Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

Phytochemical composition, antioxidant, and anti-inflammatory activities of essential oil of *Acmella uliginosa* (Sw.) Cass. grown in North India Terai region of Uttarakhand

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ABSTRACT

The plant *Acmella uliginosa* (Sw.) Cass. belonging to family Asteraceae was subjected to hydro distillation for essential oil extraction and was chemically analyzed by GC/MS for its phytochemical composition. Thirty-five compounds were identified comprising 88.1% of total essential oil composition. Limonene (12.2%) along with sesquisabinene isomer (7.3%) and caryophyllene oxide (5.9%) were the prominent compounds of the essential oil. *In-vitro* antioxidant activity of essential oil was investigated by DPPH free radical scavenging activity and metal chelating activity having IC_{50} value of 326.77 ± 5.34 µL and 14.853 ± 0.106 µL ,respectively, whereas reducing power activity having RP_{50} value of 14.011 ± 0.0446 µL. The essential oil exhibited potent anti-inflammatory activity with IB_{50} value of 5.629 ± 0.0311 µL compared to standard Diclofenac sodium salt having IB_{50} value of 23.693 ± 0.306 µg/mL. The essential oil displayed exceedingly marked anti-inflammatory as well as antioxidant activities as compared to standard marketed drugs.

ARTICLE HISTORY

Received: 06 July 2020
Revised: 22 January 2021
Accepted: 07 March 2021
ePublished: 16 March 2021

KEYWORDS

Acmella uliginosa (Sw.) Cass.
Anti-inflamatory activity
Antioxidant activity
Essential oil
Limonene

1. Introduction

Medicinal plants are considered as an affluent source of traditional medicines, and used in the preparation of modern medicine. The bioprospecting of useful natural products is a new way of systematic and sustainable exploration of natural resources for the development of valuable products which includes all bioresources such as plants, animals, and microorganism and is proved to be beneficial, not only for pharmaceutical industries but also for the host country and the native people in terms of knowledge, better education, and employment (Li et al., 2009). Medicinal plants are a rich source of natural antioxidants that are used in the prevention and treatment of various diseases. Correlating the geographical distribution and bioprospecting of flora and fauna, India is a treasure-trove in terms of biodiversity and has an abundant number of herbal plants showing medicinal properties that have been exploited in both preventive and curative medicine in traditional Indian medicinal system for centuries (Bhutani and Gohil, 2010). In Uttarakhand, more than 964 species have been documented for their various medicinal properties (Kala, 2006) and in the Trans Himalayan region, approximately 337 species of medicinal plants are reported so far (Kala and Mathur, 2002). Kumaun and Garhwal region of Uttarakhand, which is part of North India in the vicinity of the Himalayan region, is a rich repository of medicinal plants and aromatic flowers might be an interesting region for identification and exploration of other medicinal plants. *Acmella uliginosa* (Sw.) Cass. from Asteraceae (Compositae) family, is extensively distributed in tropic and subtropic regions mainly in Brazil, Africa, Indonesia, West-Indies, and Malaysia (Pandey et al., 2007). This globally distributed plant is acclimatized in India and (known) acknowledged with a different name in the distinctive region such as "Akarkara" in Kumaun

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and Garhwal region of Uttarakhand, "Gorokhbon" in Bengal, "Subhangnenek" or "Butang Baju Siti Fatimah" in Peninsular Malaysia and "Jotang" in Indonesia. The plant dwelling with pleiotropic medicinal properties is traditionally used for the treatment of various diseases in various parts of the world. The Plant being a rich source of secondary metabolites like isoflavones, flavonoids, anthocyanins, catechins, polyphenols, flavonoids have antimicrobial and antioxidant properties (Krishnaswamy et al., 1975; Dubey et al., 2013; Sana et al., 2014). *A. uliginosa* is generally used in Malaysia by the Malay community to get relief from the pain caused due to mouth ulcer, toothache, sore throat, and stomachache (Etèka et al., 2010). The whole plant part of *A. uliginosa* contains a compound known as spilanthol. Spilanthol amid with saliva inducing effect acts as a powerful insecticide and local anesthetic. It suppresses the contraction in subcutaneous muscles especially of the face and is used in anti-wrinkle products (Barbosa et al., 2016). *Spilanthes acmella* dwells with the various compounds because of which it is used as spices, antiseptic, anti-bacterial, anti-fungal, anti-malarial agent, and as a remedy for toothache, flu, cough, rabies diseases, and tuberculosis (Ramsewak et al., 1999). Japanese usually use flower head as a spice appetizer. An extract of the plant is used as a flavoring material for denitrifying and gum (Leng et al., 2011). The leaves of *A. uliginosa* are used in the treatment of skin disease and a leaf decoction is used for the treatment of diuretic and lithotriptic conditions. It is also reported that the whole plant part of *A. uliginosa* is used for the treatment of dysentery (Rao et al., 2012).

Phytochemical estimation of medicinal plants plays a significant role in revealing the new sources for therapeutically important compounds (El-Wahab et al., 2013). Bioactive compounds that are isolated from the plant materials have proven to be a beneficial source of metabolites which are difficult to get from other sources (Kinghorn, 2001; Samuelsson, 2004; Kharshiing, 2012). Analysis based on previous scientific documentation reveals that not much significant work has been done on the chemical composition and biological activities of the essential oil of *A. uliginosa* from India. However, one report has been documented for the phytochemical composition of leaves of the plant from Indonesia (Maimulyanti and Prihadi, 2016). The current study analyses the phytochemical composition of the essential oil from the whole plant part of *A. uliginosa* and investigates its antioxidant and anti-inflammatory properties.

2. Experimental

2.1. Materials and method

2.1.1. Plant collection and authentication

The plant *A. uliginosa* was collected from the Terai region of Uttarakhand in winters near sugarcane fields located at the latitude of 29° N and longitude of 79° E at an elevation of 243.8 m above the mean sea level. Herbarium specimen of the plant was submitted at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The identification of *A. uliginosa* (voucher number GBPUH-1018/1-8-2019) was established by Dr. D.S. Rawat (Assistant Professor and Plant Taxonomist), Department of Biological Science, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

2.1.2. Isolation of the essential oil

The sample of 4.94 kg fresh whole plant of *A. uliginosa* was taken and subjected to hydro distillation for 3-4 h using Clevenger apparatus. The 1.9 mL of the essential oil was separated from the aqueous solution and desiccated over anhydrous sodium sulfate. The light yellow color essential oil was stored at low temperature (4 °C) in light protected bottle for further studies.

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

To analyze and identify the phytochemical composition of the essential oil, GC/MS was carried out at GCMS-QP 2010 Plus equipment with carrier gas helium with pressure 73.3 kPa and split ratio was 10:1. During analysis, the total flow was 16.3 mL/min while the column flow rate of 1.21 mL/min. The linear velocity and purge flow were maintained at 40.1 cm/sec and 3 mL/min, respectively. Carrier gas saver, high-pressure injection, and splitter hold were off and oven temperature was initially at 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), finally hold for 11 min, flame thermionic detector (FTD).

The individual compounds were identified by comparing their Kovats indices (KI) of the peaks on Innowax fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was carried out by comparison of the fragmentation pattern of the mass spectra obtained by GC/MS analysis with those stored in the spectrometer database of NBS 54 K L, WILEY 8 libraries, and published literature (Adams, 2007). Relative amounts of identical components were based on peak areas obtained without FID response factor correction.

2.1.4. Antioxidant activities

2.1.4.1. DPPH (2,2′-Diphenylpicrylhydrazyl) free radical scavenging activity

DPPH free radical scavenging activity was analyzed with the previously reported method (Brand-Williams et al., 1995). The DPPH is a free and stable radical that can accept hydrogen radical and electron to convert them into a stable molecule that is diamagnetic. A stock solution of 0.1 mM DPPH was freshly prepared

in methanol and stored in the Amber bottle in dark at 4 °C. Test samples of the essential oil were prepared at different concentrations of 50-250 µL. Then 0.1 mL of each concentration of the essential oil was added with 2.9 mL of 0.1 mM DPPH solution and kept in dark for 30 min incubation and subjected to measurement of absorbance at 517 nm using UV spectrophotometer. Ascorbic acid was taken as standard. Percentage scavenging activity of DPPH free radical was calculated with the help of formula (Eqn.1):

$$\text{Percentage scavenging}(\%) = (1-A_t/A_0) \times 100\% \quad \text{(Eqn.1)}$$

Where A_0: absorbance of the DPPH solution and A_t: absorbance of the test sample. The percentage scavenging of DPPH free radical was then plotted against the concentration of the test sample and the IC_{50} value was calculated. The term IC_{50} is the concentration of the sample in which 50% of DPPH free radicals were scavenged. The sample having less IC_{50} value implicates better antioxidant activity. The IC_{50} value of sample was also compared with standard i.e., ascorbic acid.

2.1.4.2. Reducing power activity

Reducing power activity was done with the help of previously reported method (Yen et al., 2000). Different concentrations (5-25 µL) of the essential oil was prepared in hexane. About 2.5 mL of each concentration of the essential oil was added with the 2.5 mL of phosphate buffer (200 mM, 6.6 pH) and 2.5 mL of potassium ferricyanide (1% w/v). The mixture was then incubated in the water bath for 20 min at (50 ± 1)°C. After incubation 2.5 mL trichloroacetic acid (10% v/v in distilled water) was added and the mixture was centrifuged at 50.54 g (650 rpm) for 10 min. The 5 mL of supernatant was taken and mixed with 5 mL of distilled water. About 1 mL of ferric chloride was added to the mixture and absorbance of the resultant solution was calculated at 700 nm using a UV spectrophotometer. Gallic acid was used as a standard. Reducing power % activity of the essential oil and standard was calculated with the given formula (Eqn.2):

$$\text{Reducing power activity}\% = (A_0-A_t) \times 100/ A_0 \quad \text{(Eqn.2)}$$

Where, A_0: Absorbance of control, A_t: Absorbance of test sample. The graph of reducing power % activity against concentration was plotted to calculate the RP_{50} value of the essential oil and standard. The decreasing value of RP_{50} indicates increasing reducing activity.

2.1.4.3. Metal chelating activity

The principle of metal chelating activity is the metal chelating ability of ferrozine. Being a potent metal chelator ferrozine form a complex with ferrous ion and form red color. Different concentrations of the essential oil (5 µL-25 µL) were prepared and added with 0.05 mL of 2 mM $FeCl_2 \cdot 4H_2O$. To it, 0.2 mL of 5 mM of ferrozine was added and the volume was maintained to 5 mL by adding methanol. The mixture was incubated at room temperature for 10 min and the absorbance was calculated at 562 nm (Pavithra and Vadivukkarasi, 2015). EDTA was taken as standard. The % inhibition of metal chelation of essential oil and standard were calculated using the given formula (Eqn.3):

$$IC\% = (A_0-A_t)/A_0 \times 100 \quad \text{(Eqn.3)}$$

Where A_0: Absorbance of control A_t: Absorbance of test sample. The graph of % chelating activity against concentration was plotted to calculate IC_{50} value of the essential oil and standard. The decreasing value of IC_{50} indicates higher metal chelating activity.

2.1.5. *In-vitro* anti-inflammatory activity

Different concentrations of the essential oil (5 µL-25 µL) were prepared. About 2 mL of specific concentration of the essential oil was added with 2.8 mL of freshly prepared 1 molar phosphate buffer with pH 6.4. To it, 0.2 mL of egg albumin was added, and the final volume was maintained to 5 mL. The mixture was left for incubation for 15 min at 37°C followed by incubation for 5 min at 70°C. Absorbance was measured at 660 nm (Heendeniya et al., 2018). Diclofenac was taken as a standard. The protein denaturation % inhibition was measured with the help of a formula that is given below (Eqn. 4):

$$\text{Inhibition}\% = 100 \times (1-V_t/V_c) \quad \text{(Eqn.4)}$$

Where, V_t = Test sample absorbance and V_c= Control absorbance

2.2. Statistical analysis

Statistical analysis was done by using SPSS16.00 software for estimating the mean and standard deviation of triplicates of plant essential oil. All the results were subjected to the Duncan test for one way analysis (ANOVA) at 5% to test their significance ($p<0.05$). The significance and correlation of the essential oil were done with the help of SPSS software.

3. Results and Discussion

3.1. Chemical composition of the essential oil

The hydrodistillation of the fresh plant of *A. uliginosa* gave light yellow colored essential oil having 0.037% yield. Chromatographic analysis of the essential oil through GC/MS led to the identification of a total of 35 compounds as shown in (Table 1) and the chromatogram of the essential oil shown in (Fig. 1). The major compounds identified in the essential oil of *A. uliginosa* were limonene (12.2%), sesquisabinene isomer (7.4%), caryophylleneoxide (6%), (*E*)-caryophyllene (5.5%), 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (5.0%), myrcene (5.0%), *trans*-β-bergamotene (4.5%), spathulenol (4.5%), 1-tetradecanol (2.6%), *n*-hexacosane

(2.4%), pentadecylic acid (2.4%) and sabinene (2.3%) and others were present in minor amount. The compounds identified in the essential oil belong to the class of monoterpenes hydrocarbons, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes, and oxygenated monoterpenes. Sesquiterpenes being the dominant class of hydrocarbon comprises of 28.7% compared to monoterpenes hydrocarbon that comprises 19.5% of total compounds present. Oxygenated sesquiterpenes comprised 13.6%, whereas oxygenated monoterpenes hydrocarbon comprised the least percentage of chemical class i.e., 1.7% and the other compounds in total comprise 24.6% of total constituents as represented in (Table 2). The literature data reveals that phthalate derivatives like phthalic acid, bis (6-methylheptyl) ester present in trace amount (1.5%) in the extract might be probable contaminants (Bianco et al., 2014; Venditti, 2020).

Table 1

Chemical composition of the essential oil of the whole plant of *A. uliginosa* (AUEO).

S.N.	Compounds	Chemical formula	KI (Kovats index)	% Area	Class of compounds*
1	Sabinene	$C_{10}H_{16}$	969	2.3	MH
2	Myrcene	$C_{10}H_{16}$	988	5.0	MH
3	Limonene	$C_{10}H_{16}$	1024	12.2	MH
4	*trans*-β-Ocimene	$C_{10}H_{16}$	1050	0.7	OT
5	*Z*-Carveol	$C_{10}H_{16}O$	1207	0.7	OM
6	Carvone	$C_{10}H_{14}O$	1239	1.0	OM
7	β-Bourbonene	$C_{15}H_{24}$	1380	0.5	SH
8	β-Elemene	$C_{15}H_{24}$	1389	1.8	SH
9	*cis*-α-Bergamotene	$C_{15}H_{24}$	1415	0.6	SH
10	α-Humulene	$C_{15}H_{24}$	1489	1.3	SH
11	*E*-Caryophyllene	$C_{15}H_{24}$	1417	5.5	SH
12	Sesquisabinene isomer	$C_{15}H_{24}$	1457	7.4	SH
13	Cyclopropyl 4-ethylphenyl carbinol	$C_{12}H_{16}O$	1469	0.8	OT
14	2-Tridecanone	$C_{13}H_{26}O$	1481	0.5	OT
15	Germacrene-D	$C_{15}H_{24}$	1499	2.0	SH
16	β-Bisabolene	$C_{15}H_{24}$	1505	4.4	SH
17	*trans*-β-Bergamotene	$C_{15}H_{24}$	1513	4.5	SH
18	*E*-Nerolidol	$C_{15}H_{26}O$	1564	0.5	OS
19	Spathulenol	$C_{15}H_{24}O$	1577	4.5	OS
20	Cadin-4-en-10-ol	$C_{15}H_{26}O$	1598	0.8	OS
21	Caryophyllene oxide	$C_{15}H_{24}O$	1606	6	OS
22	1-Tetradecanol	$C_{14}H_{30}O$	1611	2.6	OT
23	Tetradecanal	$C_{14}H_{28}O$	1611	1.1	OT
24	4-(1,5Dimethylhex-4-enyl) Cyclohex-2-enone	$C_{14}H_{22}O$	1626	5.0	OT
25	3,7,11,15-Tertramethyl-, (R-(R*,R*-(*E*)-2-hexadecane 1-ol	$C_{20}H_{40}O$	1626	1.6	OT
26	*Epi*-β-caryophyllene	$C_{15}H_{24}$	1663	0.7	SH
27	Cuparenal	$C_{15}H_{20}O$	1750	0.9	OS
28	Perhydrofarnesyl acetone	$C_{18}H_{36}O$	1754	1.5	OT
29	Pentadecylic acid	$C_{15}H_{30}O_2$	1820	2.4	OT
30	9-Octadecyne	$C_{18}H_{34}$	1828	2.0	OT

Table 1 (Continued)

S.N.	Compounds	Chemical formula	KI (Kovats index)	% Area	Class of compounds*
31	Humulene oxide	$C_{15}H_{24}O$	2038	0.9	OS
32	*n*-Tetracosane	$C_{24}H_{50}$	2400	0.6	OT
33	*n*-Pentacosane	$C_{25}H_{52}$	2500	1.9	OT
34	Phthalic acid, bis(6 methylheptyl) ester	$C_{24}H_{38}O_4$	2519	1.5	OT
35	*n*-Hexacosane	$C_{26}H_{54}$	2600	2.4	OT
		88.1			

*Class of compounds: MH=Monoterpenes hydrocarbon , SH=Sesquiterpenes hydrocarbon, OM=Oxygenated monoterpenes, OS=Oxygenated sesquiterpenes, OT=Others.

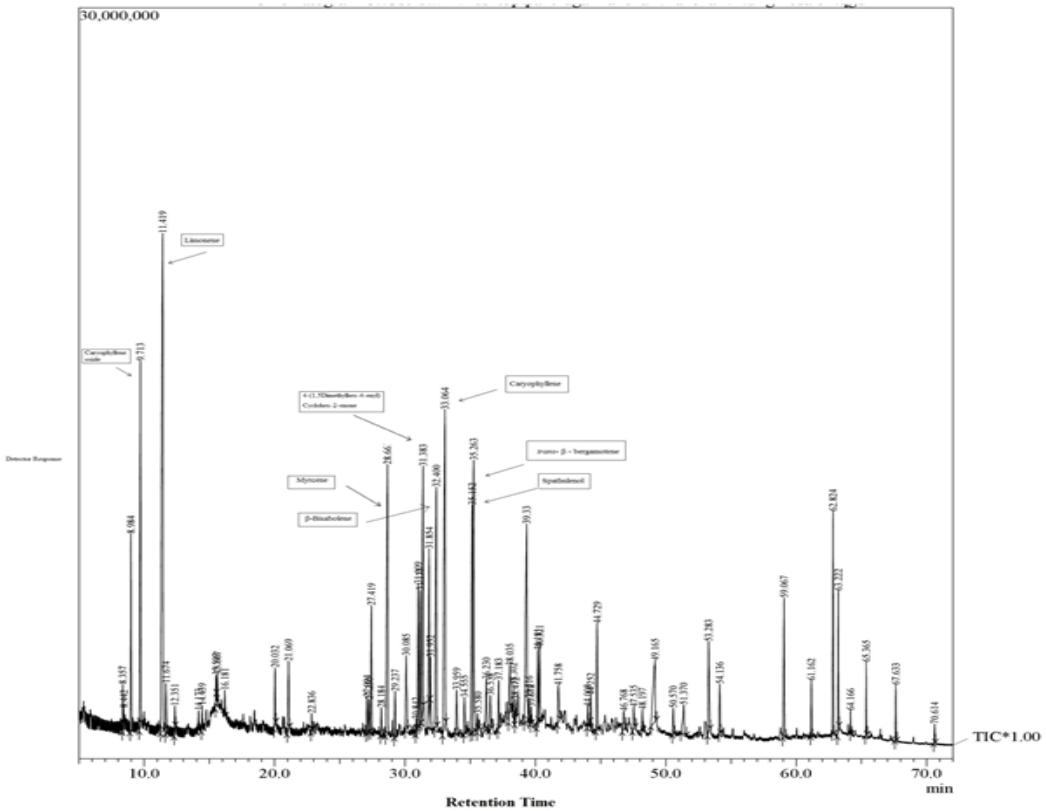

Fig. 1. Gas chromatogram of the essential oil of the whole plant of *A. uliginosa*.

Table 2
Classes of compounds present in the essential oil of the whole plant of *A. uliginosa*.

S. No.	Classes composition	% Peak area
1	Monoterpenes hydrocarbon (MH)	19.5
2	Sesquiterpenes hydrocarbon (SH)	28.7
3	Oxygenated monoterpenes (OM)	1.7
4	Oxygenated sesquiterpenes (OS)	13.6
5	Others (OT)	24.6
Total		88.1%

3.2. Antioxidant activities

The evaluation of the *in-vitro* antioxidant activity of the essential oil of the whole plant of *A. uliginosa* was performed via three different methods i.e., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, the metal chelating activity of Fe^{2+} and reducing power activity of Fe^{3+}. All the activities were evaluated by comparing the antioxidant activity of the essential oil with the standard antioxidant.

3.2.1. DPPH radical scavenging activity

DPPH free radical method is to evaluate the antioxidant activity, based on the electron transfer that brings a change in violet color. DPPH is a free radical that remains stable at room temperature and forms a diamagnetic molecule by accepting electrons or free radicals. DPPH because of the presence of its odd electron has maximum absorbance at 517 nm. The antioxidant molecule leads to quenching of DPPH free radicals. The freshly prepared DPPH solution fades and disappear from deep blue color to colorless or bleached that results into the decreasing in absorbance, hence more the briskly the absorbance decreases, the antioxidant will be more potent w.r.t. hydrogen-ion donating capacity (Amarowicz et al., 2004; Lewis et al., 2018). The DPPH free radical scavenging capacity of the essential oil was evaluated and the competence of being a potent antioxidant was tested in dose dependent manner. The concentration range of the essential oil (50-250 µL) that was considered for antioxidant evaluation possessed lesser inhibition activity on DPPH free radical in comparison to the standard. The IC_{50} value is defined as the concentration of total antioxidants required to inhibit DPPH free radicals by 50%. The IC_{50} value of DPPH free radical scavenging activity of the essential oil of *A. uliginosa* is 326.77 ± 5.34 µL which was less in comparison to the standard ascorbic acid that has IC_{50} value of 51.10 ± 0.07 µg/mL (Table 3).

Table 3

IC_{50} of DPPH free radical scavenging activity of the essential oil of the whole plant of *A. uliginosa*.

S. No.	Samples	IC_{50} values			Mean IC_{50} values
		1st	2nd	3rd	
1	AUEO	332.40	321.76	326.14	326.77 ± 5.34 µL
2	AA	51.01	51.16	51.11	51.10 ± 0.07 µg/mL

AUEO: *Acmella uliginosa* essential oil, AA: Ascorbic acid

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

Ferrozine being the most common chelating agent quantitatively form a complex with Fe^{2+} which constitutively forms red color. In the presence of other chelating agent formation of the ferrozine-Fe complex is disrupted which leads to the reduction in the intensity of red color. The estimation of the chelating activity of coexisting chelators can be depicted by measuring color reduction. The most active extract inferred with ferrous ferrozine complex suggests that the extract has chelating activity and captures ferrous ion before ferrozine (Ebrahimzadeh et al., 2008). The concentration of the essential oil considered for evaluation of metal chelating activity was 5-25 µL. The IC_{50} value of metal chelating activity of *A. uliginosa* essential oil was 14.85 ± 0.10 µL which is more significant in comparison to standard EDTA having its IC_{50} value at 45.74 ± 0.67 µg/mL (Table 4). The IC_{50} value is defined as the concentration of total antioxidant required to chelate metal ion by 50%. The essential oil is found to be a potent antioxidant.

Table 4

IC_{50} value of the metal chelating activity of the essential oil of the whole plant of *A. uliginosa*.

S. No.	Sample Name	IC_{50} values			Mean IC_{50} values
		1st	2nd	3rd	
1.	AUEO	14.84	14.96	14.75	14.85±0.10 µL
4.	EDTA	45.39	45.32	46.51	45.74±0.67 µg/mL

AUEO: *Acmella uliginosa* essential oil, EDTA: Ethylenediaminetetraacetic acid

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

Reducing power indicates electron-donating capacity of bioactive compounds and correlated with antioxidant activity. On reduction Fe^{3+} form Prussian blue color which has its maximum absorbance at 700 nm. An increase in absorbance indicates higher reducing capacity. The yellow color of test solution converts into green or blue color depending on the capacity of extract to reduce Fe^{3+} to Fe^{2+}. Higher the absorbance, higher the reducing power (Gülçin, 2015).The concentration of the essential oil considered for evaluation of reducing power activity was taken in the range of 5-25 µL. The essential oil is found to be a potent reducing agent. The RP_{50} value of reducing power activity of *A. uliginosa* is 14.01 ± 0.04 µL , whereas the standard gallic acid having its RP_{50} value at 79.90 ± 1.60 µg/mL (Table 5).

Table 5

RP_{50} of the essential oil of the whole plant of *A. uliginosa*.

S. No.	Sample Name	RP_{50} values			Mean RP_{50} values
		1st	2nd	3rd	
1.	AUEO	13.96	14.01	14.05	14.01 ± 0.04 µL
4.	GA	78.37	79.77	81.56	79.90 ± 1.60 µg/mL

AUEO: *Acmella uliginosa* essential oil, GA: Gallic acid

RP_{50} value is defined as the concentration of total antioxidants required to reduce ferric ion into a ferrous ion by 50%.

3.3 *In-vitro anti*-inflammatory activity

Denaturation is the proclaimed cause of inflammation. Protein loses tertiary structure into secondary structure due to denaturation. Albumin protein undergoes denaturation at physiological pH in presence of phosphate-buffered saline salt. The anti-inflammatory activity is a measure of the ability of plant extract to inhibit denaturation. Inhibition of heat-induced albumin denaturation was studied (Leelaprakash and Dass, 2011). The concentration of the essential oil considered for evaluation of anti-inflammatory activity was taken in the range of 5-25 µL. Essential oil is found to be a potent anti-inflammatory agent. The IB_{50} value of the anti-inflammatory activity of *A. uliginosa* of essential oil was 5.62 ± 0.03 µL, whereas the standard Diclofenac sodium salt having its IB_{50} value at 22.58 ± 0.404 µg/mL (Table 6). IB_{50} is the 50% inhibition of the denaturation of the protein.

The essential oil shows better and potent anti-inflammatory activity in comparison to the standard which may be attributed to the presence of limonene (12.2%), caryophyllene (5.5%), and caryophyllene oxide (5.9%) in the higher amount as they are reported to have good anti-inflammatory activity (Tung et al., 2008; Yu et al., 2017). The chemical compounds present in the essential oil revealed during the GC/MS analysis in the present study of whole plant extract were compared with the study of Maimulyanti and Prihadi (2016) performed on leaf extract of *A. uliginosa* from Indonesia based on the category and content of volatile component present in the essential oil. The results of GC/MS in both the analysis showed considerable variations in the chemical composition of the essential oil of plant *A. uliginosa.* The major compound present in the essential oil in the current study is limonene (12.2%) which is reported as a minor compound in the previous study i.e., 0.59% from Indonesia. Caryophyllene and caryophyllene oxide was identified as a major compound in both the study but the percentage area of both the compounds were different. In a previous study from Indonesia, it comprises 21.3% and 15.5% but in the present study from India, it was found as 5.5% and 5.9%, respectively. However, 3-careen, β-pinene was not present in the essential oil in the present study but was part of the essential oil in a previous study. Myrcene contributed to 4.9% of the total composition of the essential oil but was absent in a previous study from Indonesia (Table 7).

Table 6

IB_{50} of anti-inflammatory activity of the essential oil of the whole plant of *A. uliginosa*.

S. No.	Sample Name	IB_{50} values			Mean IC_{50} values
		1st	2nd	3rd	
1	AUEO	5.62	5.60	5.66	5.62 ± 0.03 µL
2	DF	22.74	22.12	22.88	22.58 ± 0.404 µg/mL

AUEO: *Acmella uliginosa* essential oil, DF: Diclofenac sodium

Table 7

Comparative table of the major compounds of essential oil extract with previously reported research work.

S. No.	Chemical compound	% Area	
		The present study (Uttarakhand, India)	Maimulyanti and Prihadi (2016) (Indonesia)
	Essential oil (AUEO)		
1	Caryophyllene	5.5	21.27
2	Caryophyllene oxide	5.9	15.49
3	3-Carene	-	10.73
4	β-Pinene	-	7.32
5	Sabinene	-	2.30
6	Limonene	12.2	0.59
7	4-(1,5-Dimethylhex-4-enyl) Cyclohex-2-enone	5.0	-
8	Myrcene	4.9	-
9	*trans*-β-Bergamotene	4.5	-
10	β-Bisabolene	4.4	0.30

Limonene, which is a major component of the essential oil of *A. uliginosa*, is traditionally used as a flavoring agent in cosmetics and food industries. Limonene is also utilized as an eco-friendly surfactant having lower flammability due to its solvent properties, lower odor, and VOC (Volatile Organic Compound) that make it suitable for treating contaminated surfaces in many industrial environments (Ciriminna et al., 2014). Limonene acted as an antioxidant and was reported as an excellent dietary source for cancer chemoprevention (Aggarwal and Shishodia, 2006). It has been reported to reduce the risk of skin, stomach, mouth, breast, colon, and lung cancer (Milind and Dev, 2012). Limonene acts as a potent antioxidant, and anti-inflammatory agent, natural cholesterol-lowering agent, antibacterial and antifungal agent. It also acts as a mild appetite suppressant and has an anti-anxiety effect (Mizrahi et al., 2006; Zheng et al.,1992). However, limonene is known as an allergen in the essential oil and fragrances as per European Chemical Agency (ECHA) reports and this aspect should also be considered for its use in the cosmetic and detergent industry. Caryophyllene and caryophyllene oxide exhibited several biological activities including anti-carcinogenic, anti-microbial, antioxidant, analgesic, and anti-inflammatory activity. Myrcene elicited anti-inflammatory, analgesic, antibiotic, sedative, and antimutagenic agents, and bisabolene showed anti-inflammatory, antioxidant, anti-microbial, analgesic, and anti-irritant activity (Tung et al., 2008). Due to the presence of limonene, caryophyllene, caryophyllene oxide, and other compounds that have antioxidant activity, the essential oil shows better and potent antioxidant activity in comparison to the standards.

4. Concluding remarks

The variation in the chemical composition of the essential oil of *A. uliginosa* obtained from the Terai region of Uttarakhand compared to the previous study reported from Indonesia might be due to the difference in the geographical area and climatic condition compared to different regions. Edaphic factors were different, and the plant part taken under the study was also different which might be the reason for the difference in the chemical composition of the essential oil of *A. uliginosa* growing in India and Indonesia. The essential oil of the whole plant of *A. uliginosa* showed significant antioxidant activity in terms of reducing power and metal chelating of Fe^{2+} and displayed marked anti-inflammatory activity as compared to standards. Antioxidant activity of the essential oil is comparable to standard established antioxidants like ascorbic acid, gallic acid, and EDTA. The essential oil of *A. uliginosa* can be utilized as a potent antioxidant and anti-inflammatory agent and might be commercialized further for its beneficial activities. The plant which grows as a weed is easily available and might be utilized for the production of potent antioxidant which would be commercially cost-effective for pharma as well as cosmetic industries. Furthermore, the locally grown weed can also be used as an anti-inflammatory agent as it shows better activities compared to standard drugs available in the market.

Conflict of interest

There is no conflict of interest declared by the author to publish this manuscript.

Acknowledgment

Dr. D.S. Rawat, Assistant Professor Biological sciences at G.B. Pant University of Agriculture and Technology, Pantnagar, India is thankfully acknowledged for identifying plant species.

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