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Composition, antioxidant and anti-inflammatory activities of different polarity extracts of *Anaphalis busua* from the Himalayan terrain of Uttarakhand

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ABSTRACT

The current study describes the analysis of the phytochemical composition and biological activities of various polarity extracts of the *Anaphalis busua* plant that was collected at an altitude of 1654 m in the Himalayan terrain of Uttarakhand, India. The extracts were prepared by the cold percolation method, which was then subjected to GC-MS for phytochemical analysis. A total of 31 compounds were identified that constituted 94.95% of the total methanolic extract. Mome inositol (31.03%) was identified as the main compound in the methanolic extract. Twenty-two compounds that comprise 68.24% of the total hexane extract were identified. Tetracontane (19.33%) was present in a significant proportion. The methanolic extract demonstrated potent antioxidant activity in terms of DPPH radical scavenging and metal chelating activity that have IC_{50} values of 81.71±1.334 and 11.26±0.005 µg/mL, respectively, compared to standards ascorbic acid and EDTA that have IC_{50} values at 12.71±0.02 and 11.36±0.06 µg/mL, respectively. The methanolic extract showed potent anti-inflammatory activity with an IC_{50} value of 24.10±0.09 µg/mL in comparison to standard diclofenac potassium with an IC_{50} value of 18.95±0.03 µg/mL. *In vitro* studies reveal that *A. busua* has a strong therapeutic potential and, if further explored, may prove to be a powerful antioxidant, anti-inflammatory, and cost-effective agent compared to synthetically derived agents from pharmaceutical industries.

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1. Introduction

The diverse and variable pharmacological effects of medicinal plants are primarily dependent on their phyto-chemical constituents [1]. One of the largest families of Asteraceae has several edible plants and medicinal values. *Anaphalis busua* (Buch.-Ham.) DC. or Tall Pearly Everlasting belonging to this family has various medicinal properties, *i.e.*, antioxidant activity, antibacterial, antifungal, anti-inflam-matory, and many more [2].

Medicinal plants play an important role in the livelihood worldwide and are used as raw materials for the extraction of active constituents in pure form (*eg*., alkaloids such as quinine and quinidine from cinchona bark, emetine from ipecacuanha root, glycosides from digitalis leaves, sennosides from senna leaves), as precursors for synthetic vitamins or steroids and as preparations for indigenous and herbal medicines [3]. Medici-nal plants are the localized and global heritage of natural antioxidants in addition to other significant bioactive leads that are implemented in the prevention and treatment of diseases such as atherosclerosis, heart stroke, diabetes, cancer, and the ageing process that is prevalent in both rural and urban areas [4,5]. India's forests are the fundamental repository for a plethora of fragrant plants. In India, plants are generally known to possess some medicinal properties [6,7]. Another report recorded that a total of 2500 plants are traditional medicine, of which 100 plants are used regularly. The Himalayan range has a splendid history of knowledge of plant-based therapy. Uttarakhand, near the Himalayas, which is home to many medicinally important plant families, is the centre of medicinal plant species due to its prolific biodiversity [8]. *Anaphalis* is the largest genus belonging to the Asteraceae family and has more than 110 distinct species of herbaceous plants spread pre-dominantly in central and southern Asia. It is well variegated in the Himalayas and the Qinghai-Tibet Plateau region. The fresh leaves of this plant and some other species of *Anaphalis* are

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macerated and applied to the cut wounds under a ragged bandage. When taken before meals, the leaves induce appetite; in addition, it is administered to recovering patients for its sedative and tonic properties. It exhibits antiasthmatic, anticoughing, expectorant, and antiphlogistic activity [9]. *A. busua*, an upright perennial herb, is called '*Buki Phool*' in Nepal [2]. The plant is also recognized by another name, Bugla. The cough, cold, and sore throat are treated with its flowers and leaves [10]. Discrete parts from various *Anaphalis* species are used as remedies for various diseases and wounds and may also be used as an antiseptic. The roots of *A. busua* are used as juice, which is then applied externally to the affected areas.

The Himalayan terrain has many medicinal plants with pleotropic medicinal properties because of its phytogeographical location, which are still unexplored for their medicinal properties. Therefore, the present study aims to collect the *A. busua plant* from the Himalayan terrain. The plant of study was further taken for extraction in two different polarity solvents. The phytochemical composition of the extracts was then analyzed using GC-MS. After chemical analysis, biological activities such as antioxidant and anti-inflammatory activities of the extracts were evaluated using standard protocols. *A. busua* possesses a variety of phytochemicals that make it beneficial as an antinociceptive, antioxidant, antibacterial, antifungal, and anti-inflammatory agent. The review of the literature shows that not much work has been done on *A. busua* from hilly terrain of Uttarakhand, India, for its phytochemical and medicinal properties. Therefore, it can be studied for consumption as a drug of plant origin with little or no side effects compared to synthetic drugs available on the market.

2. Experimental

2.1. Plant collection and authentication

The plant *A. busua* was collected from the Gethia and Bhowali hilly regions of Uttarakhand at an elevation of 1654 m in September 2020. Dr. Dharmendra Singh Rawat (Plant taxonomist), Department of Biological Science, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India, established the botanical identification of the plant.

2.2. Extract preparation

The whole plant of *A. busua* was shade dried and then grinded to powdered form which was subsequently dissolved in hexane and methanol. The resulting mixture was kept at room temperature for a week with intermittent shaking for successive extraction using the cold percolation method [11]. The resulting extract was filtered twice using Whatman filter paper to remove any undissolved moieties. The filtered solvent of the hexane and methanol extract was evaporated using a rotatory evaporator to obtain the dried form of the extract. The respective extracts were weighed to calculate the yield. The plant extract obtained was resinous and sticky in nature. Nonpolar compounds constituted the major part of the extract compared to the polar ones. The hexane extract had a yield of 3.33%, while the methanolic extract had a yield of 2.37%.

2.3. Analysis and identification of compounds with the help of gas chromatography mass spectrometry (GC-MS)

GC-MS was used to analyze and identify the phytochemical content of essential oil using GCMS-QP 2010 Plus equipment with helium as the carrier gas at a pressure of 73.3 kPa and a split ratio of 10:0. The overall flow rate was 16.3 mL/min during the study, with a column flow rate of 1.21 mL/min, a linear velocity of 40.1 cm/sec, and a purge flow of 3 mL/min. The carrier gas saver, high pressure injection, and splitter hold were all turned off and the oven temperature was set to 60 °C

Ramp@ 3 °C/min up to 210 °C (isotherm for 2 min), then 6 °C/min up to 280 °C (isotherm for 2 min), then hold for 11 minutes, flame thermionic detector (FTD).

The Kovats indices (KI) of the peaks in the DB-5 column were compared to the values in the literature and compared to the standard library spectra, which were constructed using pure substances and components of known essential oils. The fragmentation pattern of the mass spectra produced by GC-MS analysis was compared with those contained in the NBS 54 K L spectrometer database, the WILEY 8 library, and the published literature for further identification [12]. Peak areas obtained without FID response factor correction were used to calculate the relative amounts of identical components (Tables 1 and 2).

2.4. Antioxidant activities

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

The antioxidant activity of the methanol and hexane extracts was determined using a previously published method [13]. DPPH is a free and stable radical that can receive a hydrogen atom and an electron, transforming it into a stable diamagnetic molecule. The freshly produced DPPH solution in methanol was stored at 4 ° C in an amber bottle in the dark. A stock solution of 0.1 mM DPPH was produced in methanol. The test sample comprised varied polarity solvent extracts that were subjected to free radical scavenging activities. The test samples were developed in methanol and hexane at varying concentrations, that is, 5, 10, 15, 20, and 25 µg/mL. The absorbance was estimated at 517 nm using a UV spectrophotometer after 30 min of incubation [13]. Using the same approach, ascorbic acid was used as a reference for determining the free radical scavenging activity. The formula used to compute the percentage scavenging activity of the DPPH free radical was as follows:

$$\text{Percentage (\%) scavenging} = (1-A_t/A_o) \times 100\% \quad (1)$$

where A_o is the absorbance of the DPPH solution and A_t is the absorbance of the test sample.

The IC_{50} value was derived by plotting the percentage of DPPH free radical scavenging versus the concentration of the test sample. The IC_{50} concentration of a sample is the concentration at which 50% of the DPPH free radical is scavenged. A sample with a lower IC_{50} value has higher antioxidant activity. The IC_{50} values of the samples were compared with standard ascorbic acid.

2.4.2. Metal chelating activity

Metal chelating activity is based on the ability of ferrozine to chelate metals. Ferrozine is a powerful metal chelator that forms a ferrozine-ferrous complex with ferrous ions and gives it a red color having maximum absorbance at 562 nm [14]. Different concentrations of methanol and hexane extracts (5, 10, 15, 20, and 25 µg/mL) were prepared. The volume was increased to 5 mL by adding 0.2 mL of 5 mM ferrozine and 0.2 mL of methanol. The mixture was incubated for 10 min at room temperature and the absorbance was measured at 562 nm. The standard was EDTA, which was subjected to the same process as the plant extract sample. The following formula was used to calculate the percent inhibition of metal chelation in the extract and standard.

$$C\% = (A_0-A_t)/A_o \times 100 \quad (2)$$

where A_o = absorbance of the control, A_t = absorbance of the test sample.

Table 1. Chemical composition of the methanolic extract of the whole plant of *A. busua.*

No	Compounds	Chemical formula	Kovats index	% Area	Class of compound
1	Trans beta caryophyllene	$C_{15}H_{24}$	1494	1.95	Sesquiterpene hydrocarbon
2	1(10),4-Cadinadiene	$C_{15}H_{24}$	1523	0.91	Sesquiterpene hydrocarbon
3	Cubebol	$C_{15}H_{26}O$	1580	0.68	Sesquiterpene oxygenated
4	Mome Inositol	$C_7H_{14}O_6$	1647	31.03	Cyclitol
5	Phytone	$C_{18}H_{36}O$	1754	1.45	Sesquiterpene oxygenated
6	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	1757	0.69	Sesquiterpene oxygenated
7	1-Methyl-4-methylene-2-(2-methyl-1-propenyl)-1-vinylcycloheptane	$C_{15}H_{24}$	1796	0.53	Sesquiterpene hydrocarbon
8.	Acetylcholine	$C_7H_{17}NO_3$	1808	0.50	Ester
9	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	1814	1.49	Fatty acid methyl ester
10	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	1886	0.64	Fatty acid methyl ester
11	1,3,14,16-Nonadecatetraene	$C_{19}H_{32}$	1924	0.73	Alkene
12	Palmitic acid	$C_{16}H_{32}O_2$	1968	9.82	Fatty acid
13	Palmitic acid glycidyl ester	$C_{19}H_{36}O_3$	1984	0.53	Fatty acid ester
14	*cis*-3,14-Clerodadien-13-ol	$C_{20}H_{34}O$	2010	1.85	Diterpene oxygenated
15	Neophytadiene	$C_{20}H_{38}$	2045	1.25	Diterpene hydrocarbon
16	Phytol	$C_{20}H_{40}O$	2045	0.59	Acyclic diterpene alcohol
17	Methyl linoleate	$C_{19}H_{34}O_2$	2093	0.93	Polyunsaturated fatty acid ester
18	Stearic acid	$C_{18}H_{36}O_2$	2167	1.29	Long chain fatty acid
19	α-Linoleic acid	$C_{18}H_{32}O_2$	2173	5.63	Polyunsaturated fatty acid
20	Oleic acid	$C_{18}H_{34}O_2$	2175	10.80	Long-chain monounsaturated fatty acid
21	Propyleneglycol monooleate	$C_{21}H_{40}O_3$	2527	1.81	Fatty acid ester
22	Campesterol	$C_{28}H_{48}O$	2632	4.98	Phytosterol
23	2-Linoleoylglycerol	$C_{21}H_{38}O_4$	2713	3.76	Unsaturated fatty acid monoglyceride
24	Cycloeucalenol acetate	$C_{30}H_{50}O$	2816	0.52	Sterol ester
25	Lanosterol	$C_{30}H_{50}O$	2882	0.87	Tetracyclic triterpenoid
26	Phytyl myristate	$C_{34}H_{66}O_2$	2890	0.74	Phytyl fatty acid ester
27	Eicosyl benzoate	$C_{27}H_{46}O_2$	2949	0.82	Benzoic acid ester
28	3,5-Dihydroxy-6,7,8-trimethoxyflavone	$C_{18}H_{16}O_7$	2957	0.75	Flavonoids
29	16-Keto-26-hydroxycholesterol	$C_{27}H_{44}O_3$	3010	0.92	Cholesterol
30	6-Nitrocholest-5-en-3-ol acetate	$C_{29}H_{47}NO_4$	3108	5.08	Ester
31	Lanosteryl tosylate	$C_{37}H_{56}O_3S$	4024	0.70	Sterol
	Total			94.95	

To calculate the IC_{50} value of the extract and standard, the percentage of chelating activity against concentration was drawn. The higher metal chelating activity is indicated by a decreased IC_{50} value.

2.4.3. Reducing power activity

The reducing power activity of the extracts was investigated using a method described previously [15]. Different concentrations of methanol and hexane extract (50, 100, 150, 200, and 250 µg/mL) were prepared. Around 2.5 mL of each conc. of plant extract was mixed with 2.5 mL of phosphate buffer (200 mM, pH = 6.6) and 2.5 mL of potassium ferricyanide (1% w/v). The mixture was then incubated in a 50 °C water bath for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10% v/v in distilled water) was added to the mixture, which was further centrifuged for 10 min at 50.54 g (1000 rpm). Subsequently, 5 mL of the supernatant was combined with 5 ml of distilled water. The absorbance of the resulting solution was measured at 700 nm using a UV spectrophotometer after adding 1 ml of ferric chloride to the mixture. The experiment was carried out in triplicate. Gallic acid was used as a control and was tested in the same way. The control was subjected to the identical procedure, except that distilled water was used instead of the sample. The following formula was used to compute the extract and standard's reducing power percent activity.

$$\% \text{ Reducing power activity} = (A_o\text{-}A_t)\times 100/A_o \quad (3)$$

where A_o = absorption of the control, A_t = absorption of the test sample.

The RP_{50} value of the extract and standard was calculated by plotting a graph between the reducing power activity against the concentration. The value of RP_{50} is the significant amount of total antioxidants required to convert ferric ions into ferrous ions by 50%. A lower value of RP_{50} indicates a higher reduction property.

2.5. In vitro anti-inflammatory activity

The approach described by Kar *et al*. was used to investigate *in vitro* anti-inflammatory activity [16]. Various concentrations, that is, 5, 10, 15, 20, and 25 µg/mL of methanol and hexane extracts, were produced in their respective solvents. A volume of 2 mL of a specified quantity of plant extract was mixed with 2.8 mL of freshly prepared phosphate buffer of pH = 6.4. Subsequently, 0.2 mL of egg albumin was added to the mixture, bringing the total amount to 5 mL. After 15 min of incubation at 37 °C, the mixture was incubated for 5 min at 70 °C. At 660 nm, the absorbance was measured. The standard was subjected to the same procedure as the plant extract. In this study, diclofenac potassium was used as a standard.

$$\% \text{ Inhibition} = 100 \times \left(1 - \frac{Vt}{Vc}\right) \quad (4)$$

where V_t = test sample absorbance and V_c = control absorbance.

3. Statistical analysis

To determine the mean and standard deviation of triplicates of plant extracts, statistical analysis was performed using the SPSS16.00 program. To evaluate their significance ($p < 0.05$), all results were subjected to a 5% point Ducane test for one-way analysis (ANOVA). SPSS software was used to determine the significance and association of various extracts.

4. Results and discussion

4.1. GC-MS analysis of a methanolic extract of the whole plant of A. busua

In the methanolic extract, 31 compounds were identified using GC-MS, representing 94.95% of the total composition of the methanol extract. Some of the compounds that are present in the highest quantity are mome inositol (31.03%), oleic acid (10.80%), palmitic acid (9.82%), α-linoleic acid (5.63%) and, 6-nitrocholest-5-en-3-ol acetate. (5.08%).

Table 2. Chemical composition of the hexane extract of the whole plant of *A. busua.*

No	Compounds	Chemical formula	Kovats index	% Area	Class of compound
1	Pogostol	$C_{15}H_{26}O$	1530	0.97	Sesquiterpene oxygenated
2	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	1754	0.90	Sesquiterpene oxygenated
3	Humulene-1,6-dien-3-ol	$C_{15}H_{26}O$	1757	0.50	Sesquiterpene oxygenated
4	Palmitic acid	$C_{16}H_{32}O_2$	1968	1.20	Saturated Fatty acid
5	*cis*-3,14-Clerodadien-13-ol	$C_{20}H_{34}O$	1984	1.04	Diterpene oxygenated
6	Docosane	$C_{22}H_{46}$	2105	0.66	Hydrocarbon
7	Celidoniol deoxy	$C_{29}H_{60}$	2109	1.81	Hydrocarbon
8	Eicosane	$C_{20}H_{42}$	2113	2.88	Hydrocarbon
9	Pentacosane	$C_{25}H_{52}$	2175	3.76	Hydrocarbon
10	Heneicosane	$C_{21}H_{44}$	2208	5.10	Hydrocarbon
11	Campesterol	$C_{28}H_{48}O$	2632	0.64	Phytosterol
12	Clionasterol	$C_{29}H_{50}O$	2731	0.52	Phytosterol
13	Stigmasterin	$C_{29}H_{48}O$	2739	1.87	Phytosterol
14	2,6,10-Trimethyl,14-ethylene-14-pentadecane	$C_{20}H_{38}$	3030	1.21	Sesquiterpene oxygenated
15	2-Dotriacontanone	$C_{32}H_{64}O$	3338	0.55	Ketone
16	Decanoic acid	$C_{30}H_{58}O_2$	3400	1.89	Saturated fatty acid
17	Tetratriacontane	$C_{34}H_{70}$	3401	14.14	Hydrocarbon
18	4-*tert*-Butylcalix[4]arene	$C_{44}H_{56}O_4$	3412	2.38	Macrocyclic phenols
19	Pentatriacontane	$C_{35}H_{72}$	3500	2.64	Hydrocarbon
20	Hexatriacontane	$C_{36}H_{74}$	3600	3.20	Hydrocarbon
21	Octadecyl benzoate	$C_{25}H_{42}O_2$	3717	1.05	Ester
22	Tetracontane	$C_{40}H_{82}$	3997	19.33	Hydrocarbon
	Total			68.24	

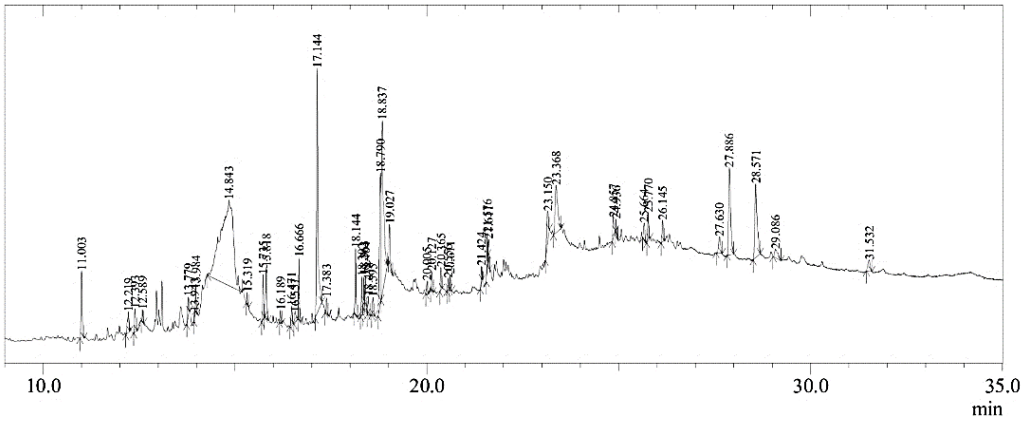

Figure 1. Gas chromatogram of the whole plant part of the *A. busua* methanolic extract (ABME).

The remaining compounds that occupy a % area less than 5% are mentioned in Table 1. Other constituents with a contribution of less than 1% were thought to be present in tiny amounts. Table 1 and Figure 1 provide detailed information on the compounds.

4.2. GC-MS analysis of the hexane extract of the whole plant of A. busua

Twenty-two compounds were identified with the help of GC-MS representing 68.24% of the total hexane extract composition. The compounds that are present in a prominent amount are tetracontane (19.33%), tetratriacontane (14.14%), heneicosane (5.10%), pentacosane (3.76%) and hexatriacontane (3.20%). The compounds occupying less than 3% of the area are given in Table 2. While the other compounds that contribute less than 1% were present in small amounts. The precise information on the compounds has been conferred in Table 2 and Figure 2.

4.3. In vitro antioxidant activity

The antioxidant activity of the hexane and methanolic extracts of the whole plant of *A. busua* was achieved using three separate methods: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating activity of Fe^{2+}, and reducing power activity of Fe^{3+}. The antioxidant activity of the hexane and methanolic extracts was compared to that of a standard reference antioxidant to assess all activities.

4.3.1. DPPH radical scavenging activity

DPPH free radical activity is used to assess antioxidant activity by observing the change in violet color caused by electron transfer. DPPH is a stable free radical that forms a diamagnetic molecule by receiving electrons or other free radicals at ambient temperature. The maximum absorbance of DPPH is at 517 nm due to the presence of its odd electron. The freshly prepared DPPH solution fades and disappears from deep blue to colorless or bleached, as antioxidant molecules quench the DPPH free radicals, decreasing the absorbance. The faster absorbance decreases, the more potent the antioxidant becomes in terms of hydrogen-ion-donating capacity [17,18]. In this study, the DPPH free radical scavenging activity of plant extracts was assessed and their ability to operate as a powerful antioxidant was examined in a dose-dependent manner. Different concentrations of plant extract (5-25 µg/mL) were used to evaluate the inhibitory activity on the DPPH free radical. The IC_{50} value is defined as the amount of total antioxidants required to inhibit the DPPH free radical by 50%. The IC_{50} value of the DPPH free radical scavenging activity of *A. busua* is in order of ABME (81.71±1.33 µg/mL) > ABHE (177.83±15.31 µg/mL) with the standard ascorbic acid having an IC_{50} value at 12.71±0.02 µg/mL (Table 3).

Table 3. Mean IC_{50} values with SD of *in vitro* antioxidant activity of the whole plant of *A. busua* *.

Sample	Mean IC_{50} values with SD (µg/mL)		
	DPPH radical scavenging activity	Metal chelating activity of Fe	Reducing power activity
ABME	81.71±1.334	11.26±0.005	10.76±0.01
ABHE	177.83±15.311	11.64±0.03	10.04±0.01
Standard	Ascorbic acid: 12.71±0.020	EDTA: 11.36±0.06	Gallic acid: 2.146±0.05

* ABME: *Anaphalis busua* methanolic extract; ABHE: *Anaphalis busua* hexane extract.

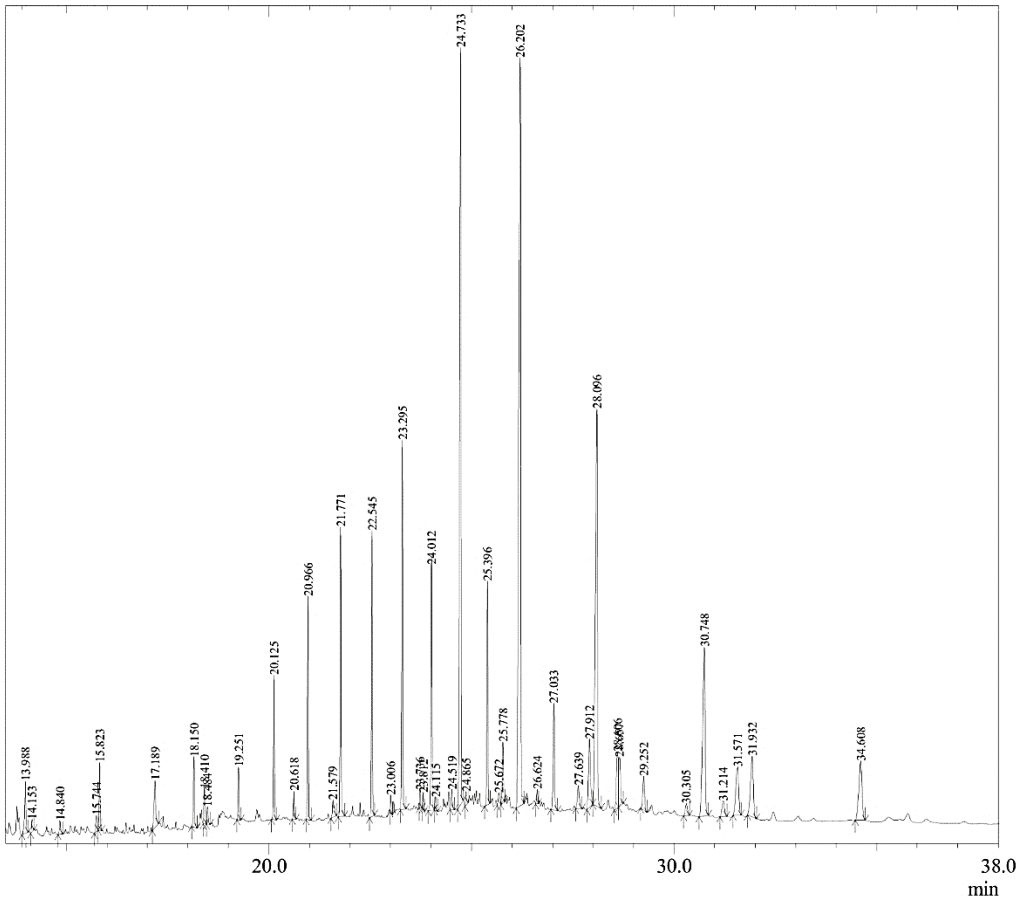

Figure 2. Gas chromatogram of the whole plant part of *A. busua* hexane extract (ABHE).

4.3.2. Metal chelating activity of Fe^{2+}

Ferrozine, the standard chelating agent, forms a quantitative complex with Fe^{2+}, leading to a red color. Formation of the ferrozine-Fe complex is hindered in the presence of other chelating agents, resulting in a reduction in the intensity of the red color. Calculating the color reduction can be used to estimate the chelating activity of coexisting chelators. The extract with the highest activity is inferred with the ferrous ferrozine complex, which implies that it has chelating activity and captures ferrous ions before ferrozine [19]. In this study, the metal chelating capacity of plant extracts as well as their potential to act as a strong antioxidant was evaluated in a dose-dependent manner. The amount of plant extract present (5-25 µg/mL), that is considered for evaluation has good metal chelating activity. The IC_{50} value is the value that is defined as the significant amount of total antioxidant required to chelate metal ions by 50%. The methanolic extract, as well as the hexane extract, showed potent antioxidant properties compared to standard EDTA. The IC_{50} value of the metal chelating activity of *A. busua* is in the order of ABME (11.26±0.005 µg/mL) > ABHE (11.64±0.03 µg/mL) with standard EDTA having its IC_{50} value at 11.36±0.06 µg/mL as shown in Table 3.

Methanol and hexane extract of *A. busua* show comparable metal chelating activity with an IC_{50} value of 11.26±0.005 and 11.64±0.03 µg/mL, respectively. The primary methanolic extract compound is mome-inositol, which has been documented to exhibit antioxidant properties, perhaps the cause of this effect [20]. Tetracontane, which occupies the highest percentage (19.33%) of constituents followed by tetratriacontane (14.14%) in the hexane extract, has been reported to exhibit antioxidant activity [21].

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

Reducing power is an evaluation of a bioactive compound's ability to donate electrons and is related to its antioxidant action. When Fe^{3+} is reduced, it produces a prussian blue color with maximum absorption at 700 nm. The higher reducing capacity is shown by an increase in absorbance. Depending on the capacity of the extract to reduce Fe^{3+} to Fe^{2+}, the yellow color of the test solution changes to green or blue. The decreasing power is proportional to the absorbance [22].

The reducing power capacity of plant extracts was investigated in this study and their ability to be a potent reducing power agent was evaluated in a dose-dependent way. The plant extract concentrations selected for the study (50-250 µg/mL) have good reducing power activity. The RP_{50} value is the significant amount of total antioxidant necessary to convert ferric ions into ferrous ions by 50%.

Table 4. IB_{50} of anti-inflammatory activity of plant extracts of the whole plant of *A. busua*.

No	Sample name	Mean IC_{50} values with SD (µg/mL)
1	ABME	24.10±0.09
2	ABHE	25.97±0.61
3	Diclofenac	18.95±0.03

The reducing power activity of methanolic and hexane extract is almost comparable; however, it has less reducing power activity compared to standard gallic acid. The RP_{50} value of reducing power activity of *A. busua* is in order of ABHE (10.04±0.01 µg/mL) > ABME (10.76±0.01 µg/mL) with the standard gallic acid having its RP_{50} value at 2.146±0.05 µg/mL (Table 3).

The methanol and hexane extract of *A. busua* show comparable reducing power activity with an IC_{50} value of 10.76±0.01 µg/mL and 10.04±0.01 µg/mL, respectively. In addition to mome-inositol, several additional substances in the methanolic extract have been linked to antioxidant activity, including campesterol, lanosterol, campesterol methyl ester, 16-keto-26 hydroxycholesterol, cholest-5-en-3-ol and 7-hexadecenoic acid [23]. The antioxidant potential of the methanolic extract of *Anaphalis busua* is completely consistent with this statement. The heneicosane present in good amounts in the hexane extract exhibited antioxidant activity [24].

4.4. In vitro anti-inflammatory activity

Inflammation is said to be attributable to protein denaturation [25]. Inflammation and its associated diseases are becoming a major health problem for most people [26]. Denaturation converts the tertiary structure of a protein to a secondary structure. In the presence of phosphate buffered saline salt, albumin protein denatures at physiological pH. The potential of plant extracts to suppress denaturation was investigated for anti-inflammatory effects. The effect of heat on albumin denaturation was evaluated [25]. In this study, the anti-inflammatory capacity of plant extracts was evaluated and their ability to be a potent anti-inflammatory was examined dose-dependently. The plant extract concentration selected for the study (5-25 µg/mL) has good anti-inflammation activity. Compared to hexane extract, the methanolic extract was found to possess better anti-inflammatory properties. IB_{50} is the 50% inhibition of protein denaturation. Table 4 shows the IB_{50} values of the anti-inflammatory activity of *A. busua,* which are in the order of ABME (24.10±0.09 µg/mL) > ABHE (25.97±0.61 µg/mL) with the standard diclofenac having its IB_{50} value at 18.95±0.03 µg/mL.

The methanolic extract with an IC_{50} value of 24.10±0.09 µg/mL shows potent anti-inflammatory activity compared to the standard diclofenac with an IC_{50} value of 18.95±0.03 µg/mL. Mome inositol has also been known to have anti-inflammatory properties [20]. A similar anti-inflammatory activity of *A. busua* methanolic extract could be possible due to the presence of mome inositol or the synergistic effect of other major/minor compounds present in the extracts. Oleic acid is another compound present in a significant amount that has shown anti-inflammatory activity [24]. The hexadecanoic acid identified in the methanolic extract is known to exhibit strong antimicrobial and anti-inflammatory activities [27].

5. Conclusions

The plant *A. busua* thrives as a weed in hilly areas of Uttarakhand and is found in abundance, however, it has not been explored much for its medicinal and therapeutic properties. Preliminary results show that *A. busua* hexane and methanol extracts were found to be effective against metal chelating activity compared to EDTA, which could be further exploited for advanced studies to be used for their therapeutic properties. Additionally, locally grown weed can also be used for its anti-inflammatory properties, as it has demonstrated effective anti-inflammatory activity compared to standard diclofenac, which is a marketed drug. Plant extracts could be used as powerful antioxidants that could be environmentally friendly and economically viable for the pharmaceutical and cosmetic sectors, as the plant is found in abundance as a weed in hilly areas.

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CRediT authorship contribution statement

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

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