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Article

Composition, Antioxidant and Anti-inflammatory activities of Hexane and Methanol extracts of *Acmella uliginosa* from Terai region of Uttarakhand

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Acmella uliginosa, an edible herb belonging to Asteraceae family, was collected from the Terai region of Uttarakhand, India. Methanol and hexane extracts of the whole plant were prepared using soxhlet apparatus. The GC-MS analysis of plant extracts identifies 22 and 35 major compounds of methanol and hexane extracts which comprises of 74.21% and 73.20% of the total composition of extracts, respectively. The major compound in hexane was 2, 4-heptadienal (7.99%) whereas trans, trans-9, 12-octadecadienoic acid propyl ester (16.96%) was major compound in methanol extract. The extracts were evaluated for antioxidant and anti-inflammatory properties. Methanol extract showed higher free radical scavenging and reducing power activities with IC_{50} value 153.82±1.69 µg/mL and RP_{50} value of 152.28±0.41 µg/mL, respectively. The metal chelating activity was higher in hexane extract as compared to methanol extract i.e., 62.08±0.25 µg/mL. The anti-inflammatory activity assessed by its ability to inhibit denaturation was higher in methanol having IB_{50} value 87.33±0.15 µg/mL. The total phenolic content (TPC), total flavonoid content (TFC) and ortho-dihydric phenol content (ODP) of methanol and hexane extracts were also evaluated. TPC, TFC and ODP was higher in methanol extract having value of 122.23±0.22, 35.01±0.29 and 8±0.86 mg/mL, respectively. *Acmella uliginosa*, might be considered as a natural source for antioxidant and anti-inflammatory properties.

Keywords: *Acmella uliginosa*. Hexane extract. Methanol extract. Antioxidant activity. Anti-inflammatory activity.

INTRODUCTION

Plants possessing therapeutic properties and having beneficial pharmacological effects are being used as a supplement of medicine since time immemorial. The utilization of medicinal plants in traditional medicine and in ethno-medicine is practiced worldwide for household treatment. About 80% of population all over the world depends on the traditional or herbal medicine mostly in Africa and other developing nations (Okoye *et al.,* 2014). In the past few decade efforts have been made for the preparation of bioactive compounds from natural resources whose pharmaceutical properties are documented and well established. The systematic and organized search for useful products derived from bioresources including plants, microorganisms and others that can be developed and refined further for commercialisation is prevailing now a day which have implications for overall benefits of the society. India has an abundant number of herbal plants showing medicinal properties. Many indigenous traditional medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy and Siddha are famous and preponderating in India from decades (Ravishankar, Shukla, 2007). A great diversity of flora in Himalayan region gives an opportunity for analysing and exploring medicinal plant for their phytochemical composition, biological activities,

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and isolation of secondary metabolites from distinctive families of kingdom plantae. It being a rich repository of medicinal and aromatic flower, and since it is located in the vicinity of the Himalaya, Kumaun and Garhwal region of Uttarakhand, have around a thousand of species being documented for having medicinal properties whereas trans himalyan region have more than 337 species reported for medicinal properties (Kala, Mathur, 2002, Kala, 2006). *Acmella uliginosa*, which generally grows as a weed belongs to Asteraceae family and mainly grows in the disturbed areas such as roadsides, cultivated fields, streams, marshes, pastures, meadows, and forests from sea level to 1200 m in elevation**.** In India it is widely distributed and acknowledged with the distinct name in different regions such as Akarkara in Kumaun and Garhwal region of Uttarakhand and Gorokhbon in Bengal. The plant is rich in different secondary metabolites such as anthocyanins, flavonoids, catechins, isoflavones and is reported to have antimicrobial, antioxidant, anti-inflammatory and antiarthritic properties (Lagnika *et al*., 2016; Paul *et al*., 2016). As *Acmella* dwells with the pleiotropic medicinal properties, it was traditionally used for treating various diseases all over the world (Leng *et al.,* 2011; Rao, Rao, Rao, 2012). The plant is a good source of many bioactive compounds and have been shown effective in pain amelioration and memory impairment in rat models (Paul *et al*., 2019; Ahmad, Yusoff, Tuan Ismail, 2020). Based on previous documentation on analysis of *Acmella uliginosa*, not much significant work has been done on the biological activities and chemical composition of the plant extracts from Terai region of Uttarakhand. In view of the aforesaid, the objective of this research is to analyse phytochemical composition of plant *Acmella uliginosa* and its biological activities.

MATERIAL AND METHODS

Plant collection and authentication

The weed *Acmella uliginosa* was collected from Terai region of Uttarakhand near sugarcane fields in winters from Pantnagar 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 plant was submitted at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The identification of *Acmella uliginosa* (voucher number GBPUH-1018/1-8-2019) was established by Dr. D.S. Rawat, Assistant professor and plant taxonomist in Department of Biological Science, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

Extract preparation

The whole plant of *Acmella uliginosa* was shade dried for 3 days, coarsely powdered, and then was further subjected to successive extraction using soxhlet apparatus. The extraction was done by using polar and non-polar solvents i.e., methanol and hexane. The extracts were further concentrated by applying established standard operating procedure i.e., vacuum distillation.

Analysis with the help of Gas Chromatography- Mass Spectrometry (GC-MS) GC-MS was carried out at GCMS-QP 2010 Plus equipment to analyse and identify the phytochemical composition of plant extract. The analysis on Gas chromatography was performed with carrier gas helium at 73.3 kPa pressure and spilt ratio was 10:1. The total flow was 16.3 mL/min while column flow rate was maintained at 1.21 mL/min during analysis. The linear velocity and purge flow was maintained at 40.1 cm/sec and 3 mL/min, respectively. The carrier gas saver, high pressure injection and splitter hold were off, and the oven temperature was initially kept at 60˚C RAMP@ 3°C/min up to 210°C (isotherm for 2 min) then at 6°C/min up to 280°C (isotherm for 2 min), and finally was hold for 11 min**.** The compounds of different polarity extracts were identified with the help of NIST-MS and FFNSC Wiley libraries.

Antioxidant activity

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

The antioxidant activity of plant extract was checked against 2, 2'- Diphenylpicrylhydrazyl (DPPH) free radical according to the previously reported method. The test samples were prepared at different concentrations i.e., 50

µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL in methanol and hexane, respectively. About 0.1 mL of each solvent extract was added with 2.9 mL of 0.1 mM DPPH i.e., freshly prepared in methanol solvent and kept for 30 min incubation in dark after mixing thoroughly. The absorbance was taken at 517 nm wavelength using UV spectrophotometer (Brand-Williams, Cuvelier, Berset, 1995). Ascorbic acid was taken as standard. The triplicates of each sample were measured and averaged. The calculation of percentage scavenging activity was done by using formula: -

Percentage (%) scavenging = $(1-A_t/A_o)$ x 100% (Adewusi, Steenkamp, 2011).

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

The graph of percentage scavenging vs concentration was plotted and IC_{50} value was calculated with the help of equation Y= mx+c by using the formula

IC_{50} = (50-c)/ m (Sebaugh, 2011).

The sample having less IC_{50} value implicates better antioxidant activity.

Reducing power activity

The reducing power activity of plant extract was determined by the method developed by Yen, and collaborators (2000).About 2.5 mL of plant extract of different concentrations (50-250 µg/mL) in their respective solvent were mixed with 2.5 mL of phosphate buffer (200 mM, 6.6 pH) and 2.5 mL of potassium ferricyanide (1% W/V). After 20 min incubation at (50 ±1) ˚C, 2.5 mL of trichloroacetic acid (10 % V/V) was added to the mixture and centrifuged at 50.54 g (650 rpm) for 10 min. After centrifugation 5 mL supernatant was taken and mixed with 5 mL of distilled water and 1 mL of ferric chloride. The absorbance was measured at 700 nm with UV spectrophotometer (Yen, Duh, Chuang, 2000). Gallic acid was considered as standard. The analysis was done in triplicates and averaged. The formula for calculating reducing power was: -

Reducing Power % activity = $(A_0-A_t) \times 100/ A_0$ (Lim, Lim, Yule, 2009).

Where, A_0 = absorbance of control, A_t = absorbance of test sample

RP_{50} value was calculated by graph of reducing power % activity against concentration of extract and standard. The decreasing value of RP_{50} indicates increasing reducing activity.

Metal chelating activity

The metal chelating activity was done according to previously reported method (Pavithra, Vadivukkarasi, 2015). A volume of 0.05 mL of 2 mM $FeCl_2 \cdot 4H_2O$ and 0.2 mL of 5 mM of ferrozine was added with varying concentration (50-250 µg/mL) of hexane and methanol extracts. The volume was made up to 5 mL by adding methanol and kept for 10 min incubation at room temperature. The absorbance was measured at 562 nm by using UV spectrophotometer. EDTA was taken as standard. The calculation of percentage inhibition of metal chelation was done with the help of the following formula: -

$$IC\% = \frac{(A_0 - A_t)}{A_0} \times 100$$ (Ebrahimzadeh *et al*., 2008)

Where, A_0=absorbance of control A_t = absorbance of test sample.

IC_{50} value was determined by plotting the graph between % chelating activity and concentration. The decreasing value of IC_{50} indicated higher metal chelating activity.

In- vitro anti-inflammatory activity About 2.8 mL of freshly prepared phosphate buffer (6.4 pH, 1M) was added with 2 mL of different concentrations (50-250 µg/mL) of the methanol and hexane extract. About 0.2 mL of egg albumin was added with the mixture and the volume was made up to 5 mL. The mixture was left for incubation for 15 min at 37˚C followed by incubation of 5 min at 70˚C. The absorbance was measured at 660 nm (Heendeniya, Ratnasooriya, Pathirana, 2018). Diclofenac

was taken as a standard. The formula for measuring protein denaturation % inhibition was: -

% inhibition =100 × (1-V_t/V_c) (Chandra *et al*., 2012).

Where, V^t = test sample absorbance and V^c = control absorbance.

Biochemical assays

Quantitative estimation of the extracts with different polarity for different content was analysed with the help of different biochemical assay by spectrophotometer. Different biochemical assay analysed were total phenol, orthodihydric phenols and total flavonoid.

Total phenols estimation Folin-ciocalteu method was used for the determination of total phenol content (Chaubey *et al.,* 2017). About 1 mL of sample extract was added with 1 mL of 95% (V/V) of ethanol followed by addition of 5 mL of distilled water. To each sample extract 0.5 mL of 50% (V/V) FCR (Folin-ciocalteu reagent) was added. After that the mixture was left for 3 min and the Na_2CO_3 (20%) in the amount of 2 mL was added and left for 60 min of incubation. The absorbance was taken at 650 nm. Gallic acid was taken as a standard for total phenols. For determining the actual phenolic content in the sample extract, the standard curve was plotted by using varied concentration of gallic acid and the result was calculated in terms of mg/gm gallic acid equivalent.

Total flavonoid estimation Total flavonoid estimation was done with the help of aluminium chloride colorimetric method (Hossain *et al*., 2012). The 10 mg/mL stock solution of different polarity extract was prepared and 1 mL of extract of different polarity solvents from stock solution was added with 3 mL of methanol. The volume of 0.2 mL of $AlCl_2$ (10% V/V) was added followed by 0.2 mL of 1M solution of potassium acetate. After adding 2.8 mL of distilled water, the mixture was incubated at room temperature for 6 minutes. Absorbance was taken at 415 nm. Methanol was considered as a blank and catechin was taken as the standard. For determination of actual flavonoid content in sample extract, the standard curve of catechin was plotted and the result was calculated in term of mg/gm catechin equivalent.

Ortho-dihydric phenol content Orthodihydric phenol content estimation was done with the help of previously reported method (Mahadevan, Sridhar, 1986). The 10 mg/mL stock solution of extract was prepared by dissolving 100 mg of extract in 10 mL of 80% aqueous methanol. About 0.1 mL of extract was added in 0.4 mL of water. To it, 1 mL of 0.05N HCl, 1 mL of Arnow's Reagent (10 gm sodium nitrite and 10 gm of sodium molybdate in 100 mL Distilled water**),** 10 mL of water and 2 mL of 1N NaOH were added and mixed thoroughly. Appearance of pink colour determined the presence of ortho dihydric phenols. Absorbance was taken at 515 nm**.** The presence of orthodihydric phenol was calculated with the help of calibration curve of standard catechol at different concentration and the concentration was expressed in mg/gm catechol equivalent.

Statistical Analysis

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

RESULTS AND DISCUSSION

Chemical composition

The types of solvents used for extraction determine bioavailability, differential solubility having compatibility between properties of drugs and solvent used for its extraction for speed of action of bioactive compounds. Dry sample (1.9 kg) of the whole plant was subjected to soxhlet extraction for the preparation of extracts. The yield of *Acmella uliginosa* plant extract in methanol and hexane was 2.02 and 1.09% respectively. The difference in yield of different polarity solvent may be attributed to the

availability of different compound present in respective solvent. GC chromatogram of the hexane and methanol extracts are shown in Figures 1 and 2 and the constituents of plant extracts are listed in Tables I and II. A total of 22 compounds were identified in methanol extract and 35 compounds in n-hexane extract. The major compounds present in hexane extract are 2,4-heptadienal (7.99%), β-sitosterol (7.75%), oleyl alcohol (6.82%), phytol isomer (4.60%), 1-pentadecanol (3.35%), tetrapentacontane (3.32%), 2,4-heptadienal, *(E,E)* (2.84%), tetracontane (2.73%), 24-norursa-3,12-diene (2.56%), squalene (2.55%), β-sesquiphellandrene (2.42%), tetracontane (2.42%) and the major compound present in methanol extract are *trans*-9,12-Octadecadienoic acid, propyl ester (16.96%), 2,4-heptadienal, *(E,E)* (10.38%), octadecanoic acid (6.22%), stigmast-5-en-3-ol,(3β) (5.68%), 2-hexadecen-1-ol,3,7,11,15-tetramethyl-(2E,7R,11R) (4.42%), stearicacid-1-monoglyceride (3.77%), bis (2-ethylhexyl) phthalate (3.45%), 9,12-octadecadienoic (3.34%), 5,8,11-eicosatriynoicacid, methylester (2.82%) and N-(2methylbutyl)(2E,4E,8Z,10E)-dodecatetraenamide (2.50%).

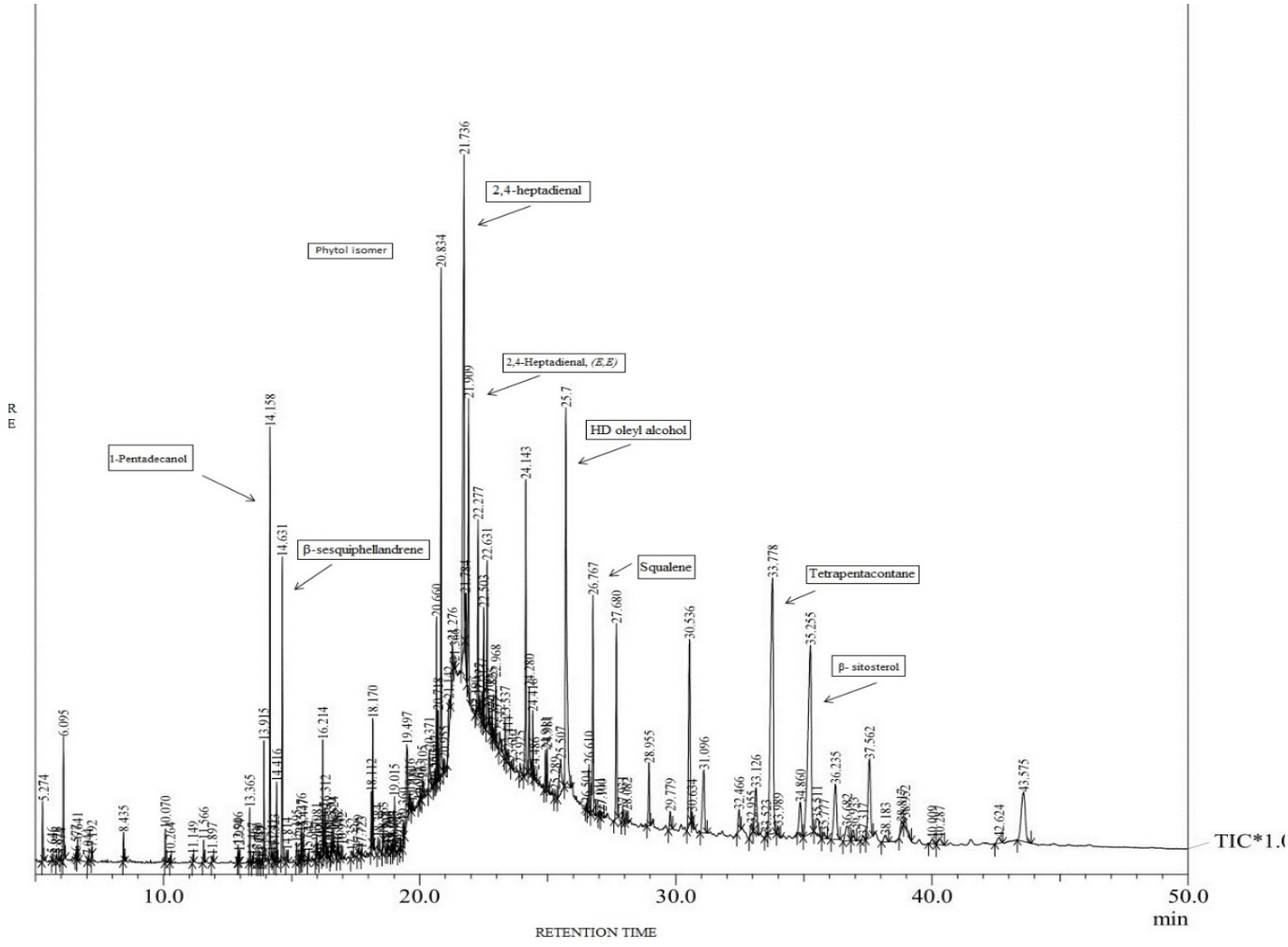

FIGURE 1 – Hexane extract chromatogram.

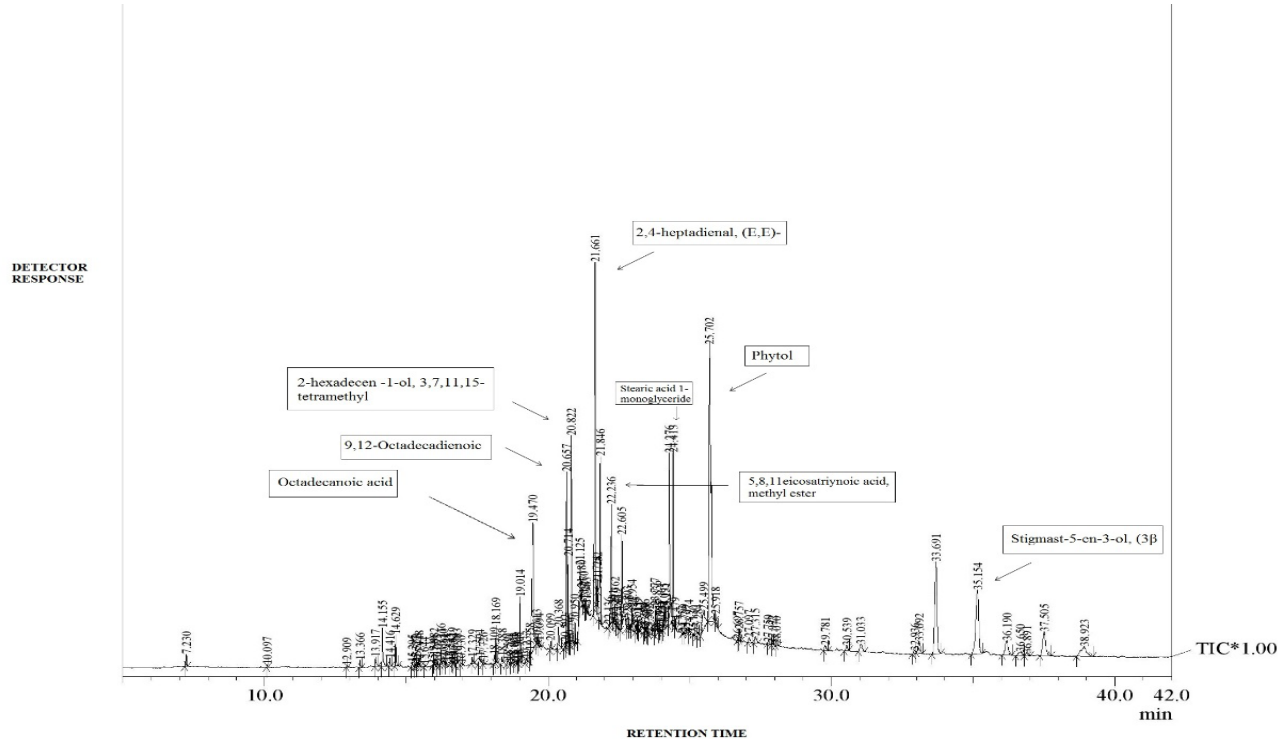

FIGURE 2 – Methanol extract chromatogram.

TABLE I - Chemical composition of hexane extract of whole plant of *A. uliginosa*

S. No.	Chemical compounds	Chemical formula	R. time	% Area	Method of identification
1.	1-pentadecanol	$C_{15}H_{32}O$	14.15	3.35	M+: - 228 M/Z: - 55,83,97,41
2.	β-sesquiphellandrene	$C_{15}H_{24}$	14.63	2.42	M+: - 204 M/Z -69,41,93,120
3.	l-(+)-ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	19.49	1.49	M+: - 652 M/Z- 57,73,43,41,129
4.	linoleic acid, methyl ester	$C_{19}H_{34}O_2$	20.66	1.18	M+: -294 M/Z- 67, 81,55,41
5.	phytol isomer	$C_{20}H_{40}O$	20.83	4.60	M+: - 296 M/Z-71,57,43,41
6	2,4-heptadienal	$C_7H_{10}O$	21.73	7.99	M+: - 110 M/Z- 81,41,53,67
7	2,4-heptadienal, *(E,E)*	$C_7H_{10}O$	21.90	2.84	M+: - 110 M/Z- 81,53,41,
8	(2E,6Z,8E)-N-(2-Phenylethyl)-2,6,8-decatrienamide	$C_{18}H_{23}NO$	22.63	1.51	M+: -269 M/Z- 81,68,53,40
9	tetracontane	$C_{40}H_{82}$	24.14	2.73	M+: - 562 M/Z- 57,71,43,99

TABLE I - Chemical composition of hexane extract of whole plant of *A. uliginosa*

S. No.	Chemical compounds	Chemical formula	R. time	% Area	Method of identification
10	cachalot O-8	$C_{18}H_{36}O$	25.50	1.07	M+- 268 M/Z- 55,82,67,41
11	oleyl alcohol	$C_{18}H_{36}O$	25.71	6.82	M+- 268 M/Z- 55,67,82,41
12	squalene	$C_{30}H_{50}$	26.76	2.55	M+- 410 M/Z- 69,81,95,41
13	tetracontane	$C_{40}H_{82}$	27.68	2.42	M+: - 562 M/Z- 57,71,85,43
14	tetrapentacontane	$C_{54}H_{110}$	30.53	3.32	M+: - 758 M/Z: - 57,71,85,43
15	γ-tocopherol	$C_{29}H_{50}O_2$	31.09	1.68	M+: - 430 M/Z- 165,430,205
16	24-epicampesterol	$C_{28}H_{48}O$	33.12	1.24	M+: - 400 M/Z: - 43,5741,213
17.	β- sitosterol	$C_{29}H_{50}O$	35.25	7.75	M+: - 414 M/Z: - 43,41,57
18.	olean-12-en-3-one	$C_{30}H_{48}O$	36.23	1.93	M+: - 424 M/Z: - 218, 203,189,175
19.	24-norursa-3,12-diene	$C_{29}H_{46}$	37.56	2.56	M+: - 394 M/Z: - 218,203, 161,
20	hex-3-en-2-one	$C_6H_{10}O$	6.095	0.98	M+: - 98 M/Z: - 83,55,98,29
21	cis-9-tetradecen-1-ol	$C_{14}H_{28}O$	13.91	0.92	M+: - 212 M/Z: - 67,55,81,41
22	3,7,11-trimethyl-2,6,10-dodecatrien -1-ol	$C_{15}H_{26}O$	14.41	0.57	M+: - 222 M/Z: - 69,93,41,119
23	dihydro-aplotaxene	$C_{17}H_{30}$	16.21	0.81	M+: - 234 M/Z: - 67,81,55.95
24	perhydrofarnesyl acetone	$C_{18}H_{36}O$	18.17	0.90	M+: - 268 M/Z: -58,43,71,85
25	1,1":1',1"'-bivanadocenium (2+), bis[hexafluorophosphate (1-)]	$C_{20}H_{16}V_2$	20.37	0.61	M+: - 358 M/Z: - 128,57,77
26	α.-glyceryl linolenate	$C_{21}H_{36}O_4$	20.71	0.51	M+: - 352 M/Z: - 79,67,95
27	cyclopropane, 1-bromo-1-(3-methyl-1-pentenylidene)-2,2,3,3-tetramethyl-	$C_{13}H_{21}Br$	21.14	0.62	M+: - 256 M/Z: - 55,105
28	14-methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	21.27	0.61	M+: - 252 M/Z: - 81,67,55,41

TABLE I - Chemical composition of hexane extract of whole plant of *A. uliginosa*

S. No.	Chemical compounds	Chemical formula	R. time	% Area	Method of identification
29	2-heptylfuran	$C_{11}H_{18}O$	21.78	0.73	M+: - 166 M/Z: - 81, 53,109
30	heneicosane	$C_{22}H_{44}$	22.50	0.86	M+: - 296 M/Z: - 57,71, 43,85
31	glycerol, 2-palmitate	$C_{19}H_{38}O_4$	24.28	0.94	M+: - 330 M/Z: - 98,112,74,84
32	tetracontane	$C_{40}H_{82}$	26.61	0.51	M+: - 562 M/Z: - 57,71, 85, 43
33	tetracontane	$C_{40}H_{82}$	28.95	0.90	M+: - 562 M/Z: - 57,71, 85, 43
34	tetracontane	$C_{40}H_{82}$	34.86	0.87	M+: - 562 M/Z: - 57,71, 85, 43
35	methyl commate D	$C_{31}H_{54}O_4$	36.68	0.61	M+: - 486 M/Z: - 218, 203,189, 133
	Total			**73.2%**	

TABLE II - Chemical composition of methanolic extract of whole plant of *A. uliginosa*

S. No.	Chemical compounds	Molecular formula	Retention time	% Area	Method of identification
1.	(Z)-3-hexadecene	$C_{16}H_{32}$	14.155	1.10	M+: - 224 M/Z: - 69, 55,41
2.	pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	19.01	1.24	M+: -256 M/Z: - 87,74,57, 41
3.	octadecanoic acid	$C_{18}H_{36}O_2$	19.470	6.22	M+: -284 M/Z: - 73,60,41,43
4.	9,12-octadecadienoic	$C_{19}H_{34}O_2$	20.657	3.34	M+: -294 M/Z: - 81,67,41,55
5.	methyl 11,14,17-eicosatrienoate	$C_{21}H_{36}O_2$	20.714	1.59	M+: - 320 M/Z: - 79, 67,108,55
6.	2-hexadecen -1-ol, 3,7,11,15-tetramethyl -, (2E,7R,11R)-	$C_{20}H_{40}O$	20.822	4.42	M+: -296 M/Z: - 71,57,43,95
7.	1-tetradecyne	$C_{14}H_{26}$	21.125	1.71	M+: -194 M/Z: - 81, 67,41
8.	2,4-heptadienal, *(E,E)*	$C_7H_{10}O$	21.66	10.38	M+: - 110 M/Z: - 81, 67, 41,53
9.	5,8,11-eicosatriynoic acid, methyl ester	$C_{21}H_{30}O_2$	22.23	2.82	M+: - 314 M/Z: - 129, 91, 41
10.	N-(2-methylbutyl) (2E,4E,8Z,10E)- dodecatetraenamide	$C_{17}H_{27}NO$	22.60	2.50	M+: - 261 M/Z: - 81,41,95

TABLE II - Chemical composition of methanolic extract of whole plant of *A. uliginosa*

S. No.	Chemical compounds	Molecular formula	Retention time	% Area	Method of identification
11.	stearic acid 1-monoglyceride	$C_{21}H_{42}O_4$	24.27	3.77	M+: - 358 M/Z: - 98,84,74, 57
12.	bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	24.413	3.45	M+: -390 M/Z: - 149,167,57,43
13.	*trans, trans*-9,12-octadecadienoic acid, propyl ester	$C_{21}H_{38}O_2$	25.702	16.96	M+: -322 M/Z: - 81,67,95,55
14.	stigmast-5-en-3-ol, (3β)	$C_{29}H_{50}O$	35.154	5.68	M+: - 414 M/Z: -43,81,95,41
15.	methyl commate c	$C_{31}H_{50}O_4$	36.190	1.35	M+: -486 M/Z: -218,203,189,175
16.	24-norursa-3,12-diene	$C_{29}H_{46}$	37.505	2.28	M+: - 394 M/Z: - 218,203,55,41
17.	γ.-tocopherol, methyl ether	$C_{29}H_{50}O_2$	38.923	1.69	M+: - 430 M/Z: - 165,43,151,57
18	sesquisabinene isomer	$C_{15}H_{24}$	14.629	0.72	M+: - 204 M/Z: - 69, 77,55,41
19	perhydrofarnesyl acetone	$C_{18}H_{36}O$	18.169	0.71	M+: - 268 M/Z: - 58,43,71,41
20	(5-methylhepta-1,3-dienyl) benzene	$C_{14}H_{18}$	20.368	0.88	M+: - 186 M/Z: - 129,115,91,77
21	4-butylbenzoic acid	$C_{15}H_{23}NO_2$	23.836	0.55	M+: - 249 M/Z: - 58,71,91,103
22	δ- 5-24-isoergosten -3β-ol (campesterol)	$C_{28}H_{48}O$	33.092	0.85	M+: - 400 M/Z: - 213,107,159,145
	Total			**74.21%**	

***In- vitro* antioxidant activity** Antioxidants prevent tissue damage and thus are related with normal functioning of many metabolic pathways directly or indirectly. Three different methods were considered for the evaluation of *in-vitro* antioxidant activity of hexane and methanol extracts of *Acmella uliginosa* i.e., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, metal chelating activity of Fe^{2+} and reducing power activity of Fe^{3+}. The evaluation of both the extracts for different antioxidant activity was done by comparing them with the standard antioxidant.

DPPH radical scavenging activity

DPPH free radical method is to evaluate the antioxidant activity, based on electron transfer that brings change in violet colour. DPPH is a free radical that remains stable at room temperature and forms a diamagnetic molecule by accepting electron or free radical. DPPH because of the presence of its odd electron have maximum absorbance at 517nm. As antioxidant molecule leads to quenching of DPPH free radicals, the freshly prepared DPPH solution fades and disappear from deep blue colour to colourless or bleached that results into decreasing in absorbance, hence more briskly the absorbance decreases, antioxidant will be more potent w.r.t. hydrogen-ion donating capacity (Amarowicz *et al.,* 2004).

The evaluation of DPPH free radical scavenging capacity of plant extracts and the competence of being a potent antioxidant was tested in terms of % inhibition or % scavenging DPPH. The IC_{50} value is the value that

is defined as the significant amount of total antioxidant required to inhibit DPPH free radical by 50%. Methanol extract is found to be a potent antioxidant in comparison to hexane extract. The IC_{50} value of DPPH free radical scavenging activity of *Acmella uliginosa* methanol and hexane extracts are 153.82±1.69 µg/mL and 204.37±0.41 µg/mL respectively with the standard ascorbic acid having its IC_{50} value, 51.56±0.44 µg/mL. (Table III).

TABLE III - IC_{50} of DPPH free radical scavenging activity plant extracts of whole plant of *A. uliginosa*

S. No.	Samples	IC_{50} values in triplicate			Mean IC_{50} values (µg/mL)
		1st	2nd	3rd	
1.	AUME	153.72	153.13	153.56	153.82±1.69 µg/mL
2.	AUHE	204.83	204.28	204.06	204.37±0.41 µg/mL
3.	AA	51.11	51.99	51.58	51.56±0.44 µg/mL

AUME-*Acmella uliginosa* methanolic extract**, AUHE**-*Acmella uliginosa* hexane extract, **AA-** Ascorbic acid

Metal chelating activity of Fe^{+2} Ferrozine form complex with Fe^{2+} which constitutively form red colour. In the presence of other chelating agent formation of Ferrozine-Fe complex is disrupted. The estimation of chelating activity of coexisting chelators can be depicted by measuring colour reduction**.** The most active extract inferred from ferrous ferrozine complex suggest that the extract have chelating activity and capture ferrous ion before ferrozine (Ebrahimzadeh, Pourmorad, Bekhradnia, 2008).

The metal chelating capacity of plant extracts was evaluated and their competence of being a potent antioxidant was tested in a dose dependent manner. The concentration of plant extracts (50-250 µg/mL), that is considered for evaluation possess good metal chelating activity. The IC_{50} value of metal chelating activity of *Acmella uliginosa* of hexane and methanol extracts are 62.08±0.25 µg/mL and 83.26±0.07 µg/mL with the EDTA having its IC_{50} value, 45.74± 0.67 µg/mL (Table IV). IC_{50} value is the value that is defined as the significant amount of total antioxidant required to chelate metal ion by 50%.

TABLE IV - IC_{50} value of metal chelating activity of plant extracts of whole plant of *A. uliginosa*

S. No.	Sample Name	IC_{50} values (µg/mL) in triplicate			Mean IC_{50} values with standard deviation (µg/mL)
		1st	2nd	3rd	
1.	AUME	83.02	83.13	83.62	83.26± 0.07
2.	AUHE	61.65	62.01	62.58	62.08±0.25
3.	EDTA	45.39	45.32	46.51	45.74±0.67

AUME- *Acmella uliginosa* methanolic extract**, AUHE**- *Acmella. uliginosa* hexane extract
EDTA- Ethylenediaminetetraacetic acid

Reducing power activity of Fe^{+3} The reducing power indicates electron donating capacity of bioactive compounds and its correlation with antioxidant activity. On reduction Fe^{3+} form Prussian blue colour which has its maximum absorbance at 700 nm. An increase in absorbance indicates higher reducing capacity. Yellow colour of test solution converts into green or blue colour depending on the capacity of extract to reduce Fe^{3+} to Fe^{2+}. The higher the absorbance, the higher is the reducing power (Gülçin, 2015).

The concentration of plant extracts that is considered for evaluation is 50-250 µg/mL which possess good reducing power activity. The value that is defined as the significant amount of total antioxidant required to reduce ferrous ion into ferric ion by 50% is RP_{50} value. Methanol extract is found to be a potent reducing agent in comparison to hexane extract. The RP_{50} value of reducing power activity of *Acmella uliginosa* of hexane and methanol extracts are 152.28±0.41 µg/mL and 158.42±2.96 µg/mL respectively with the standard gallic acid having its RP_{50} value at 79.90 ± 1.60 µg/mL (Table V).

TABLE V - RP_{50} value of reducing power of plant extracts of whole plant of *A. uliginosa*

S. No.	Sample Name	RP_{50} values (µg/mL) in triplicate			Mean RP_{50} values (µg/mL)
		1st	2nd	3rd	
1.	AUME	152.31	151.85	152.69	152.28 ±0.41 µg/mL
2.	AUHE	156.16	157.33	161.77	158.42 ± 2.96 µg/mL
3.	Gallic acid	78.37	79.77	81.56	79.90 ± 1.60 µg/mL

AUME- *A. uliginosa* methanolic extract**, AUHE**- *A. uliginosa* hexane extract, **GA-** Gallic Acid

Correlation of antioxidant activity with various parameters In the present analysis, the correlation of total phenolic content with IC_{50} of different antioxidant activity was investigated (Table VI A). There was negative correlation between TPC and IC_{50} of DPPH radical scavenging and reducing power activity at 5% level of significance **(**α= 0.05) (Table VI B**)**.

The negative correlation illustrates the relation between phenolic content with antioxidant activity. More the phenolic content lesser the concentration required to inhibit the free radical. The correlation between TPC and IC_{50} of metal chelating activity was positive (Table VI B). The different relationships between the antioxidant activity and the total phenolic content can be due to many factors; in fact, the total phenolic content does not incorporate all the antioxidants. Also, it must be considered that the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants (Piluzza*,* Bullitta, 2011).

TABLE VI A - IC_{50} of antioxidant activities and TPC of plant extracts of whole plant of *A. uliginosa*

Plant extracts	IC_{50} of various antioxidant activities			
	DPPH radical scavenging activity	Metal chelating activity of Fe^{+2}	Reducing power activity of Fe^{+3}	TPC (Total Phenolics Content)
AUME	153.82±5.34	83.26±0.07	152.28±0.41	122.23±0.22 mg/gm GAE
AUHE	204.37±0.41	62.08±0.25	158.42±2.96	83.66±0.33 mg/gm GAE

AUME- *A. uliginosa* methanolic extract**, AUHE**- *A. uliginosa* hexane extract

TABLE VI B - Correlation coefficient between various parameters

Parameter	DPPH radical scavenging activity	Metal chelating activity of Fe^{2+}	Reducing power of Fe^{3+}
TPC (Total phenolic content)	-0.99959	0.999307	-0.90292

In- vitro anti-inflammatory activity Denaturation is the proclaimed cause of inflammation. In anti-inflammatory activity, the ability of plant extract to inhibit denaturation was assessed (Chandra *et al,* 2012). In the present investigation, the anti-inflammatory of capacity plant was evaluated and its competence of being a potent anti-inflammatory was tested in dose dependent manner**.** The concentration of plant extracts that is considered for evaluation possessing good anti- inflammation activity is 50-250 µg/mL. Methanol extract is found to be a potent antioxidant in comparison to hexane extract. The % inhibition with linear regression is shown in Figure 3. The IB_{50} is the 50% inhibition of denaturation of protein. The IB_{50} of anti-inflammatory activity of *Acmella uliginosa* for methanol extract is 87.33±0.15 µg/mL and for hexane extract 139.44±1.01 µg/mL with the standard Diclofenac sodium having its IB_{50} value, 23.69± 0.30 µg/mL (Table VII and Figure 4).

TABLE VII - IB_{50} of anti-inflammatory activity of plant extracts of whole plant of *A. uliginosa*

S. No.	Sample Name	IB_{50} values in triplicate			Mean IC_{50} values
		1st	2nd	3rd	
1.	**AUME**	87.24	87.24	87.51	87.33± 0.154 µg/mL
2.	**AUHE**	140.30	138.33	139.68	139.44± 1.012 µg/mL
3.	**Diclofenac sodium**	22.74	22.12	22.88	22.58±0.404 µg/mL

AUME- *A. uliginosa* methanolic extract, AUHE- *A. uliginosa* hexane extract

Biological activities

Total phenols content (TPC), total flavonoid content (TFC), total ortho dihydric phenol content (ODP) and total antioxidant content (TAC) of plant extracts is shown in Table VIII.

TPC, TFC, ODP and TAC of methanol and hexane plant extracts was evaluated with the help of calibration curve of standard and the results was expressed in term of mg/gm gallic acid equivalent (mg/gm of GAE), mg/gm catechin equivalent (mg/gm of CNE), mg/gm catechol

equivalent (mg/gm of CLE) and mg/gm ascorbic acid equivalent (mg/gm of AAE) respectively.

The total phenolic content, flavonoid content, ortho dihydric phenol and total antioxidant content is better in methanol in comparison to hexane extract as phenolic and flavonoid derivatives are more soluble in polar solvents compared to non-polar solvents (Deighton *et al.,* 2000). Many scientific studies have conclusively established correlation between phenolic content and antioxidant activity (Hossain, Shah, 2015) which may be attributed to increased antioxidant activity in methanolic extract.

CONCLUSION

Hexane and methanol extracts of *Acmella uliginosa* shows potent antioxidant and anti-inflammatory activities. The total phenolic and flavonoid content was maximal in polar solvent attributing high antioxidant activity. The locally growing weed has its antioxidant and anti-inflammatory activities comparable to the standards like Gallic acid, Ascorbic acid and Diclofenac sodium. Further investigation of plant for other mechanism based biological activities could lead to utilization of plant for the purpose of therapeutic formulae in pharmaceuticals, cosmetic industries and food recipes.

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