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Fatty acid composition and antioxidant activity of *Angelica glauca* and *Chenopodium album* seed extracts from Kashmir

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SUMMARY: *Angelica glauca* Edgew. and *Chenopodium album* Linn. are medicinally important plants with aromatic, nutritious and flavor-enhancing properties. In this study the fatty acid composition of petroleum ether seed extracts (PE) of processed plants were analyzed by gas chromatography-mass spectrometry (GC-MS). The PE consisted mainly of unsaturated fatty acids, such as petroselinic acid 74.26% and oleic acid 7.37% for *A. glauca* and linoleic acid 53.05% and oleic acid 20.74% for *C. album*. The de-fatted seed extracts were screened for their antioxidant activities using 2,2-di-phenyl-1-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), hydrogen peroxide (H_2O_2) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay. Almost all the assays resulted in defatted seed extracts showing powerful radical scavenging activity. These findings suggest that the processed plants could be used as ingredients (as a source of natural antioxidants and unsaturated fatty acids) for the formulation of functional foods.

KEYWORDS: *Angelica glauca; Antioxidant activity; Chenopodium album; Fatty acid composition; Linoleic acid; Petroselinic acid*

RESUMEN: ***Composición en ácidos grasos y actividades antioxidantes de extractos de semillas de* Angelica glauca *y de* Chenopodium álbum *de Cachemira.*** *Angelica glauca* Edgew. y *Chenopodium album* Linn. son plantas medicinales importantes que tienen propiedades aromáticas y nutritivas y se usan como condimento. En este estudio, la composición en ácidos grasos de los extractos de éter de petróleo (EP) de semillas de la planta se analizó por cromatografía de gases-espectrometría de masas (GC-MS). El EP consiste principalmente en ácidos grasos insaturados, especialmente ácido petroselínico 74,26% y ácido oleico 7,37% para *A. glauca* y ácido linoleico 53,05% y ácido oleico 20,74% para *C. album*. Además, a los extractos de semillas desgrasados se estudió sus actividades antioxidantes utilizando 2,2-di-fenil-1-picrylhydrazyl (DPPH), nitroazul de tetrazolio (NBT), peróxido de hidrógeno (H_2O_2) y 2,2'-azino-bis (3-etilbenzotiazolina-6-ácido sulfónico) (ABTS) ensayos de eliminación de radicales. Casi todos los ensayos sugieren que los extractos de semillas desgrasados mostraron potentes actividades de eliminación de radicales. Por último, los resultados sugieren que las plantas estudiadas podrían usarse como ingrediente (como fuente de antioxidantes naturales y ácidos grasos insaturados) para la formulación de alimentos funcionales.

PALABRAS CLAVE: *Ácido linoleico; Ácido petroselínico; Actividad antioxidante; Angelica glauca; Chenopodium album; Composición en ácidos grasos*

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1. INTRODUCTION

Lipids are found in all parts of plants but are mostly dominant in seeds. They are nutritional sources of food (Chen and Chuang, 2002). Monounsaturated fatty acids, such as oleic acid, are highly stable against oxidation and when blended with other oils have been found to have benefitial protective effects (Lampi and Kamal-Eldin, 1998). The antioxidants in plants prevent lipid oxidation, and the deterioration of color, flavor and nutritional quality of various foods (Kozlowska *et al*., 2014). They may also be an alternative to toxic synthetic antioxidants (Koleva *et al*., 2012). The extraction and preservation of natural antioxidants to replace the synthetic ones is the main target of the food, pharmaceutical and cosmetic industries (Binic *et al*., 2013).

Angelica glauca Edgew is an ornamental, perennial herb which is native to the temperate northern region from Kashmir to Uttarakhand. It is locally known as "Chora". Traditionally it is used in medicines, aromatic spices and condiments. It is used for various drug formulations at the domestic and international levels. The roots of *A. glauca* contain valeric acid, angelic acid and angelisine resin and have various medicinal properties such as stimulant, cardioactive, carminative, digestive, sudorific and expectorant (Chopra *et al*., 1956). Essential oil from the whole plant possesses antioxidant, antimicrobial and phytotoxic activity (Irshad *et al*., 2011). *Chenopodium album* Linn. (Chenopodiaceae) locally known as "Bathu" is an erect, annual plant growing up to 150 cm tall in the temperate zone. Traditionally it is used to cure arthritis and rheumatism (Prajapati *et al*., 2003). It is a medicinally important plant as it possesses anticancer (Ankita and Chauhan, 2012), anti-inflammatory (Usman *et al*., 2010), hepatoprotective (Pal *et al*., 2011), spasmolytic and analgesic activity (Ahmad *et al*., 2012). Our laboratory is also currently working on the analysis of novel seed oils for fatty acid composition (Sharma *et al*., 2009). A scanning of the literature has revealed that the whole plant extracts of *A. glauca* and *C. album* possess antioxidant activity (Irshad *et al*., 2011; Lone *et al*., 2017). However, no work on seed extracts for fatty acid composition and antioxidant activity from Kashmir (India) have been reported. Moreover, these plants possess tremendous nutritional and medicinal properties, less input care cost and high natural abundance. Therefore, this study was carried on *A. glauca* and *C. album* to determine their fatty acid composition and antioxidant activity, in order to evaluate them as potential natural sources of antioxidants and fatty acids used for nutritional purposes in the food industry.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

The seeds of *A. glauca* and *C. album* used in this work were collected from local nurseries in the Sher-e-Kashmir University of Agricultural Sciences and Technology (SKAUST-K) in 2019. 2,2-di-phenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), hydrogen peroxide (H_2O_2) and nitroblue tetrazolium (NBT) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). All other chemicals were of analytical grade.

2.2. Successive soxhlet extraction

The dried seeds were crushed separately into powder with a grinder. The seed powder from each plant was mixed with sodium sulphate and kept in an oven for about 2 h at 60-70 °C to remove any remaining moisture. A 50 g sample (Raaman, 2006) from each plant was put in a soxhlet apparatus along with 180 mL of solvent in a 250 mL round-bottom flask. Extractions were then performed using the petroleum ether solvent (PE), chloroform (CF), ethyl acetate (EA), acetone (AT) and methanol (MT). The extraction time ranged from 4-6 hours. The extracted oil (PE) was passed through anhydrous sodium sulphate (Na_2SO_4). All seed extracts were kept at 4 °C until further analysis. The oil characteristics were determined according to the standard AOCS procedures (Link, 1973) and the data are presented in Table 1.

2.3. Fatty acid methyl esters (FAMEs) preparation

One gram oil (PE) was saponified with 0.5N alcoholic potassium hydroxide (KOH). The unsaponifiable matter was removed with diethyl ether and the aqueous layer was acidified with 6N hydrochloric acid (HCl) followed by extraction with diethyl ether to get mixed fatty acids (MFAs) which were further treated with excess absolute methanol with a few drops of sulphuric acid (H_2SO_4) as catalyst and the reaction was refluxed

TABLE 1. Saponification and iodine values of petroleum ether extracts of of *A. glauca* and *C. album* and yield (%, *w/w*) of different extracts.

Name of plant	Seed extracts (%, *w/w*)					S.V (PE)	I.V (PE)
	PE	CF	EA	AT	MT		
A. glauca	14.62± 0.08^{c}	7.61± 0.29^{e}	10.54± 0.35^{d}	15.61 ± 0.09^{b}	19.82 ± 0.70^{a}	155.18± 1.37	73.80± 0.93
C. album	15.01± 0.69^{b}	9.32± 0.40^{e}	11.91± 0.03^{d}	13.32 ± 0.20^{c}	22.73 ± 0.48^{a}	145.59 ± 0.97	118.04 ± 1.43

PE: petroleum ether extract; CF: chloroform extract; EA: ethyl acetate extract; AT: acetone extract: MT: methanol extract. Values are arranged as means ± S.D. (n=3)

Different letters in each row are statistically significant different at ($p< 0.05$) according to Duncan's test.

for 1-2 hours. After completion of the reaction, as monitored by thin layer chromatography (TLC), the resulting mixture was diluted with water and continuously extracted with diethyl ether. The combined extracts were washed with 5% aqueous sodium bicarbonate and dried over anhydrous sodium sulphate to yield FAMEs which were further purified with n-hexane and diethyl ether (98/2, *v/v*) by column chromatography.

2.4. Fourier-transform infrared spectroscopy (FTIR) analysis

Transmittance spectra were obtained using a Fourier-transform infrared spectroscopy (FTIR) (Frontier, Perkin-Elmer Ltd, UK) fitted with an Attenuated total reflectance (ATR) crystal of zinc selenide and the length was recorded in cm^{-1}. The solvent used in the FTIR analysis was carbon tetrachloride (CCl_4) because of its transparent nature in the main region of the IR spectrum. The samples were put on ATR crystal kept at 65 °C so as to fully cover the surface of the crystal. A small amount of sample (50-100 µL) was taken. The samples were measured in duplicate. The spectra were continuously measured over a range of 3500-500 cm^{-1} and data resolution of 4 cm^{-1} and air were taken as reference background material. After each scan the ATR crystal was removed, cleaned and dried with tissue paper and ethanol.

2.5. Gas chromatography mass spectrometry analysis

The fatty acid composition was determined by using Gas chromatography, Perkin Elmer (GC, Clarius 600) coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, D.E). An Elite-5MS capillary column (0.25mm × 30mm) with a flame ionization detector was used. Helium was used as carrier gas at a flow rate of 0.5 mL/min. The injector, column and detector temperatures were 180 °C, 260 °C and 280 °C, respectively. The oven temperature was programmed as follows: 180 °C for 2 min, then raised to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, then raised to 215 °C at 2 °C/min, and held for 10 min. The injector and detector temperatures were maintained at 260 and 280 °C, respectively. Individual fatty acids were recognized with typical mass spectra from The National Institute of Standards and Technologies (NIST) library of the GC-MS.

2.6. Antioxidant activity

2.6.1. Radical scavenging activity (DPPH assay)

The antioxidant activity of seed extracts was determined by using DPPH following the procedure of (Shimada *et al*., 1992) with some modifications. Briefly, 200 µL of each extract (25-200 µg/mL) with 3.8 mL DPPH solution were incubated in the dark at room temperature for 1 h. The absorbance of the mixture was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (I\%)} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Where, A control is the absorbance of the DPPH radical (without the test sample), and A sample is the absorbance of the DPPH radical with the different extract samples of various concentrations.

2.6.2. Nitroblue tetrazolium assay (NBT assay)

Superoxide anion scavenging activity was determined as described (Vyas and Kumar, 2005). The reaction was performed in 50 mM/L phosphate buffer (PH 7.8) containing concentrations of (25-200) µg/mL of the extract, 50 mM/Lnitroblue tetrazolium (NBT), 10 mmol/L D,L-methionine, and 0.025% (*v/v*) Triton X-100. The reaction was initiated by illuminating the reaction mixture, the absorbance of formazan was recorded at 560 nm, and the percentage scavenging activity was described as the inverse of the produced formazan. BHT was used as a positive control. The percentage scavenging of NBT radicals was calculated using the equation described in the DPPH assay. The results were compared with standard drug BHT.

2.6.3. Hydrogen peroxide (H_2O_2) scavenging activity

The ability of the extracts to scavenge H_2O_2 radicals was determined according to the method described by (Ruch *et al*., 1989). A hydrogen peroxide solution of 40 mM was prepared in phosphate buffer (pH 7.4). A spectrum was produced using a UV-Visible spectrophotometer with phosphate buffer solution as a blank. The extracts at different concentrations (25-200 µg/mL) in 3.4 mL phosphate buffer were added to 0.6 mL of H_2O_2 solution (0.6 mL, 43 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with BHT, the reference compound.

The percentage scavenging of H_2O_2 radicals was calculated using the equation described in the DPPH assay. The results were compared with BHT.

2.6.4. Radical scavenging activity (ABTS assay)

The antioxidant activity of the seed extracts was determined by ABTS according to (Re *et al*., 1999). The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, and stored in the dark at room temperature for 16 h. Various concentrations (25-200 µg/mL) of the plant extracts were mixed with 1 mL of $ABTS^{\bullet+}$ solution and homogenized. Absorbance was then recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were made after at least 6 min. The reaction mixture of the standard group was prepared by mixing 950 µL of $ABTS^{\bullet+}$ solution and 50 µL of BHT. As for the antiradical activity, the ABTS scavenging ability was expressed as IC_{50} (µg/mL). The reaction mixture of the standard group was obtained by mixing 950 µL of $ABTS^{\bullet+}$ solution and 50 µL of BHT. The ABTS scavenging ability was expressed as IC_{50} (µg/mL). The percentage scavenging of ABTS radicals was calculated using the equation described in the DPPH assay. The results were compared with standard drug BHT.

2.7. Stastical analysis

The antioxidant parameters were expressed as the mean ± SD with three replicates each. The results of all the antioxidant activities were analyzed with one-way analysis of variance (ANOVA). Duncan's post hoc test was applied for comparisons of means and differences were considered significant at 95% statistical significance using IBM SPSS Statistics 20.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of the extracts

The percentage (%, *w/w*) of various de-fatted extracts of *A. glauca and C. album* is depicted in Table 1. The yield of almost all the extracts was found to be significantly good, but seed extraction through methanol (MT) was found to be predominantly high for *C. album* 22.73% and *A. glauca* 19.82% and through petroleum ether (PE) 15.01% and 14.62% for *C. album* and *A. glauca,* respectively. The order of extracted yield through other solvents was acetone (AT) > ethyl acetate (EA) > chloroform (CF). The saponification value (SV) is the indicator of the average molecular weight and hence chain length (Nehdi *et al*., 2012). The higher the saponification value, the smaller is the chain length of fatty acids in a triacylglycerol. The saponification value (PE) of *A. glauca* at 155.18 is higher than *C. album* at 145.59, mainly because of the dominance of low molecular weight fatty acids (Table 1). The iodine value (IV) gives an indication of the degree of

TABLE 2. Saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids of petroleum ether extracts of *A. glauca* and *C. album.*

Common and systematic names	Carbon numbers	Chemical formula	Area (%)	
			A. glauca	*C. album*
Decanoic acid	C10:0	$C_{10}H_{20}O_2$	-	1.67 ± 0.02
Myristic acid	C14:0	$C_{14}H_{28}O_2$	0.32 ± 0.01	1.15 ± 0.02
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	0.61 ± 0.35	6.32 ± 0.21
Stearic acid	C18:0	$C_{18}H_{36}O_2$	0.79 ± 0.20	2.79 ± 1.37
Petroselinic acid	C18:1 Δ6	$C_{18}H_{34}O_2$	74.26 ± 0.33	-
Oleic acid	C18:1 Δ9	$C_{18}H_{34}O_2$	7.37 ± 0.55	20.74 ± 0.12
Linoleic acid	C18:2	$C_{18}H_{32}O_2$	0.65 ± 0.07	53.05 ± 0.05
Linolenic acid	C18:3	$C_{18}H_{32}O_2$	-	2.01 ± 0.04
Eicosanoic acid	C20:0	$C_{20}H_{40}O_2$	-	1.11 ± 0.16
Eicosenoic acid	C20:1	$C_{20}H_{38}O_2$	0.59 ± 0.08	0.83 ± 0.13
Docosanoic acid	C22:0	$C_{22}H_{44}O_2$	0.40 ± 0.01	-
Docosenoic acid	C22:1	$C_{22}H_{42}O_2$	-	0.94 ± 0.03
Unidentified acids			1.39 ± 0.15	1.80 ± 0.23
∑TSFA[a]			2.12 ± 0.36	13.04 ± 1.38
∑TUFA[b]			82.28 ± 0.26	77.57 ± 0.04

∑TSFA[a]: total unsaturated fatty acids; ∑TUFA[b]: total saturated fatty acids.
Values are arranged as mean ± S.D. (n=3).

unsaturation and could be used to determine the oxidative stability of oils. The iodine value of *A. glauca* is lower at 73.80; while as *C. album* was found to be 118.04 higher than that of its earlier study (Ahmad *et al*., 1986). This may be mainly due to presence of a high percentage of polyunsaturated acids (linoleic acid) Table 2.

3.2. Functional group analysis by FTIR

The FTIR spectrum is one of the most important and powerful tools for the determination of functional groups in various plant extracts. This technique works on the basis of functional groups and gives information in the form of peak values. In this work the ATR-FTIR analysis was used to observe mainly the ester peak in the FAMEs of *A. glauca* and *C. album* as compared to its respective petroleum ether (PE) seed extracts from which they are synthesized. As displayed in Figure 1, the FAME of *A. glauca* showed a sharp, strong transmittance band at 1742 cm^{-1} as compared to the PE extract. Similarly, the FAME of *C. album* displayed a sharp peak at 1741 cm^{-1} as compared to its respective PE extract. In general, apart from TLC, the FTIR analysis gives valuable information regarding the formation of FAMEs from PE extracts, which is compulsory for GC-MS analysis.

3.3. Fatty acid composition

The fatty acid composition is a good indicator of the quality and stability of the oil. From the GC graphs as given in (Figure 2) and mass spectra as given in (Figure 3), the total numbers of fatty acids identified for *A. glauca* and *C. album* were 8 and 10, respectively. The analyzed (PE) plant seed extracts were rich in unsaturated fatty acids (TUFA) with 82.28% for *A. glauca* and 77.57% for *C. album*. Petroselinic acid at 74.26% was found to be the dominant monounsaturated acid in *A. glauca* compared to *Petroselinium crispum* (Ngo-Duy *et al*., 2009), but higher than other species in the Apiaceae family (Knothe and Steidley, 2019). The petroselinic acid composition found in *A. glauca* is similar to that of *Coriandrum sativum,* which is the valuable raw material for the pharmaceutical and food

industries (Delbeke *et al*., 2016); while 7.37% oleic acid was found, which is comparatively similar to other species in its family (Knothe and Steidley, 2019). 0.61% palmitic acid and 0.40% docosanoic acid were found. Linoleic acid was found to be dominant in the seed oil of *C. album* at 53.05% and oleic acid was found at 20.74%, which is similar to the Ayani variety of *C. album* from Mantaro valley (Peru) (Peiretti and Tassone, 2013). It also contained 2.01% linolenic acid, 6.32% palmitic acid, 1.67% decanoic acid, 1.11% eicosanoic acid and 0.94% docosenoic acid. Other fatty acids were found in smaller amounts, such as myristic acid (0.32 and 1.15%), stearic acid (0.79 and 2.79%), and eicosenoic acid (0.59 and 0.83%) for *A. glauca* and *C. album,* respectively.

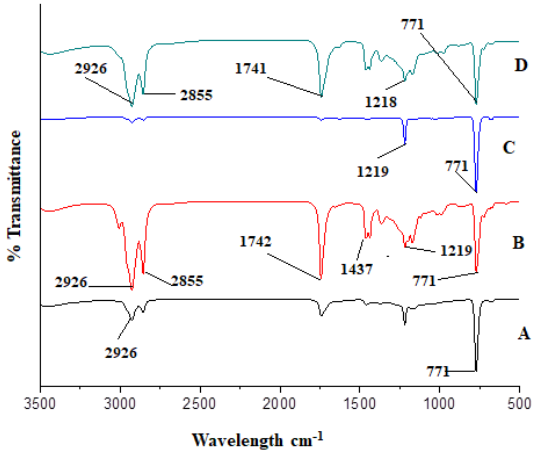

FIGURE 1. FTIR analyses of A: petroleum ether extract (PET) of *A. glauca*; B: FAME of *A. glauca*; C: petroleum ether extract (PET) of *C. album*; D: FAME of *C. album*.

3.4. Antioxidant activity

The antioxidant activities of the de-fatted seed extracts of *A. glauca* and *C. album* were determined using the DPPH radical scavenging, hydrogen peroxide, superoxide anion radical and NBT radical assay. The radical scavenging activity assay provides information about the antiradical activity of the extracts. The seed extracts were compared with standard antioxidant drug butylated hydroxyltoluene (BHT).

3.4.1. DPPH radical scavenging activity

DPPH radical scavenging assay is based on the fact that when the antioxidants present in de-fatted seed extracts react with DPPH radicals, they convert them into the yellow-colored compound di-phenyl hydrazine. The lower the absorbance of the reaction mixture, the higher is the free radical scavenging activity. As shown in Table 3, all the de-fatted seed extracts showed the good results for DPPH radical activity but the most enhanced effect was shown at 100 µg/mL and 200 µg/mL concentrations of extracts. The EA extracts of *A. glauca* at 25 µg/mL and 50µg/mL showed better results for radical scavenging compared to other extracts of the same plant ($p < 0.05$) at the same concentration with reference to BHT. All the extracts of *A. glauca* showed comparatively similar radical scavenging at 100 µg/mL and 200 µg/mL. However, at 100 µg/mL AT showed a dominant effect (81.26%) followed by EA (80.15%) and CF (78.81%); while MT showed the least inhibition (77.29%) compared to BHT (88.31%). At 200 µg/mL EA (92.73%) showed a dominant effect followed by AT (90.30%), MT (89.73%) and CF (85.84) with BHT (95.60%), which is similar to the inhibition shown by the essential oil of whole *A. glauca* plant in an earlier study with respect to BHT (Irshad *et al*., 2011). All the MT extracts of *C. album* at all concentrations showed good results compared to other extracts of the same plant. However, a dominant effect was shown by MT (79.49%), followed by AT (77.01%); while EA (65.26%) showed the least inhibition with BHT (88.31) at 100 µg/mL. At 200 µg/mL, the MT extract showed (87.73%) inhibition of radicals better than earlier reports of the whole plant (Lone *at al*., 2017), followed by EA (86.41%) and AT (84.55%) and CF (80.44%) showed the least inhibition compared to standard BHT (95.60%) at ($p < 0.05$) significance.

3.4.2. NBT radical scavenging assay

Superoxide scavenging activity was determined by the NBT assay and is depicted in Table 4. In general, the de-fatted seed extracts of *A. glauca* showed slightly better NBT radical scavenging compared to *C. album*. At 25µg/mL and 100 µg/mL the MT and EA extracts of *A. glauca* showed dominant effects followed by CF; while the least effect was shown by AT ($p < 0.05$) with respect to BHT. At concentrations of 50 µg/mL and 200 µg/mL MT and CF extracts showed dominant NBT scavenging activity followed by EA and AT. Earlier studies (Noh *et al*., 2014) found that the SOD activity of *A. gigas* (apiaceae) was 34%, which is lower than the one found in

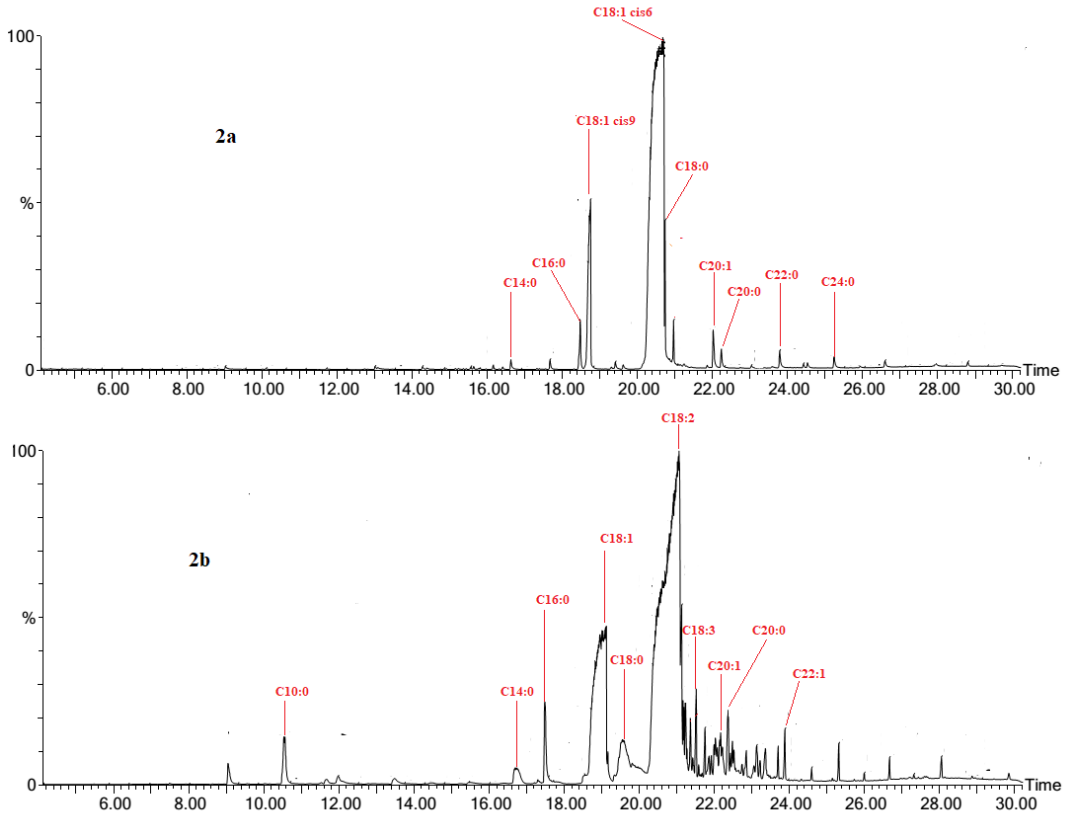

FIGURE 2. GC-MS chromatogram of the fatty acid methyl esters isolated from 2a:*A. glauca*; 2b:*C. album* seeds.

this study. This may be due to the difference in extraction method, different plant material used and contents of extracts taken. In the case of *C. album* at 25 µg/mL, MT and AT predominated; while at 50 µg/mL EA showed a dominant effect followed by MT and CF and the least inhibition of radicals was shown by AT extract compared to positive standard BHT. At 100 µg/mL, MT showed a dominant inhibition of radicals followed by EA; while the least effect was shown by AT and CF. However, at 200 µg/mL, EA and MT showed dominant scavenging followed by the CF and AT extracts of *C. album* ($p < 0.05$) with respect to BHT (Table 4). These results are comparatively better than those shown by *Chenopodium murale* extracts at similar concentrations (Khan *et al*., 2019).

3.4.3. H_2O_2 radical scavenging assay

The results for H_2O_2 radical scavenging activity of extracts is shown in Table 5. At 25 µg/mL and 50 µg/mL, the dominant radical scavenging was shown by the MT (31.05% and 55.79%) and AT (28.80 and 54.97%) seed extracts of *A. glauca* at ($p < 0.05$), respectively, with respect to BHT. AT displayed dominant (78.96 and 87.63%) inhibition followed by MT (75.49 and 83.12%) with BHT, which showed (79.06 and 92.44%) at 100 µg/mL and 200 µg/mL, respectively ($p < 0.05$), which is comparably similar to the earlier results shown by the Methanolic plant extract of *Withania somnifera* 84.49% and better than *Petroselinium crispum* 60.27% (Tupe *et al*., 2013). The CF extracts of *A. glauca* showed better inhibition and the least inhibition was observed in EA at 100 µg/mL and 200 µg/mL ($p < 0.05$) with respect to BHT. In *C. album* at 25 µg/mL and 50 µg/mL concentrations of extracts radical scavenging was shown in the following increasing order of magnitude: MT > EA > AT > CF. At 100 µg/mL and 200 µg/mL MT was shown to be dominant (72.42 and 87.67%) with BHT (79.06 and 92.44%) inhibition followed by CF and EA and AT showed the least inhibition ($p < 0.05$). Lone *et al.,* (2017) reported that the Methanol extract of *C. album* showed the highest H_2O_2 radical scavenging inhibition of 94% at 300 µg/mL with respect to BHT.

3.4.4. ABTS radical scavenging activity

Radical scavenging activity (ABTS) is shown in (Table 6). At 25 µg/mL and 100 µg/mL EA and AT extracts of *A. glauca* showed powerful radical inhibition effect while as CF and MT extracts of same plant showed normal inhibition ($p < 0.05$) with respect to BHT. At 50 µg/mL EA and MT extracts of *A. glauca* showed dominant effect followed by AT while CF shows least inhibition. At 200 µg/mL almost all seed extracts of *A. glauca* showed comparatively same results with the following increasing order of radical scavenging EA (91.24%) > MT (90.41) > AT (89.72) > CF (80.19) with BHT showed (93.12) ($p < 0.05$). Earlier reports on ABTS radical scavenging have found that the plant extracts of *Terminalia chebula*, *Salacia reticulate*, *Hemidesmus indicus*, *Aegle marmelos* and *Terminalia arjun* showed 100, 99.95, 99.43, 98.91 and 98.91 % inhibition respectively (Tupe *et al.*, 2013). This increase in % inhibition is mainly due to high concentration of plant extracts taken.

For *C. album* at 25 µg/mL and 200 µg/mL all seed extracts show good ABTS scavenging activity with the increasing order of their radical scavenging CF > EA > MT > AT ($p < 0.05$). At 50 µg/mL the radical scavenging results are almost similar with CF (61.64%), MT (60.17%), EA (59.99%) and AT (57.90%) with BHT (71.46%) and at 100 µg/mLEA, MT and CF showed dominant effect while AT showed least inhibition (Table 6). Adedapo *et al.,* (2011) in earlier studies on this plant found that the acetone leaf extract possesses 52-53% and 66% inhibition of ABTS radicals at 50 µg/mL and 100 µg/mL respectively, while they found methanol extracts have relatively better inhibition capacity.

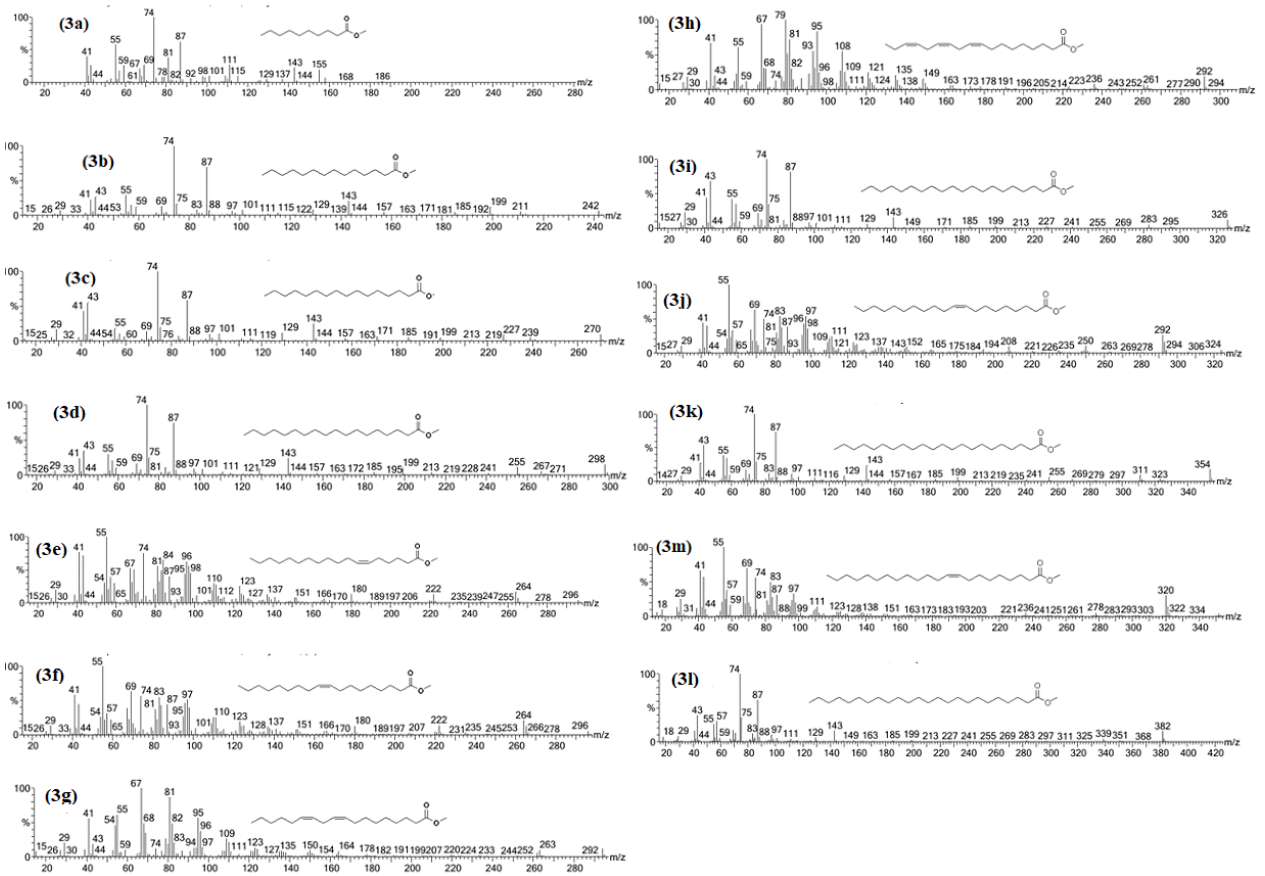

FIGURE 3. GC-MS spectra of 3a: Methyl decanoate; 3b: Methyl tetradecanoate; 3c: Methyl palmitate; 3d: Methyl stearate; 3e: Methyl petroselinate; 3f: Methyl oleate; 3g: Methyl linoleate; 3h: Methyl linolenate; 3i: Methyl eicosanoate; 3j: Methyl 9-eicosenoate; 3k: Methyl docosanoate; 3m: Methyl docosenoate, 3l: Methyl tetracosanoate.

TABLE 3. 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of defatted seed extracts of *A. glauca* and *C. album*.

Seed extracts	*A. glauca* Concentration (µg/mL)				*C. album* Concentration (µg/mL)			
	25	**50**	**100**	**200**	**25**	**50**	**100**	**200**
Chloroform	30.12 ± 0.02^{e}	61.62 ± 0.04^{e}	78.81 ± 0.12^{d}	85.84 ± 0.04^{e}	21.60 ± 0.22^{e}	44.21 ± 0.07^{e}	69.92 ± 0.09^{d}	80.44 ± 0.06^{e}
Ethyl acetate	36.58 ± 0.03^{b}	68.03 ± 0.07^{b}	80.15 ± 0.02^{c}	92.73 ± 0.07^{b}	28.55 ± 0.14^{c}	49.02 ± 0.16^{c}	65.26 ± 0.12^{c}	86.41 ± 0.08^{c}
Acetone	33.52 ± 0.01^{c}	64.23 ± 0.09^{d}	81.26 ± 0.16^{b}	90.30 ± 0.07^{c}	24.63 ± 0.17^{d}	47.13 ± 0.26^{d}	77.01 ± 0.09^{c}	84.55 ± 0.12^{d}
Methanol	31.55 ± 0.07^{d}	66.08 ± 0.17^{c}	77.29 ± 0.03^{e}	89.73 ± 0.07^{d}	30.69 ± 0.16^{b}	63.01 ± 0.10^{b}	79.49 ± 0.04^{b}	87.73 ± 0.09^{b}
BHT	39.33 ± 0.03^{a}	74.80 ± 0.02^{a}	88.31 ± 0.05^{a}	95.60 ± 0.06^{a}	39.33 ± 0.03^{a}	74.80 ± 0.02^{a}	88.31 ± 0.05^{a}	95.60 ± 0.06^{a}

Data are expressed as means ± S.D (n=3).
The different letters in each column are statically significant different at (p< 0.05) by one way ANOVA and Duncan's test.BHT: Butylated hydroxytoluene.

TABLE 4. Nitroblue tetrazolium (NBT) radical scavenging activity of defatted seed extracts of *A. glauca* and *C. album*.

Seed extracts	*A. glauca* Concentration (µg/mL)				*C. album* Concentration (µg/mL)			
	25	**50**	**100**	**200**	**25**	**50**	**100**	**200**
Chloroform	22.70 ± 0.03^{d}	37.15 ± 0.04^{c}	51.31 ± 0.03^{d}	64.37 ± 0.03^{c}	16.67 ± 0.01^{d}	31.20 ± 0.05^{d}	43.76 ± 0.07^{c}	64.04 ± 0.03^{d}
Ethyl acetate	24.63 ± 0.03^{b}	36.49 ± 0.07^{d}	52.79 ± 0.02^{c}	62.64 ± 0.08^{d}	14.04 ± 0.13^{e}	34.81 ± 0.20^{b}	50.94 ± 0.15^{c}	68.29 ± 0.01^{b}
Acetone	21.96 ± 0.20^{e}	34.69 ± 0.16^{e}	46.14 ± 0.05^{e}	60.14 ± 0.04^{e}	19.79 ± 0.03^{c}	28.53 ± 0.02^{e}	45.24 ± 0.02^{d}	60.35 ± 0.04^{e}
Methanol	24.18 ± 0.02^{c}	38.67 ± 0.03^{b}	54.27 ± 0.01^{b}	68.64 ± 0.01^{b}	20.57 ± 0.09^{b}	32.27 ± 0.25^{c}	53.12 ± 0.02^{b}	66.79 ± 0.01^{c}
BHT	30.96 ± 0.02^{a}	51.18 ± 0.08^{a}	73.39 ± 0.08^{a}	82.21 ± 0.05^{a}	30.96 ± 0.02^{a}	51.18 ± 0.08^{a}	73.39 ± 0.08^{a}	82.21 ± 0.05^{a}

Data are expressed as means ± S.D (n=3).
The different letters in each column are statically significant different at (p< 0.05) by one way ANOVA and Duncan's test.
BHT: Butylated hydroxytoluene.

TABLE 5. Hydrogen peroxide (H_2O_2) radical scavenging activity of defatted seed extracts of *A. glauca* and *C. album*.

Seed extracts	*A. glauca* Concentration (µg/mL)				*C. album* Concentration (µg/mL)			
	25	**50**	**100**	**200**	**25**	**50**	**100**	**200**
Chloroform	20.16 ± 0.09^{e}	39.99 ± 0.09^{d}	69.37 ± 0.06^{d}	82.10 ± 0.01^{d}	14.86 ± 0.10^{e}	36.32 ± 0.11^{e}	65.18 ± 0.07^{c}	85.57 ± 0.01^{c}
Ethyl acetate	24.77 ± 0.03^{d}	38.38 ± 0.34^{e}	62.27 ± 0.08^{e}	76.08 ± 0.05^{e}	22.48 ± 0.09^{c}	48.99 ± 0.10^{c}	64.95 ± 0.10^{cd}	78.86 ± 0.06^{d}
Acetone	28.80 ± 0.11^{c}	54.97 ± 0.05^{c}	78.96 ± 0.04^{ab}	87.63 ± 0.01^{b}	20.94 ± 0.06^{d}	41.39 ± 0.05^{d}	61.81 ± 0.06^{c}	75.85 ± 0.04^{e}
Methanol	31.05 ± 0.09^{b}	55.79 ± 0.08^{b}	75.49 ± 0.07^{c}	83.12 ± 0.09^{c}	28.08 ± 0.08^{b}	58.12 ± 0.09^{b}	72.42 ± 0.05^{b}	87.67 ± 0.04^{b}
BHT	35.18 ± 0.10^{a}	68.68 ± 0.07^{a}	79.06 ± 0.06^{a}	92.44 ± 0.01^{a}	35.18 ± 0.10^{a}	68.68 ± 0.07^{a}	79.06 ± 0.06^{a}	92.44 ± 0.01^{a}

Data are expressed as means ± S.D (n=3).
The different letters in each column are statically significant different at (p< 0.05) by one way ANOVA and Duncan's test.
BHT: Butylated hydroxytoluene.

TABLE 6. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity of defatted seed extracts of *A. glauca* and *C. album*.

Seed extracts	*A. glauca* Concentration (µg/mL)				*C. album* Concentration (µg/mL)			
	25	50	100	200	25	50	100	200
Chloroform	28.55 ± 0.03^{d}	53.58 ± 0.05^{e}	73.38 ± 0.09^{e}	80.19 ± 0.01^{e}	32.15 ± 0.03^{b}	61.64 ± 0.05^{b}	76.71 ± 0.07^{d}	88.22 ± 0.03^{b}
Ethyl acetate	34.73 ± 0.10^{b}	59.42 ± 0.43^{b}	78.03 ± 0.02^{b}	91.24 ± 0.10^{b}	31.79 ± 0.01^{c}	59.99 ± 0.02^{cd}	78.51 ± 0.01^{b}	87.17 ± 0.04^{c}
Acetone	32.84 ± 0.06^{c}	56.48 ± 0.10^{d}	77.04 ± 0.05^{c}	89.72 ± 0.03^{d}	29.06 ± 0.06^{e}	57.90 ± 0.46^{e}	72.19 ± 0.07^{e}	84.77 ± 0.04^{e}
Methanol	27.83 ± 0.06^{e}	58.73 ± 0.12^{c}	73.62 ± 0.08^{d}	90.41 ± 0.10^{c}	30.53 ± 0.06^{d}	60.17 ± 0.14^{c}	77.28 ± 0.06^{c}	85.70 ± 0.11^{d}
BHT	44.24 ± 0.15^{a}	71.46 ± 0.02^{a}	83.57 ± 0.09^{a}	93.12 ± 0.01^{a}	44.24 ± 0.15^{a}	71.46 ± 0.02^{a}	83.57 ± 0.09^{a}	93.12 ± 0.01^{a}

Data are expressed as means ± S.D (n=3).

The different letters in each column are statically significant different at ($p< 0.05$) by one way ANOVA and Duncan's test.

BHT: Butylated hydroxytoluene.

4. CONCLUSIONS

The present study indicates that *A. glauca* and *C. album*are potent medicinal plants with their seeds being rich in unsaturated fatty acids especially petroselinic acid (an uncommon positional isomer of oleic acid) and linoleic acid respectively. They also contain significant oleic acid. Moreover, due to their powerful DPPH, NBT, H_2O_2 and ABTS radical scavenging activities which suggest that the seed extracts of worked plants could be used as non-exploited, natural source in future food and pharmaceutical industries. However, more work is needed to isolate target product. Also, green extraction methods, cost effective analysis and safety measurements should be taken in concentration to determine the commercial potential of these plants. Anyhow this encouraging work adds more knowledge to existing literature of these plants from Kashmir.

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