamethyl $C_{g}H_{g}O_{g}O_{g}$ 0.433125Hydrocarbon21Eicosanal- $C_{g}H_{g}O_{g}O_{g}$ 0.191999	5	Hexanoic acid 3 5 5-trimethyl- 2	C H O	0.13	1575	Ester
$ \begin{array}{c} \mbode{linear} \begin{tabular}{lllllllllllllllllllllllllllllllllll$	5	7-dimethyloct-1-en-3-yn-yl ester	C_{19} , C_{32} , C_{2}	0.15	1575	Ester
35555122003monocarboxylic acids monocarboxylic acids7hexadec-2-en-1-ol $C_gH_uO_c$ 0.092045Diterpenoid8Palmitaldehyde, diallyl acetal $C_{u}H_uO_c$ 0.14Acetals99-Octadecent-1ol, (Z)- $C_{u}H_uO_c$ 0.312061Unsaturated fatty alcohol10Hexadecanoic acid, butyl ester $C_{u}H_uO_c$ 0.052177Fatty acid esters11Docosare $C_{u}H_uO_c$ 0.22258Diterpene lactones13Eicosane $C_{u}H_uO_c$ 0.341808Carboxylic ester and an epoxide14Glycidyl oleate $C_{u}H_uO_c$ 0.341808Carboxylic ester and an epoxide15Heneicosane $C_{u}H_uO_c$ 0.442704Dicarboxylic acid161, 2benzenedicarboxylic acid $C_{u}H_uO_c$ 0.3430171611-Tetradecen-1-ol, acetate, (Z)- $C_uH_uO_c$ 0.34301717Fatty aldehydesAlkane110e1811-Tetradecen-1-ol, acetate, (Z)- $C_uH_uO_c$ 0.34301719Furazanoj3.4-bjpyrazine, 5-(2,3) $C_uH_uO_c$ 0.361787Carboxylic ester19Licosanal $C_uH_uO_c$ 0.191999Fatty aldehydes22,610,15,19,23-Hexamethyl $C_uH_uO_c$ 0.133125Hydrocarbon22,610,14,48,22Cha_uO0.193600Alkane233-Methyloctacosane $C_uH_uO_c$ 0.243600	6	cis-13-Octadecenoic acid methyl ester	СНО	0.12	2085	Unsaturated acyclic
7hexadec-2-en-1-ol $C_{sl}H_{sl}O$ 0.092045Ditergenonid8Palmitaldehyde, diallyl acetal $C_{sl}H_{sl}O$ 0.14Acetals99-Octadecen-1-ol, (Z)- $C_{tl}H_{sl}O$ 0.312061Unsaturated fatty alcohol10Hexadecanoic acid, butyl ester $C_{sl}H_{sl}O$ 0.052177Fatty acid esters11Docosane $C_{21}H_{sl}O$ 0.222258Ditergene lactones124,8,12,16-Tertamethylheptadec $C_{sl}H_{sl}O_2$ 0.242258Ditergene lactones13Eicosane $C_{sl}H_{sl}O_2$ 0.341808Carboxylic ester and an epoxide14Glycidyl oleate $C_{sl}H_{sl}O_2$ 0.661787Carboxylic ester15Heneicosane $C_{sl}H_{sl}O_2$ 0.661787Carboxylic acid161,2benzenedicarboxylic acid $C_{sl}H_{sl}O_2$ 0.34301717Celidoniol, deoxy $C_{sl}H_{sl}O_2$ 0.661787Carboxylic ester1811-Tetradecen-1-ol, acetate, (Z)- $C_{sl}H_{sl}O_2$ 0.661787Carboxylic ester19Furzanol3, 4-blyrazine, 5-(2,3) $C_{sl}H_{sl}O_2$ 0.161835Unsaturated hydrocarbon2,6,10,15,19,23-Hexamethyl $C_{sl}H_{sl}O$ 0.191999Fatty aldehydes22Hexacosane, 1-iodo- $C_{sl}H_{sl}O$ 0.133125Hydrocarbon233-Methyloctacosane $C_{sl}H_{sl}O$ 0.92046Ketone24Hexatriacontane $C_{sl}H_{sl}O$		els 15 Octudecenole deld, methyrester	C ₁₉ 36 2	0.12	2005	monocarboxylic acids
Product L L + 101 $C_{g0} H_{g0} O$ $LordActetals8Palmitaldehyde, diallyl acetalC_{g1} H_{g0} O0.14Acetals99-Octadecen-1-0, (2)-C_{g1} H_{g0} O0.312061Unsaturated fatty alcohol10Hexadecanoic acid, butyl esterC_{g1} H_{g0} O0.052177Fatty acid esters11DocosaneC_{21} H_{g0} O0.222258Diterpene lactones124,8,12,16-TetramethylheptadecC_{21} H_{g0} O_20.222258Diterpene lactones13EicosaneC_{g1} H_{g0} O_20.341808Carboxylic ester and an epoxide14Glycidyl oleateC_{21} H_{g0} O_20.142704Dicarboxylic acid15HeneicosaneC_{21} H_{g0} O_20.341808Carboxylic ester and an epoxide161,2benzenedicarboxylic acidC_{g4} H_{g0} O_20.0661787Carboxylic ester19Furzazno[3,4-b]pyrazine, 5-(2,3)C_{10} H_{20} O_20.161835Unsaturated hydrocarbon2,6,10,15,19,23-HexamethylC_{20} H_{20} O_20.161835Unsaturated hydrocarbon22,610,15,19,23-HexamethylC_{20} H_{20} O_20.233600Alkane233-MethyloctacosaneC_{20} H_{20} O_20.213600Alkane24HexatocaneneC_{20} H_{20} O_20.233600Alkane252-NonadecanoneC_{20} H_{20} O_20.2433600$	7	hexadec-2-en-1-ol	СНО	0.09	2045	Diterpenoid
8Palmitaldehyde, diallyl acetal 9 $C_{22}H_{40}O_{2}$ 0.14 Acetals99-Octadecen-1-ol, (2)- (Oleyl alcohol) $C_{3}H_{30}O_{2}$ 0.31 2061 Unsaturated fatty alcohol10Hexadecanoic acid, butyl ester $C_{31}H_{30}O_{2}$ 0.05 2177 Fatty acid esters11Docosane $C_{22}H_{40}O_{2}$ 0.22 2258 Diterpene lactones $4,8,12,16$ -Tertamethylheptadec $C_{21}H_{40}O_{2}$ 0.2 2258 Diterpene lactones13Eicosane $C_{20}H_{40}O_{2}$ 0.34 1808 Carboxylic ester and an epoxide14Glycidyl oleate $C_{21}H_{40}O_{3}$ 0.34 1808 Carboxylic acid15Heneicosane $C_{22}H_{40}O_{2}$ 0.06 1787 Carboxylic acid16 $1,2benzenedicarboxylic acidC_{41}H_{40}O_{4}0.142704Dicarboxylic acid17Celidoniol, deoxyC_{20}H_{40}O_{2}0.061787Carboxylic ester1811-Tetradecen-1-ol, acetate, (Z)-C_{40}H_{40}O_{4}0.161835Unsaturated hydrocarbon202,6,10,14,18,22 tetracosahexaen,C_{30}H_{40}O_{2}0.161835Unsaturated hydrocarbon21Eicosanal-C_{20}H_{40}O_{40}0.191999Fatty aldehydes22Hexacosane, 1-iodo-C_{30}H_{40}O_{40}0.133125Hydrocarbon233-MethyloctacosaneC_{40}H_{40}O_{40}0.223801Fatty aldehyde24$	ĺ		C ₂₀ H ₄₀ O	0.05	2045	Bitelpenold
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	8	Palmitaldehyde, diallyl acetal	СНО	0.14		Acetals
Oleyl alcohol Cash and all all all all all all all all all al	9	9-Octadecen-1-ol. (Z)-	C H O	0.31	2061	Unsaturated fatty alcohol
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	-	(OlevLalcohol)	-18 - 36 -			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	10	Hexadecanoic acid, butyl ester	СНО	0.05	2177	Fatty acid esters
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	11	Docosane	C_{20}^{11} H_{40}^{2}	1 32	2109	Alkanes
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	12	4 8 12 16-Tetramethylbentadec	C H O	0.2	2258	Diterpene lactones
13 Eicosane $C_{20}H_{42}$ 1.82 2009 Alkane 14 Glycidyl oleate $C_{21}H_{40}O_3$ 0.34 1808 Carboxylic ester and an epoxide 15 Heneicosane $C_{22}H_{40}O_3$ 0.14 2704 Dicarboxylic acid 1.81 15 Heneicosane $C_{22}H_{40}O_4$ 0.14 2704 Dicarboxylic acid 1.81 16 1.2benzenedicarboxylic acid $C_{21}H_{40}O_4$ 0.14 2704 Dicarboxylic acid 1.81 17 Celidoniol, deoxy $C_{20}H_{40}O_4$ 0.14 2704 Dicarboxylic acid 1.81 18 11-Tetradecen-1-ol, acetate, (Z)- $C_{16}H_{40}O_2$ 0.06 1787 Carboxylic ester 19 Furzanol3.4-Bjpyrazine, 5-(2,3) $C_{18}H_{22}N_{9}O$ 0.34 3017 - 20 2,6,10,15,19,23-Hexamethyl $C_{20}H_{30}O$ 0.16 1835 Unsaturated hydrocarbon 21 Eicosanal- $C_{20}H_{40}O$ 0.19 1999 Fatty aldehydes 22 Hexacosane, 1-iodo- $C_{20}H_{40}O$ 0.019 2046 Ketone 23<	12	an-4-olide	$C_{21} \Gamma_{40} C_2$	0.2	2250	Ditelpene lactories
13 Encode $C_{33}^{0} H_{20}^{0}$ 1.02 1.03 Mathematical constraints 13 Glycidyl oleate $C_{11}^{0} H_{20}^{0}$ 0.34 1808 Carboxylic ester and an epoxide 15 Heneicosane $C_{21}^{0} H_{20}^{0}$ 0.66 2109 Alkane 16 1,2benzenedicarboxylic acid $C_{24}^{0} H_{20}^{0}$ 0.14 2704 Dicarboxylic acid 16 1,2benzenedicarboxylic acid $C_{24}^{0} H_{20}^{0}$ 0.16 1.83 4395 Alkane 18 11-Tetradecen-1-ol, acetate, (Z)- $C_{16}^{0} H_{20}^{0} O_{2}$ 0.06 1787 Carboxylic ester 19 Furazano[3,4-b]pyrazine, 5-(2,3 $C_{18}^{0} H_{20}^{0} O_{2}$ 0.16 1835 Unsaturated hydrocarbon 2,6,10,14,18,22 tetracosahexaen, $C_{20}^{0} H_{20}^{0} O_{20}^{0}$ 0.16 1835 Unsaturated hydrocarbon 21 Eicosanal- $C_{20}^{0} H_{20}^{0} O_{20}^{0} 0_{11}^{0}$ 3600 Alkane 23 -Methyloctacosane $C_{19}^{0} H_{20}^{0} O_{20}^{0} 0_{22}^{0} 3600 Alkane 25 -Nonadecanone C_{19}^{0} H_{20}^{0} O_{2}^{0} 0_{2}^{0} 3600 Alkane 26<$	13	Ficosane	СН	1.82	2009	Alkane
In order of the system Carl Parts	14	Glycidyl oleate	C_{20}^{-1}	0.34	1808	Carboxylic ester and an enoxide
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	15	Heneicosane	$C_{21} H_{38} C_{3}$	2.66	2109	Alkane
10 1,221:130 G ₄ 0,14 2704 Difference 10 1,221:130 G ₄ 0,14 2704 Difference 11 Celidoniol, deoxy $C_{g4}H_{g0}$ 1.83 4395 Alkane 18 11-Tetradecen-1-ol, acetate, (Z)- $C_{10}H_{30}O_2$ 0.06 1787 Carboxylic ester 19 Furazano[3,4-b]pyrazine, 5-(2,3 $C_{10}H_{22}N_6O$ 0.34 3017 -dimethylphenylamino)-6-(perhy - Aniline - 20 2,6,10,14,18,22 tetracosahexaen, $C_{30}H_{30}$ 0.16 1835 Unsaturated hydrocarbon 2,6 i0,15,19,23-Hexamethyl - - - Aniline 21 Eicosanal- C $_{20}H_{40}O$ 0.19 1999 Fatty aldehydes 22 Hexacosane, 1-iodo- C $_{20}H_{40}O$ 0.05 3600 Alkane 23 3-Methyloctacosane C $_{20}H_{30}O$ 0.09 2046 Ketone 24 Hexatriacontane C $_{10}H_{30}O$ 0.11 3340 Ketone 25 2-Nonadecanone C $_{10}H_{30}O$ 0.2 3801 Fatty aldehyd	16	1 2benzenedicarboxylic acid	C_{22}^{11}	0.14	2704	Dicarboxylic acid
$\begin{array}{ccc} C_{29}^{+60} & 1.55 &$	17	Celidoniol deoxy	$C_{24} \Gamma_{38} O_4$	1.83	4395	Alkane
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	18	11-Tetradecen-1-ol acetate (7)-	C_{29}^{11}	0.06	1787	Carboxylic ester
13 Initiazanity, y-type y and the second secon	10	Furazano[3.4 blowrazino 5.(2.3)]	$C_{16}H_{30}O_{2}$	0.34	3017	Carboxyne ester
Aniline202,6,10,14,18,22 tetracosahexaen, 2,6,10,15,19,23-Hexamethyl $C_{30}H_{50}$ 0.161835Unsaturated hydrocarbon21Eicosanal- 2,6,10,15,19,23-Hexamethyl $C_{20}H_{30}O$ 0.191999Fatty aldehydes22Hexacosane, 1-iodo- $C_{20}H_{30}O$ 0.133125Hydrocarbon233-Methyloctacosane $C_{20}H_{40}O$ 0.092046Ketone24Hexatriacontane $C_{30}H_{30}O$ 0.092046Ketone252-Nonadecanone $C_{19}H_{30}O$ 0.23801Fatty aldehydes26n-Octacosan-1-al $C_{20}H_{50}O$ 0.23801Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.113340Ketone28Pentatriacontane $C_{30}H_{22}$ 1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{40}O$ 0.342739Phytosterols31Tetracontane $C_{40}H_{42}$ 14.73997Alkanes32Phytyl tetradecanoate $C_{34}H_{6}O_2$ 1.225525Phytyl ester342-Pentadecanone, 6,10,14-trimethyl- $C_{44}H_{6}O_2$ 0.351754Sequiterpene35Phytyl tetradecanoate $C_{34}H_{6}O_2$ 0.372168Fatty acid phytyl ester	15	-dimethylphenylamino)-6-(perhy	$C_{18} \Gamma_{22} \Gamma_{6} O$	0.34	5017	
and bit Praze printCan bit Praze printAthene20 $2, 6, 10, 14, 18, 22$ tetracosahexaen, $2, 6, 10, 15, 19, 23$ -HexamethylCao bit Praze printCao bit Praze print21Eicosanal- Eicosanal-Cao bit Praze printCao bit Praze printCao bit Praze print233-Methyloctacosane Hexacosane, 1-iodo-Cao bit Praze print0.133125Hydrocarbon24Hexatriacontane Cao bit Praze printCao bit Praze print0.053600Alkane252-Nonadecanone n-Octacosan-1-alCao bit Praze print0.092046Ketone26n-Octacosan-1-alCao bit Praze printCao bit Praze printFatty aldehyde272-pentadecanone Prata printCao bit Praze print1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- Cao bit Praze printCao bit Praze print1.683500Alkanes30a-SitosterolCao bit Praze print Cao bit Praze printCao bit Praze print Cao bit Praze printPhytosterols31Tetracontane Cao bit Praze print Cao bit Praze printCao bit Praze print Cao bit Praze print1.225525Phytyl ester335, 11, 17, 23-tetratert-butyl pentacycloCa bit Praze print Cao bit Praze print Cao bit Praze print1.225525Phytyl ester342-Pentadecanone, 6, 10, 14-trimethyl-Ca bit Praze print Cao bit Praze print Cao bit Praze print Cao bit Praze print1.225525Phytyl ester35Phytyl tetradecanoateCa bit Praze print<		dro 1 azopinyl)				Anilino
202,6,10,15,19,23-HexamethylC0.101033CCC21Eicosanal-CCCH0.053600Alkane233-MethyloctacosaneCC0.133125Hydrocarbon24HexatriacontaneCC0.133600Alkane252-NonadecanoneC0.190.092046Ketone26n-Octacosan-1-alC0.143340Ketone272-pentadecanoneC1919000.113340Ketone28PentatriacontaneC1919000.342739Phytosterols29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)-C2914.773997Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)-C14.773997Alkanes21TetracontaneC14.773997Alkanes29Phytyl tetradecanoateC14.763997Alkanes31TetracontaneC14.763997Alkanes32Phytyl tetradecanoateC14.763997Alkanes342-Pentadecanone, 6, 10, 14-trimethyl-C18.600.351754Sesquiterpene35Phytyl tetradecanoateC18.600.351754Sesquiterpene	20	$2.6 \pm 10.14 \pm 18.22 $ tetracosaheyaen	СН	0.16	1835	Unsaturated hydrocarbon
21Ercosanal- Ercosanal- $C_{20}H_{40}O$ 0.191999Fatty aldehydes21Ercosanal- $C_{26}H_{33}I$ 0.053600Alkane233-Methyloctacosane $C_{29}H_{60}$ 0.133125Hydrocarbon24Hexatriacontane $C_{36}H_{74}$ 20.433600Alkane252-Nonadecanone $C_{19}H_{38}O$ 0.092046Ketone26n-Octacosan-1-al $C_{28}H_{56}O$ 0.23801Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.113340Ketone28Pentatriacontane $C_{35}H_{72}$ 1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{40}O$ 0.342739Phytosterols30 2 -Sitosterol $C_{29}H_{60}O$ 1.473997Alkanes31Tetracontane $C_{4}H_{60}O_2$ 1.225525Phytyl ester335,11,17,23-tetratert-butylpentacyclo $C_{4}H_{50}O_4$ 0.565294Alkyl aryl ethers342-Pentadecanone, 6,10,14-trimethyl- $C_{18}H_{30}O$ 0.351754Sesquiterpene35Phytyl tetradecanoate $C_{34}H_{6}O_2$ 0.372168Fatty acid phytyl ester	20	2.6.10.15.19.23 Hovemathyl	C ₃₀ H ₅₀	0.10	1055	Chisaturated hydrocarbon
21Elcosatral $C_{20}h_{40}$ 0.13 1999 Fatty aldenydes22Hexacosane, 1-iodo- $C_{26}H_{53}I$ 0.05 3600 Alkane233-Methyloctacosane $C_{29}H_{60}$ 0.13 3125 Hydrocarbon24Hexatriacontane $C_{36}H_{74}$ 20.43 3600 Alkane252-Nonadecanone $C_{19}H_{30}O$ 0.09 2046 Ketone26n-Octacosan-1-al $C_{28}H_{50}O$ 0.2 3801 Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.11 3340 Ketone28Pentatriacontane $C_{35}H_{72}$ 1.68 3500 Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{40}O$ 0.96 2731 Phytosterols30 2 -Sitosterol $C_{29}H_{50}O$ 0.96 2731 Phytosterols31Tetracontane $C_{40}H_{82}$ 14.7 3997 Alkanes32Phytyl tetradecanoate $C_{34}H_{60}O_2$ 1.22 5525 Phytyl ester33 $5,11,17,23$ -tetratert-butylpentacyclo $C_{44}H_{60}O_4$ 0.56 5294 Alkyl aryl ethers342-Pentadecanone, $6,10,14$ -trimethyl- $C_{18}H_{30}O$ 0.37 2168 Fatty acid phytyl ester	21	Ficosanal	СНО	0.10	1000	Eatty aldobydos
22Hexacosane, Hodo $C_{29}H_{51}^{-1}$ 0.05^{-1} 3000^{-1} 7 Wane233-Methyloctacosane $C_{29}H_{60}$ 0.13 3125 Hydrocarbon24Hexatriacontane $C_{36}H_{74}$ 20.43 3600 Alkane252-Nonadecanone $C_{19}H_{38}O$ 0.09 2046 Ketone26n-Octacosan-1-al $C_{28}H_{56}O$ 0.2 3801 Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.11 3340 Ketone28Pentatriacontane $C_{35}H_{72}$ 1.68 3500 Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{48}O$ 0.34 2739 Phytosterols30 ² -Sitosterol $C_{29}H_{50}O$ 0.96 2731 Phytosterols31Tetracontane $C_{34}H_{60}O_2$ 1.22 5525 Phytyl ester33 $5,11,17,23$ -tetratert-butylpentacyclo $C_{44}H_{56}O_4$ 0.56 5294 Alkyl aryl ethers342-Pentadecanone, $6,10,14$ -trimethyl- $C_{18}H_{36}O$ 0.37 2168 Fatty acid phytyl ester	21	Hoxacosano 1 jodo		0.15	3600	Alkano
233-Methyloctacosane $C_{29}H_{60}$ 0.133125Hydrocarbon24Hexatriacontane $C_{36}H_{74}$ 20.433600Alkane252-Nonadecanone $C_{19}H_{38}O$ 0.092046Ketone26n-Octacosan-1-al $C_{28}H_{56}O$ 0.23801Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.113340Ketone28Pentatriacontane $C_{39}H_{72}$ 1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{48}O$ 0.342739Phytosterols30 ² -Sitosterol $C_{29}H_{50}O$ 0.962731Phytosterols31Tetracontane $C_{40}H_{82}$ 14.73997Alkanes32Phytyl tetradecanoate $C_{34}H_{60}O_2$ 1.225525Phytyl ester335,11,17,23-tetratert-butylpentacyclo $C_{44}H_{56}O_4$ 0.565294Alkyl aryl ethers342-Pentadecanone, 6,10,14-trimethyl- $C_{18}H_{36}O$ 0.372168Fatty acid phytyl ester	22	Tiexacosarie, 1-1000-	$C_{26} \Gamma_{53}$	0.05	5000	Aikane
24Hexatriacontane $C_{36}H_{74}$ 20.433600Alkane252-Nonadecanone $C_{19}H_{30}O$ 0.092046Ketone26n-Octacosan-1-al $C_{28}H_{56}O$ 0.23801Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.113340Ketone28Pentatriacontane $C_{35}H_{72}$ 1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{48}O$ 0.342739Phytosterols30 ² -Sitosterol $C_{29}H_{50}O$ 0.962731Phytosterols31Tetracontane $C_{40}H_{82}$ 14.73997Alkanes32Phytyl tetradecanoate $C_{34}H_{66}O_2$ 1.225525Phytyl ester335,11,17,23-tetratert-butylpentacyclo $C_{44}H_{56}O_4$ 0.565294Alkyl aryl ethers342-Pentadecanone, 6,10,14-trimethyl- $C_{18}H_{36}O$ 0.372168Fatty acid phytyl ester	23	3-Methyloctacosane	C. H.	0.13	3125	Hydrocarbon
252-Nonadecanone $C_{19}H_{30}O$ 0.092046Ketone26n-Octacosan-1-al $C_{28}H_{56}O$ 0.23801Fatty aldehyde272-pentadecanone $C_{15}H_{30}O$ 0.113340Ketone28Pentatriacontane $C_{35}H_{72}$ 1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}H_{48}O$ 0.342739Phytosterols30 ² -Sitosterol $C_{29}H_{50}O$ 0.962731Phytosterols31Tetracontane $C_{40}H_{82}$ 14.73997Alkanes32Phytyl tetradecanoate $C_{34}H_{60}O_2$ 1.225525Phytyl ester335,11,17,23-tetratert-butylpentacyclo $C_{44}H_{56}O_4$ 0.565294Alkyl aryl ethers342-Pentadecanone, 6,10,14-trimethyl- $C_{18}H_{36}O$ 0.372168Fatty acid phytyl ester	24	Hexatriacontane	CH	20.43	3600	Alkane
26n-Octacosan-1-al $C_{19}^{+} H_{30}^{-}$ 0.023801Fatty aldehyde272-pentadecanone $C_{15}^{+} H_{30}^{-}$ 0.23801Fatty aldehyde28Pentatriacontane $C_{35}^{+} H_{72}^{-}$ 1.683500Alkanes29Stigmasta-5,22-dien-3-ol, (3.beta.,22e)- $C_{29}^{-} H_{48}^{-}$ 0.342739Phytosterols30 ² -Sitosterol $C_{29}^{-} H_{50}^{-}$ 0.962731Phytosterols31Tetracontane $C_{40}^{-} H_{82}^{-}$ 14.73997Alkanes32Phytyl tetradecanoate $C_{34}^{-} H_{60}^{-}$ 1.225525Phytyl ester335,11,17,23-tetratert-butylpentacyclo $C_{44}^{-} H_{56}^{-} O_4^{-}$ 0.565294Alkyl aryl ethers342-Pentadecanone, 6,10,14-trimethyl- $C_{18}^{-} H_{36}^{-} O_4^{-}$ 0.372168Fatty acid phytyl ester	25	2-Nonadecanone	C H O	0.09	2046	Ketone
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342-Pentadecanone, 6,10,14-trimethyl- $C_{18}H_{36}O$ 0.351754Sesquiterpene35Phytyl tetradecanoate $C_{34}H_{66}O_2$ 0.372168Fatty acid phytyl ester	33	5.11.17.23-tetratert-butylpentacyclo	$C_{}^{34}H_{}^{66}O_{}^{-2}$	0.56	5294	Alkyl aryl ethers
35 Phytyl tetradecanoate $C_{34}H_{66}O_2$ 0.37 2168 Fatty acid phytyl ester	34	2-Pentadecanone, 6,10,14-trimethyl-	$C_{10}H_{10}O$	0.35	1754	Sesquiterpene
	35	Phytyl tetradecanoate	$C_{18}^{18}H_{10}^{36}O_{10}$	0.37	2168	Fatty acid phytyl ester
l otal 51.88		Total	34 bb 2	51.88		, , , ,

Table 3: IC₅₀ values of different antioxidant activities and anti-inflammatory activity.

Extracts	DPPH radical scavenging activity	IC_{50} of different antioxidant The metal chelating activity of Fe ²⁺	activities Reducing power activity of Fe ³	Anti-inflammatory activity
AMHE	141.42±0.73 μg/mL	146.64±1.25 μg/mL	107.0682±0.76 μg/mL	46.929±0.674 μg/mL
AMME	75.94±0.66 μg/mL	73.29±0.96 μg/mL	79.6497±0.96 μg/mL	37.586±1.050 μg/mL
Standard	Ascorbic acid	Ascorbic acid	Gallic acid	Diclofenac sodium
	57.66 \pm 0.40 μ g/mL	$60.18 \pm 0.39 \ \mu m g/mL$	74.37± 0.29 μg/mL	51.595±1.180 μg/mL

will produce a ferrous-ferrozine complex, indicating that it has chelating properties and may absorb ferrous ions (Olorundare *et al.*, 2020). The metal chelating power of different polarity extracts was ascertained and their capability to behave as strong antioxidant was also evaluated using sequential concentrations. The plant extracts concentrations in the range of 10–100 μ g/mL were taken into consideration for evaluation and displayed powerful metal chelating potential. The total antioxidant concentration required to chelate metal ions by 50% represents the IC₅₀ value. Comparably, methanolic extract demonstrated strong antioxidant effects having IC₅₀ value 72.90±0.096 µg/mL compared to AMHE having IC₅₀ 141.42±1.25 µg/mL while the standard, EDTA had its IC₅₀ value of 60.18±0.39 µg/mL.

The better metal chelation in the methanolic extract may be due to the presence of heneicosane (2.52%) which has earlier been notified to exhibit antioxidant activity (Bahuguna *et al.*, 2023).

Reducing power activity of Fe⁺³

Reducing power is frequently employed to assess the antioxidant capacity. It is frequently associated with the presence of reductants, which function as antioxidants by donating a hydrogen atom to break the chains of free radicals. Thus, the development of Perl's Prussian blue at 700 nm can be used to monitor the sample's reducing power (Santos et al., 2013). In this test, the Fe+3/ferricyanide complex was reduced to Fe⁺²/ferrous form when reductants were present in the antioxidant sample. The amount of plant extract used was in concentrations of 10 μ g/mL to 100 μ g/mL. These were found to exhibit good reducing power activity. The amount of total antioxidants required to convert Fe³⁺ into Fe²⁺ by 50% is known as the RP50 value. The reducing power activity of methanolic and standard Gallic acid was almost similar however, hexane extract displayed lower reducing power activity. The RP₅₀ value of the standard (Gallic acid) at 74.37 \pm 0.2973 µg/mL was commensurable to methanolic extract (AMME) at $79.64 \pm 5.360 \ \mu g/mL$ whereas, hexane extract at 107.06 \pm 1.34 $\mu g/mL$ had higher $RP_{_{50}}$ value. The better reducing power activity in the methanolic extract may be due to the presence of neophytediene, and phytyl palmitate, which has been previously reported to show antioxidant activity.

In vitro anti-inflammatory activity

Protein denaturation has been regarded as one factor contributing to inflammation. Protein denaturation occurs when many linkages in the tertiary structure of the protein are perturbated. Due to their high antioxidant content, natural substances and their components have a special ability to alleviate inflammation (Yadav et al., 2017). Albumin protein, which is often used to evaluate in vitro anti-inflammatory efficacy, is used in this test. When albumin protein is exposed to phosphate buffer saline at physiological pH, it partially denatures, and at high temperatures, it denatures entirely. This test assessed the avoidance of heat-induced albumin denaturation based on prior reports (Carrasco-Castilla et al., 2012). The Methanolic plant extract taken in concentrations of 10-100 μ g/mL had better activity when compared with hexane extract. IB₅₀ is the concentration at which 50% of protein denaturation is inhibited. The IB₅₀ of A. margaritacea methanolic extract (AMME) was found to be lower $(37.58 \pm 1.050 \ \mu g/ \text{ mL})$ when compared to hexane extract (AMHE) with $46.92 \pm 0.674 \ \mu g/mL$. The IB50 of standard Diclofenac sodium was $51.59 \pm 1.180 \,\mu$ g/mL. The lower IB50 value of extracts indicates better inflammatory activity compared to the standard. AMME (A. margaritacea Methanolic extract) surpassed AMHE (A. margaritacea Hexane extract) as an anti-inflammatory agent. This might be due to the high amount of celidoniol deoxy (5.47%) present in methanolic extract (Subin et al., 2021). The comparable activity observed in the hexane extract may be due to the presence of hexatricontane (20.43%), and eicosane (1.82%) which has been formerly reported to show anti-inflammatory action (Chuah et al., 2018).

Following table 3 gives information about IC₅₀ values of different antioxidant activities and anti-inflammatory activity.

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