Research Article

Fatty acid composition, antibacterial and antioxidant potential of *Atropa belladonna* and *Thymus linearis* seeds grown in Kashmir

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Abstract

In this study fatty acid composition of petroleum ether (AP and TP) and biological potential (Antioxidant and antibacterial) of chloroform (AC and TC), methanol (AM and TM) extracts of *Atropa belladonna* L. and *Thymus linearis* Benth. respectively was obtained by Soxhlet extraction technique from seeds were investigated. Fatty acid profile was obtained by gas chromatography mass spectrometry, antioxidant potential (DPPH-2,2-di-phenyl-1-picrylhydrazyl; ABTS-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and antibacterial activity against (*Pseudomonas aeruginosa*, *Serratia marcescens*, *Escherichia coli* and *Klebsiella pneumonia*) was also performed. Linoleic acid was dominantly found with 74.42% in AP and 84.39% in TP. In antioxidant assays, the dominant inhibition was shown by AM (84.98% and 83.90%) and TM (85.27% and 83.29%) as compared to BHT (93.72% and 90.87%) for DPPH and ABTS radical scavenging respectively at 200 µg/mL. Moreover, AM and TM extracts showed good antibacterial activity. In conclusion, these plants could be selected as an adequate species in agricultural system, in food and pharmaceutical industries.

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Graphic abstract

Keywords Soxhlet extraction · Fatty acid composition · Linoleic acid · Antioxidant activity · Antibacterial activity

1 Introduction

Antioxidants are very useful by preventing or delaying the formation of free radicals and lipid peroxidation in animals and human bodies, two well-known causes of aging in humans [1]. It also protects us from diseases related to heart, lungs, muscle and brain [2]. The use of antioxidants is to strength the body and prevent from various diseases. The approach towards natural synthesis is very much because of harmful side effects of overuse of synthetic antioxidants [3]. The great abundance and overuse of synthetic antimicrobial drugs leads to serious implications and formation of microbes resistant to these drugs [4]. The vast demand of new antimicrobial drugs, leads to search of new drugs from plant sources because of their sustainability, ecofriendly, inexpensive nature [5]. Fatty acids (FAs) are very important constituents in variety of foods, such as in salads, cooking oils, bakery, confectionary, ice-creams, emulsions and many specially tailored products [6]. Fats are medium for transferring of heat to various foods [7]. Besides their vast use in foods, FAs have got wide application in industries such as in soap and detergents, ink, varnish, lubricants,

paints etc. Thus oilseed crops have got both nutritional and industrial importance in ever expanding market. *Atropa belladonna* L., belonging to *Solanaceae* family locally known as “Sangagur” is an important medicinal plant from Kashmir. Its name come from Greek “Atropos” meaning three fates that cuts the life and “Belladonna” means beautiful women in Italian [8]. It is used in facial ornaments and to increase pupil size in women [9]. It possesses atropine which is used to inhibit multiplication of virus [10]. The plant contains various alkaloids such as atropine, hyoscyamine and scopolamine due to this it acts as anti-cholinergic [11]. *Thymus linearis* Benth. Locally known as “Jawind” belongs to “Lamiaceae” family has got wide distribution in Kashmir. The plant acts as antiviral [12], anti-cancer activities [13].

In order to continue the screening of novel seed oils in our laboratory [14], seeds of *A. belladonna* and *T. linearis* were taken for fatty acid composition. Moreover, the chloroform and methanol extracts of *A. belladonna* and *T. linearis* seeds were screened for radical scavenging assay and antibacterial activity against four multi-drug resistant bacteria, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Escherichia coli*, *Klebsiella pneumoniae* were done.

2 Materials and methods

2.1 Seed sources and chemicals

Seeds of *A. belladonna* and *T. linearis* were collected from seed nurseries of Ganderbal (Kashmir) in 2020. The plants were authenticated by Dr. Sajad Gangoo. All the chemicals and reagents were purchased from Sigma-Aldrich, USA. Standard strains of *Pseudomonas aeruginosa* ATCC 27,853, *Serratia marcescens* MTCC 97, *Escherichia coli* ATCC 25,922, *Klebsiella pneumonia* ATCC 13,883 were collected from Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh. Plant extracts were reconstituted in 0.5% v/v DMSO for biological analysis.

2.2 Successive Soxhlet extraction

The dried seeds of each plant were ground into powdered form by using mechanical grinder. The coarsely powdered seeds 51 g of *A. belladonna* and 15 g of *T. linearis* were mixed with 180 mL of solvent and put in a Soxhlet apparatus. Extractions were successively performed by using the solvent petroleum ether (40–60 °C), chloroform and methanol to obtain respective (AP and TP), chloroform (AC and TC), and methanol (AM and TM) extracts *A. belladonna* and *T. linearis* respectively. The extraction time ranges from 3–4 h. The extracts were through anhydrous sodium sulphate (Na_2SO_4) to remove any moisture left. All seed extracts were kept at 4 °C until further analyses.

2.3 Iodine value (I.V)

1 g of oil was taken in a conical flask. To it 10 mL of chloroform was added and 30 mL of Hanus solution (Hanus solution is prepared by dissolving 18 g of iodine in 1 L of glacial acetic acid and then 3 mL of bromine water was added to increase halogen concentration). The solution was shaken and kept in dark for about an hour. To this solution 10 mL of potassium iodide (15%) and 100 mL of distill water was added. This solution was titrated against sodium thiosulphate till yellow color formed, then 3–4 drops of freshly prepared starch solution was added. End point was taken blue color changes to colorless upon continuous titration. A blank titration was carried out simultaneously.

$$\text{I.V} = \frac{(B - S) \times 0.1 \times 0.127 \times 100}{\text{wt. of sample}}$$

where B and S are titre values of blank and samples respectively.

2.4 Saponification value (S.V)

Weighed about 2 g of fat or oil was saponified with 0.5 N alcoholic potassium hydroxide in round bottom flask, attached with reflux condenser in water bath for about 1 h. with occasional shaking. When the solution is still hot 3 drops of phenolphthalein indicator was added. Finally this solution was titrated with 0.5 N HCl. The similar procedure was followed for blank. The S.V was calculated by using the formula:

$$\text{S.V} = \frac{56.1 (B - S) \times N \text{ HCl}}{\text{wt. of sample}}$$

where B and S are titre values of blank and samples respectively.

2.5 Fatty acid methyl esters (FAME) preparation

In first step of the reaction, one gram oil of AP and TP was saponified separately, while using alcoholic potassium hydroxide (0.5 N). About 1/3rd part of the solvent is evaporated and diethyl ether was used to extract unsaponifiable part. The aqueous layer was acidified with 6 N hydrochloric acid to obtain mixed fatty acids (MFA). In second step MFA was treated with excess amount of absolute methanol in presence of sulphuric acid (H_2SO_4) as catalyst. On completion of reaction, the resulting mixture was diluted with ice chilled water up to cloud point formation. Finally the resulting mixture was extracted with continuously ether to obtain fatty acid methyl esters (FAMES). The FTIR of respective FAMES is given in (Fig S1, Supplementary file). The purification and characterization of FAMES and extracts was done by Column chromatography, Fourier transform infrared spectroscopy (FTIR) and Gas chromatography mass spectrometry analysis (GC–MS) respectively fully described in (Supplementary file).

2.6 Antioxidant activity determination

2.6.1 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of (AC, AM) and (TC, TM) extracts of *A. belladonna* and *T. linearis* respectively was obtained by using the method described by Shimada et al. (1992) with little modifications [15]. The concentration of extracts (25–200) µg/mL was added with 3.8 mL DPPH solution in 50% ethanol, and were incubated in the dark at room temperature for 1 h. Decoloriaation of DPPH donated H^+ was followed by measuring absorbance of the mixture at 517 nm. The radical scavenging of each extract was determined by comparing its absorbance with that of a blank solution (no

sample). Butylated hydroxytoluene (BHT) was used as positive control. The capacity to scavenge the DPPH radical was calculated using the following equation:

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

where A blank is absorbance of DPPH radical (blank), and A extract is the absorbance of DPPH radical with the different extract samples of various concentrations.

2.6.2 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity

The ABTS assay was used to determine radical scavenging capacity by following the procedure of Re et al. (1999) [16]. The ABTS⁺ were obtained by the reaction between 5 mL of 14 mM ABTS solution with 5 mL of 4.9 mM potassium persulphate (K₂S₂O₈) solution, stored in the dark at room temperature for 16 h. The seed extracts at various concentrations (25–200 µg/mL) with 1 mL of ABTS⁺ solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. The reaction mixture of standard group was obtained by mixing same ABTS⁺ solution and in place of extracts the different concentrations of BHT were taken. The ABTS radical inhibition (%) was calculated using similar equation as given in the DPPH assay. All the results were compared with BHT.

2.7 Antimicrobial activity of extracts

Agar well diffusion method was employed for screening the antimicrobial activity of extracts against the test microorganisms [17]. The tested bacterial strains were sub-cultured for 18 h; working bacterial samples were prepared and adjusted to be equivalent to 0.5 McFarland (Approximately 1–2 × 10⁸ CFU/mL). 100 µL from the bacterial culture was loaded on the Muller-Hinton agar plates and swapped with a sterile cotton swab. Wells were bored into the agar medium. One hundred micro-liters of the extracts were dispensed in each well in the plate. The seeded plate was incubated at 37° C for 24 h, the test was repeated twice, and the mean inhibition zone and standard error of means were calculated.

2.8 Determination of minimum inhibitory concentration (MIC)

MIC was performed for only those extract which showed a zone of inhibition against bacteria. To determination of MIC of extracts, broth micro-dilution method was performed with slight modifications. 96-well ELISA plate was

used and two-fold serial dilution of plant extract was prepared containing Muller-Hinton broth to obtain various concentrations. Bacteria were grown in various concentra-

tions of plant extract and incubated at 37 ± 2 °C for 24 h. After incubation, 20 micro-liter of Triphenyltetrazolium chloride (TTC, HiMedia, India) (3 mg/mL) per well was added and leave for 20 min at room temperature, and growth was indicated by pink color change and read visually. The MIC was determined as the minimum concentration at which no color change was visible i.e., no metabolically active cell [18].

2.9 Statistical analysis

The results were evaluated by using analysis of variance (ANOVA) and statistical software (IBM SPSS Statistics 20). Tukey's multiple range tests were performed to determine the differences between groups at (P < 0.05) significance.

3 Results and discussions

3.1 Physiochemical analysis of seed extracts

The % yields of various extracts as extracted through Soxhlet extraction is given in (Table 1). The yield of *T. linearis* extracts were found to be was more as compared *A. belladonna*. The highest yield was observed by chloroform extract of *T. linearis* 83.23% and *A. belladonna* 39.29% followed by methanol extract (75.34% and 37.23%) for *T. linearis* and *A. belladonna* respectively (P < 0.05). The extraction through petroleum ether showed normal yields of seed extracts. Saponification value (SV) is a measure of average chain length of all the fatty acids in triglycerides. Higher the SV lower the fatty acid average chain length which means lighter mean molecular weight of triglyceride [19]. Moreover, it is also used to determine the amount of free fatty acid present which is very important in industrial consumer. As shown in (Table 1), the S.V of PE extract of *T. linearis* is comparably more with 164.39 as compared to *A. belladonna* 147.23. Iodine value (IV) determines the degree of unsaturation, hence could be used to determine the oxidative stability of oils. Higher is IV higher is the degree of unsaturation and vice versa. As depicted in Table 1, IV of *T. linearis* was 153.49 while as *A. belladonna* was found to have 135.42. This difference may be mainly

Table 1 Saponification and iodine values of petroleum ether extracts of *A. belladonna* and *T. linearis* and percentage yield (% w/w) of other seed extracts

Name of plant	Seed extracts			Saponification value	Iodine value
	PE	CF	MT		
<i>A. belladonna</i>	10.88 ± 1.41 ^c	39.29 ± 0.71 ^a	37.23 ± 1.32 ^b	147.23 ± 1.91	135.42 ± 1.44
<i>T. linearis</i>	9.71 ± 1.21 ^c	83.23 ± 1.44 ^a	75.34 ± 0.48 ^b	164.91 ± 1.60	153.49 ± 1.18

PE petroleum ether extract, CF chloroform extract, MT methanol extract. Data is expressed as mean ± SD (n = 3). Different letters in each row are significantly different (P < 0.05) by Tukey's test

due to high content of polyunsaturated fatty acids in *T. linearis*.

3.2 Composition of Fatty acids in the AP and TP

The quality and stability of vegetable oil depends on fatty acid composition. Hence its determination is very important [20]. Seven and nine fatty acids were identified in *A. belladonna* and *T. linearis* respectively. The (Fig S2 Supplementary file) shows chromatograms of the identified fatty acids with their mass spectra in (Fig S3, Supplementary file) and Table 2 shows their composition. The unsaturated fatty acids (UFAs) were found dominantly with 77.51% in *A. belladonna* and 86.91% in *T. linearis*. The total saturated fatty acids (TSFAs) percentage was in the range of 17.60% and 8.78% in *A. belladonna* and *T. linearis* respectively. Linoleic acid was the most abundant UFA in both seed oils with 77.42% and 84.39% in *A. belladonna* and *T. linearis* respectively. The high percentage of linoleic acid in *A. belladonna* is comparably similar with other seed oils of solanaceae family [21]. Similarly the high composition of linoleic in *T. linearis* is almost similar with other plants in Lamiaceae such as that of *Hyptis suaveolens* which contain linoleic acid in concentration of 85.8% [22]. The linoleic acid comprises of about 20% fatty acids in human diet. Moreover, linoleic acid helps to protect from various cardiovascular

diseases in humans [23]. Amongst SFAs, Palmitic acid was found in moderate amount with 15.40% in *A. belladonna* and 7.99% in *T. linearis*.

3.3 Antioxidant activity

The antioxidant assay revealed the presence of antioxidant potential of AC, AM, TC and TM extracts. The percentage of inhibition was detected in all the antioxidant models that free radicals were scavenged by the seed extracts which depends on particular concentration of extracts.

3.4 DPPH assay

Figure 1A showed at 100 µg/mL, the methanol extracts viz., AM and TM showed dominant inhibition of free radicals with 78.22% and 77.22% respectively with respect to BHT 82.83%. The chloroform extracts TC showed 76.98% inhibition while as AC displayed 75.26% inhibition. However, dominant scavenging was observed at 200 µg/mL concentration of seed extracts. The TMT showed 85.27% inhibition followed by AM 84.98%, TC 83.78% and AC 82.01% with respect to BHT which showed 93.72 (P < 0.05). These results are comparatively similar with DPPH activity

Table 2 Fatty acid composition of petroleum ether (PE) extracts of *A. belladonna* and *T. linearis*

Common and systematic names	Carbon numbers	Chemical formula	Area (%)	
			<i>A. belladonna</i>	<i>T. linearis</i>
Palmitic acid	C16:0	C ₁₆ H ₃₂ O ₂	15.40 ± 0.23	7.99 ± 1.19
Palmitoleic acid	C16:1	C ₁₆ H ₃₀ O ₂	–	1.05 ± 0.41
Myristic acid	C18:0	C ₁₈ H ₃₆ O ₂	0.88 ± 0.57	0.42 ± 0.21
Oleic acid	C18:1	C ₁₈ H ₃₄ O ₂	2.36 ± 1.01	0.56 ± 1.10
Linoleic acid	C18:2	C ₁₈ H ₃₂ O ₂	74.42 ± 0.38	84.39 ± 0.98
Linolenic acid	C18:3	C ₁₈ H ₃₀ O ₂	–	0.49 ± 0.29
Eicosanoic acid	C20:0	C ₂₀ H ₄₀ O ₂	0.95 ± 0.67	0.37 ± 0.11
Eicosenoic acid	C20:1	C ₂₀ H ₃₈ O ₂	0.73 ± 1.12	0.42 ± 0.19
Docosanoic acid	C22:0	C ₂₂ H ₄₄ O ₂	0.37 ± 0.67	–
Total saturated acids			17.60 ± 0.76	8.78 ± 0.27
Total unsaturated acids			77.51 ± 1.16	86.91 ± 0.83

Data is expressed as mean ± SD (n = 3)

Fig. 1 **A** DPPH and **B** ABTS radical scavenging activity of chloroform (AC and TC), and methanol (AM and TM) extracts of *A. belladonna* and *T. linearis* respectively. BHT: butylated hydroxytoluene.

Different letters (a, b, c, d and e) at each concentration indicate significant difference at ($P < 0.05$) by Tukey's test

shown by some methanol plant extracts from *Solanaceae* family [24].

3.5 ABTS assay

The radical scavenging of ABTS by the extracts was found to be relatively higher than that of DPPH. There are various factors such as selectivity of the radicals, solubility of extracts etc. which may affect the quenching of different radicals [25]. It has also been found several compounds which can scavenge ABTS^{•+} more than DPPH radicals [26]. ABTS scavenging of various extracts of *A. belladonna* and *T. linearis* showed a concentration dependent rise in the scavenging of the ABTS free radicals (Fig. 1B). The maximum activity for methanol AMT and TMT at 200 µg/mL concentration with 83.90% and 83.29% scavenging as compared to BHT 90.87% ($P < 0.05$). At 100 µg/mL TMT extract showed 77.27% scavenging better than AMT 76.21 with respect to BHT 83.91%.

3.6 Antimicrobial activity

3.6.1 Determination of zone of inhibition

The antimicrobial properties of Petroleum ether (2:AP and 4:TP), Chloroform (1:AC and 5:TC) and Methanol (3:AM and 6:TM) extracts of *A. belladonna* and *T. linearis* respectively against *Pseudomonas aeruginosa*, *Serratia marcescens*, *E. coli*, *K. pneumonia* have been assessed in this study. The results revealed that the extract 1, 3, 5 and 6 efficiently suppress the growth of pathogenic bacteria. Zone of inhibition of extracts ranged from 8.87 to 20.625 mm. As displayed in Table 3 and (Fig S4, Supplementary file), in case of *P. aeruginosa* the highest inhibition was shown by Methanol extracts AM with 17.75 mm and TM 16.75 mm with respect to standard drug Gentamicin which showed 19.62 T ($P < 0.05$). Against *S. marcescens* the maximum effect was observed in AM 20.62 mm followed by TM 17.37 as compared to Gentamicin 22.37 mm. Similarly the methanol extracts showed dominant inhibition against

Table 3 Antimicrobial Activity of seed extracts

Microorganism	Zone of Inhibition (mm)						
	AC	AP	AM	TP	TC	TM	Gentamicin
<i>P. aeruginosa</i>	11.88 ± 0.53 ^e	–	17.75 ± 0.35 ^b	–	12.87 ± 0.17 ^d	16.75 ± 0.35 ^c	19.62 ± 0.88 ^a
<i>S. marcescens</i>	10.75 ± 0.70 ^e	–	20.62 ± 0.53 ^b	–	13.50 ± 0.35 ^d	17.37 ± 0.17 ^c	22.37 ± 0.53 ^a
<i>E. coli</i>	10.87 ± 0.17 ^e	–	20.5 ± 0.70 ^b	–	12.25 ± 0.35 ^d	18.75 ± 0.35 ^c	20.87 ± 0.17 ^a
<i>K. pneumoniae</i>	8.87 ± 0.17 ^e	–	16.87 ± 0.53 ^c	–	12.62 ± 0.53 ^d	19.0 ± 0.35 ^b	21.00 ± 0.35 ^a

AC and TC, Chloroform extract; AP and TP, Petroleum ether; AM and TM, Methanol extracts of *A. belladonna* and *T. linearis* respectively

Note not available. Diameter of zone of inhibition is a mean of triplicate ± SD. Values are the means ± standard deviation. Different letters in superscript in each row are significantly different at ($P < 0.05$) by Tukey's test

Table 4 Minimal Inhibitory Concentration (MIC) of the extracts against pathogenic bacteria

Microorganism	Minimal inhibitory concentration(mg/mL)				
	AC	AM	TC	TM	Gentamicin
<i>P. aeruginosa</i>	2 ^b	1 ^c	2.5 ^a	0.75 ^d	0.07 ^e
<i>S. marcescens</i>	1 ^b	0.5 ^c	1.25 ^a	0.375 ^d	0.05 ^e
<i>E. coli</i>	1 ^b	0.5 ^c	1.25 ^a	0.375 ^d	0.05 ^e
<i>K. pneumoniae</i>	2 ^a	1 ^c	2 ^a	1.5 ^b	0.08 ^d

AC and TC, chloroform extract; AM and TM, methanol extracts of *A. belladonna* and *T. linearis* respectively. Different letters in superscript in each row are significantly different at ($P < 0.05$) by Tukey's test

E. coli and *K. pneumoniae*. The petroleum extracts AP and TP showed very less effect against given microbes.

3.7 Determination of MIC

The microbial sensitivity to the different extracts represented by the mean MIC values ranged from 0.375 to 2 mg/mL (Table 4). *Escherichia coli*, and *Serratia marcescens* most sensitive species (MIC = 0.375 mg/mL) by methanol extract of *T. linearis* TM and 0.5 mg/mL by AM. The *P. aeruginosa* (MIC = 0.75 mg/mL) by TM and 1 mg/mL by AM while Gentamicin showed 0.07. The lowest MIC against *K. pneumoniae* was shown by TM 1.5 mg/mL. Lower MIC means higher effectively of extract. MIC analysis result presented in Table 4.

4 Conclusion

The results obtained from the present study indicated that high yield of extracts were obtained by Soxhlet extraction. Extracts of chloroform AC 39.29%, TC 83.23% and methanol AM 37.23%, TM 75.34% w/w were found to possess highest yield. Also the, petroleum ether extracts of *A. belladonna* and *T. linearis* seeds contained a high source of unsaturated fatty acids notably linoleic acid with 74.42% and 84.39% respectively. These results also showed that, the methanol extracts of these plants contained significant antioxidant and antibacterial activities. Based on these results, these plants could have great significance in food and pharmaceutical industries.

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Author's contribution MA Conceptualization and methodology ZRN Software, writing and supervision MOAK Antibacterial activity ASG material collection, software validation.

Availability of data and material Supplementary material available.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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