The Bioscan 18(2): 75-80, 2023
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

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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KEYWORDS
Anaphalis margaritacea
Antioxidant
Anti-inflammatory

Received on :
24.02.2023

Accepted on :
18.05.2023

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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 revealed lower IC_{50} 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 IB_{50} 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, anti-inflammatory, 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 from10-100 μg/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-Hydroxy-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-3-oicacidmethylester (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, 5-di (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-diphenyl-1-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 IC_{50} value is the concentration required to decrease the absorbance of DPPH by 50%. The DPPH free radical scavenging activity (IC_{50}) value of *A. margaritacea* in AMME was found to be (75.94 ± 0.66 μg/mL) and in AMHE was (141.42 ± 0.73 μg/mL) with standard ascorbic acid showing (57.66 ± 0.40 μg/mL). Methanol extract had an IC_{50} 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 the whole plant of *A. margaritacea* (AMME)

S.No.	Compound name	Chemical formula	%area	Ki value	Class of compound
1	1-Hexadecanol	$C_{16}H_{32}$	0.58	1854	Fatty alcohol
2	Neophytadiene	$C_{20}H_{38}$	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	$C_{17}H_{34}O_2$	2.81	1916	Fatty acid methyl esters
5	n-Hexadecanoic acid	$C_{16}H_{30}O_2$	2.57	1943	Saturated Fatty acid
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{18}H_{32}O_2$	2.82	1960	Unsaturated fatty acid
7	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	5.46	2069	Fatty acid
8	(2E,7R,11R)-3,7,11,15-Tetramethylhexadec -2-en-1-ol	$C_{20}H_{40}O$	1.5	2085	Diterpenoid
9	Methyl stearate	$C_{21}H_{42}O_2$	1.72	2093	Fatty acid methyl ester
10	Oleic acid	$C_{18}H_{34}O_2$	11.31	2100	Long-chain fatty acid
11	9,12-octadecadienoic acid (z,z)-, methyl ester	$C_{19}H_{32}O_2$	0.85	2111	Unsaturated fatty acid
12	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	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)-	$C_{23}H_{44}O_2$	3.52	2333	Fatty acid methyl ester
15	5R,8R,9S,10R)-2-Formyl-3-Hydroxy-5-Isopropeny -8-8Methyl(3a 10)-octahydronaptho	$C_{15}H_{22}O_2$	13.15	2475	Sesquiterpenoids
16	Heneicosane	$C_{21}H_{44}$	2.52	2483	Alkane
17	9(11)-Dehydroergosteryl benzoate	$C_{35}H_{46}O_2$	1.9	2667	Monocarboxylic acid
18	Tetracyclo[7.3.0.1e2,8.0e3,7]tridec-10-en, 5-(di	$C_{26}H_{26}$	1.92	2873	Sesquiterpenoids
19	Squalene	$C_{30}H_{50}$	0.75	2914	Triterpene
20	.alpha.-Tocospiro B	$C_{29}H_{50}O_4$	1.16	3374	Sesterterpenoids
21	17-Oxo-6.alpha.-pentyl-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	$C_{36}H_{74}$	1.39	3600	Alkane
25	³-Sitosterol	$C_{29}H_{50}O$	3.96	2731	Plant steroid
26	±- Amyrin	$C_{30}H_{50}O$	0.96	2873	Pentacyclic triterpenoid
27	4-Isopropyl-1,7,11-trimethyl-2,7,11 -cyclotetradecatrien-1-ol	$C_{19}H_{32}O_3$	0.73	1754	Monocyclic diterpene alcohol
28	Phytyl palmitate	$C_{38}H_{74}O_2$	1.43	2045	Saturated long-chain fatty acid
	Total		83.29		

Table 2 : Chemical composition of hexane extract of whole plant of *A. margaritacea* (AMHE)

S.No.	Compound name	Chemical formula	%Area	KI value	Class of compound
1	Neophytadiene	$C_{20}H_{38}$	0.1	2168	Diterpene
2	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	0.35	1754	Sesquiterpene
3	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca -6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	0.19	2081	Ketone
4	Methyl palmitate	$C_{17}H_{34}O_2$	0.15	1878	Fatty acid methyl esters
5	Hexanoic acid, 3,5,5-trimethyl-, 2, 7-dimethyloct-1-en-3-yn-yl ester	$C_{19}H_{32}O_2$	0.13	1575	Ester
6	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.12	2085	Unsaturated acyclic monocarboxylic acids
7	hexadec-2-en-1-ol	$C_{20}H_{40}O$	0.09	2045	Diterpenoid
8	Palmitaldehyde, diallyl acetal	$C_{22}H_{42}O_2$	0.14		Acetals
9	9-Octadecen-1-ol, (Z)- (Oleyl alcohol)	$C_{18}H_{36}O$	0.31	2061	Unsaturated fatty alcohol
10	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	0.05	2177	Fatty acid esters
11	Docosane	$C_{22}H_{46}$	1.32	2109	Alkanes
12	4,8,12,16-Tetramethylheptadec an-4-olide	$C_{21}H_{40}O_2$	0.2	2258	Diterpene lactones
13	Eicosane	$C_{20}H_{42}$	1.82	2009	Alkane
14	Glycidyl oleate	$C_{21}H_{38}O_3$	0.34	1808	Carboxylic ester and an epoxide
15	Heneicosane	$C_{22}H_{46}$	2.66	2109	Alkane
16	1,2benzenedicarboxylic acid	$C_{24}H_{38}O_4$	0.14	2704	Dicarboxylic acid
17	Celidoniol, deoxy	$C_{29}H_{60}$	1.83	4395	Alkane
18	11-Tetradecen-1-ol, acetate, (Z)-	$C_{16}H_{30}O_2$	0.06	1787	Carboxylic ester
19	Furazano[3,4-b]pyrazine, 5-(2,3 -dimethylphenylamino)-6-(perhy dro-1-azepinyl)-	$C_{18}H_{22}N_6O$	0.34	3017	Aniline
20	2,6,10,14,18,22 tetracosahexaen, 2,6,10,15,19,23-Hexamethyl	$C_{30}H_{50}$	0.16	1835	Unsaturated hydrocarbon
21	Eicosanal-	$C_{20}H_{40}O$	0.19	1999	Fatty aldehydes
22	Hexacosane, 1-iodo-	$C_{26}H_{53}I$	0.05	3600	Alkane
23	3-Methyloctacosane	$C_{29}H_{60}$	0.13	3125	Hydrocarbon
24	Hexatriacontane	$C_{36}H_{74}$	20.43	3600	Alkane
25	2-Nonadecanone	$C_{19}H_{38}O$	0.09	2046	Ketone
26	n-Octacosan-1-al	$C_{28}H_{56}O$	0.2	3801	Fatty aldehyde
27	2-pentadecanone	$C_{15}H_{30}O$	0.11	3340	Ketone
28	Pentatriacontane	$C_{35}H_{72}$	1.68	3500	Alkanes
29	Stigmasta-5,22-dien-3-ol, (3.beta.,22e)-	$C_{29}H_{48}O$	0.34	2739	Phytosterols
30	^{2}-Sitosterol	$C_{29}H_{50}O$	0.96	2731	Phytosterols
31	Tetracontane	$C_{40}H_{82}$	14.7	3997	Alkanes
32	Phytyl tetradecanoate	$C_{34}H_{66}O_2$	1.22	5525	Phytyl ester
33	5,11,17,23-tetratert-butylpentacyclo	$C_{44}H_{56}O_4$	0.56	5294	Alkyl aryl ethers
34	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	0.35	1754	Sesquiterpene
35	Phytyl tetradecanoate	$C_{34}H_{66}O_2$	0.37	2168	Fatty acid phytyl ester
	Total		51.88		

Table 3: IC_{50} values of different antioxidant activities and anti-inflammatory activity.

	IC_{50} of different antioxidant activities			
Extracts	DPPH radical scavenging activity	The metal chelating activity of Fe^{2+}	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 ± 0.40μg/mL	60.18 ± 0.39 μg/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 μg/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 IC_{50} value. Comparably, methanolic extract demonstrated strong antioxidant effects having IC_{50} value 72.90 ± 0.096 μg/mL compared to AMHE having IC_{50} 141.42 ± 1.25 μg/mL while the standard, EDTA had its IC_{50} 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 μg/mL to 100 μg/mL. These were found to exhibit good reducing power activity. The amount of total antioxidants required to convert Fe^{3+} into Fe^{2+} 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 RP_{50} value of the standard (Gallic acid) at 74.37 ± 0.2973 μg/mL was commensurable to methanolic extract (AMME) at 79.64 ± 5.360 μg/mL whereas, hexane extract at 107.06 ± 1.34 μg/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 μg/mL had better activity when compared with hexane extract. IB_{50} is the concentration at which 50% of protein denaturation is inhibited. The IB_{50} of *A. margaritacea* methanolic extract (AMME) was found to be lower (37.58 ± 1.050 μg/ mL) when compared to hexane extract (AMHE) with 46.92 ± 0.674 μg/mL. The IB50 of standard Diclofenac sodium was 51.59 ± 1.180 μg/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 IC_{50} values of different antioxidant activities and anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors wish to thank the Advance Instrument Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India for facilitating the GC-MS profiling of both extracts.

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