Determination of Fatty Acid Composition by GC-MS and Potential Antioxidant and Antibacterial Properties of *Rumex hastatus* and *Cytisus scoparius* Seeds Grown in Kashmir

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Abstract: *Rumex hastatus* D. Don and *Cytisus scoparius* Linn. are medicinally important plants used in Ayurveda and food, are enriched with remarkable nervine, diuretic, sedative and various other therapeutic properties. In this study Soxhlet extraction produces higher yield of seed extracts. Gas chromatography-mass spectrometry was used to determine fatty acid composition of petroleum ether extracts (PE). The major fatty acids were Linolenic acid 53.53% in *R. hastatus* and linoleic acid 59.42% in *C. scoparius*. Palmitic acid was also found in significant amount (16.58% and 19.33%) in *R. hastatus* and *C. scoparius* respectively. The chloroform (RCF, CCF), ethyl acetate (REA, CEA), acetone (RAT, CAT) and methanol (RMT, CMT) extracts of *R. hastatus* and *C. scoparius* respectively, were examined for their antioxidant activity using 2,2-di-phenyl-1-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), hydrogen peroxide (H₂O₂), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay and antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by Agar disc diffusion method. All the extracts showed good radical scavenging and antibacterial activities compared to butylated hydroxytoluene and streptomycin respectively. However, dominant radical scavenging effect was observed at 100 µg/mL and 200 µg/mL concentrations of extracts and dominant zone of inhibition was shown by CAT, CMT, RAT and REA extracts. This study exhibited beneficial properties of these plants as an ingredient (unsaturated fatty acids) for the formulation of various functional foods and a source of natural antioxidant and antibacterial agents for drug, design and development.

Key words: Soxhlet extraction, Linoleic acid, Linolenic acid, Antibacterial activity, Antioxidant activity.
1. Introduction

The seed oils of plants contain lipids which are nutritionally important sources of food [1]. They can provide oils with high concentration of monounsaturated fatty acids such as oleic acid which protects from various cardiovascular diseases [2]. The reactive oxygen (RO) and reactive nitrogen (RN) are highly reactive species produced by various metabolic processes and by external factors such as pollution, radiation or some poor diet habits. Their high concentration in absence of endogenous oxidant defenses can cause various oxidative stress or related problems that may lead to chronic and degenerative diseases such as cancer, heart disease and Alzheimer’s disease [3]. Therefore antioxidants play a big role in order to scavenge or repair these harmful free radicals [4, 5]. However some studies found that the use of synthetic antioxidants is harmful to human health [6]. Consequently there is search of alternative antioxidants from plants or plant products [7, 8]. Rumex hastatus D. Don is one of the important members of polygonaceae family which includes about 200 species distributed around the world. It has got wide distribution in Kashmir. Traditionally its leaves are used as food (sour soups), sauces and salads. Medicinally it is used to treat cough, headache and fever [9]. The juice of R. hastatus is used as flavoring agent, carminative and diuretic [10] and is used to treat blood pressure [11], tonsillitis and sore throat [12]. The methanol extracts of this plant possesses antioxidant and anticholinesterase activities [13].

Cytisus scoparius Linn. is predominantly found in Kashmir region especially afforested bank of Manasbal lake, Kashmir-India [14]. Traditionally it is used as diuretic [15], sedative and hypnotic [16], antidiabetic [17]. Pharmacologically it possesses antiviral, antitumor, cytotoxic [18], antifungal [19] and insecticidal [20] activities. It also shows diuretic, hypotension, antispasmodic and respiration effects [21]. Depending upon various ethanopharmacological uses, great abundance with less input and care cost, these plants were selected for this current study. Therefore to continue the work on seed oils [22, 23], we processed our work on C. scoparius and R. hastatus for fatty acid composition, further antioxidant and antibacterial activities of their chloroform, ethyl acetate, acetone and methanol extracts was carried out in order to evaluate their potential as natural antioxidant and antibacterial agents in food and pharmaceutical industry.

2. Materials and Methods

2.1 Seed Sources and Chemicals

R. hastatus and C. scoparius seeds were collected from seed nurseries in Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKAUST-K) in 2019. Dr. Sajad Gangoo authenticated the plants. The herbarium specimens were placed at Kashmir University Herbarium (KASH) under 1265 and 1269 of Voucher Specimen Numbers [Ref. No. F1 (Specimen voucher, CBT) KU/2019]. All the chemicals and reagents were purchased from Sigma-Aldrich, USA. Bacterial strains: two Gram-negative bacteria Escherichia coli (ATCC® 25922™), and Pseudomonas aeruginosa ATTC PAO1, one Gram-positive bacteria Staphylococcus aureus (ATCC® 23235™) were collected from Agricultural Microbiology Department, Aligarh Muslim University.

2.2 Successive Soxhlet Extraction

The dried seeds of each plant were ground into into powdered form by using mechanical grinder. The coarsely powdered seeds (50 g) of each plant 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, ethyl acetate, acetone and methanol to obtain respective (RPE and CPE), chloroform (RCF and CCF), ethyl acetate (REA and CEA), acetone (RAT and CAT) and methanol (RMT and CMT)
extracts of *R. hastatus* and *C. scoparius* respectively. The extraction time ranges from 2-3 hrs. The extracts were through anhydrous sodium sulphate (Na\(_2\)SO\(_4\)) to remove any moisture left. All seed extracts were kept at 4 °C until further analyses.

2.3 Fatty Acid Methyl Ester (FAME) Preparation

In first step of the reaction, one gram oil of RPE and CPE was saponified separately, while using alcoholic potassium hydroxide (0.5N). About 1/3\(^{rd}\) part of the solvent is evaporated and diethyl ether was used to extract unsaponifiable part. The remaining aqueous layer was acidified with 6N hydrochloric acid, and finally it was extracted with ether to obtain mixed fatty acids (MFA). In second step, The MFA was treated with excess amount of absolute methanol in presence of sulphuric acid (H\(_2\)SO\(_4\)) as catalyst. The reaction was allowed to run in refluxed condensor for about 1-2 hrs. On completion of reaction, the resulting mixture was diluted with ice chilled water up to cloud point formation. Finally resulting mixture was extracted continuously with diethyl ether. About 5% aqueous sodium bicarbonate was used to wash combined ether extracts which were dried over anhydrous sodium sulphate to yield Fatty acid methyl ester (FAME). Column chromatography with eluent petroleum ether-diethyl ether (98:2, v/v) was used to purify FAMEs. The ester peak in FAMEs were observed by Fourier transform infrared spectroscopy (FTIR) as given in (Fig. 1). The peaks at 1746 cm\(^{-1}\) and 1750 cm\(^{-1}\) for FAMEs of *C. scoparius* and *R. hastatus* respectively confirmed ester formation.

2.4 Antioxidant Activity Determination

2.4.1 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of (RCF, REA, RAT, RMT and CCF, CEA, CAT, CMT) extracts of *R. hastatus* and *C. scoparius* respectively was estimated by using the method described by Shimada et al [24] with further modifications. Simply, 200 µL of each extract with concentration range from (25-200) µg/mL was added with 3.8 mL DPPH solution, were incubated in the dark at room temperature for 1 hour. The absorbance of the mixture was then measured 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 ability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH radical scavenging activity (1%) } = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100
\]

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

![Fig. 1](image-url) Fourier transform infrared spectroscopic analyses of fatty acid methyl esters of *R. hastatus* and *C. scoparius*. 
2.4.2 Nitroblue Tetrazolium (NBT) assay
Superoxide anion scavenging activity was determined according to Vyas and Kumar [25]. Briefly, reaction was performed in 50 mmol/L phosphate buffer (pH 7.8) containing concentrations of (25-200 µg/mL) of the extract, 50 mmol/L nitroblue 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. The NBT radical scavenging percentage were calculated using the equation as described in DPPH assay. All the results were compared with BHT.

2.4.3 Hydrogen Peroxide (H₂O₂) Scavenging Activity
H₂O₂ radical scavenging assay was determined according to the method given by Ruch et al [26]. Hydrogen peroxide solution of 40 mM was prepared in phosphate buffer (pH 7.4). Spectrum was taken by UV-Visible spectrophotometer while using phosphate buffer solution as blank. The extracts at different concentrations (25-200 µg/mL) in 3.4 ml phosphate buffer were added to 0.6 ml of H₂O₂ solution (0.6 ml, 43 mM). Absorbance of H₂O₂ was determined after 10 min at 230 nm against a blank solution containing phosphate buffer with BHT as positive control. The percentage scavenging of H₂O₂ radicals were obtained by using the equation described in DPPH assay.

2.4.4 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging activity
The ABTS assay was used to determine free radical scavenging capacity using the ABTS radical action Re et al [27]. The ABTS was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulphate (K₂S₂O₈) solution, stored in the dark at room temperature for 16 h. The plant 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.

2.5 Antibacterial Activity
The antibacterial activity of seed extracts against *P. aeruginosa*, *S. aureus* and *E. coli* was done by Agar well diffusion method [28, 29] with little modification. Briefly, test strains were grown overnight at 37 °C and 120 RPM to achieve 10⁶ CFU. These cultures were inoculated on sterile Nutrient agar plates in a laminar flow hood by spread plate method. 8mm wells were cut out of the plates using sterile micropipette tips and sealed with 10 µL soft agar. 100 µL of each sample was dispensed in allotted wells of each test strain plate. A 10 µg streptomycin disc was placed on each plate as control. The plates were then incubated for 16 hours at 37 °C and zone of inhibition was measured using a scale.

2.6 Functional Group Analysis
Transmittance spectra of RCF, REA, RAT, RMT of *R. hastatus* and CCF, CEA, CAT, CMT of *C. scoparius* were obtained using a Perkin–Elmer Spectrum One FTIR spectrometer (UK) fitted with an Attenuated Total Reflectance (ATR) crystal of zinc selenide. ATR crystal was maintained at 65 °C temperature, so that it will cover whole crystal completely when sample is taken in it. The extracts in small amount (50-100 µL) dissolve in chloroform and acetone depending on their solubility were used to cover surface area of the ATR crystal. All the samples were measured in duplicate. The spectra were observed continuously over a wavelength range of 500–3500 cm⁻¹ with a data resolution of 4 cm⁻¹ and air was taken as background reference material. After
as the recognized material), in different (P < 0.05)

## 2.7 Statistical Analysis

Treatment results were statistically evaluated by using analysis of variance (ANOVA) by using the statistical software (IBM SPSS Statistics 20). Duncan’s multiple range tests were performed to determine the differences between groups at 5% significance level.

## 3. Results and Discussion

### 3.1 Physio-Chemical analysis of Seed Extracts

Soxhlet extraction is one of the versatile, simple, effective and well established which permits unattended extraction. In general the yield (%, w/w) of all extracts of C. scoparius was found to better than *R. hastatus* (Table 1). However, dominant yield was found in AT (73.62%, 48.81%), MT (66.93%, 45.94%) followed by CF (45.48%, 29.28%) and EA (42.24%, 26.09%) of *C. scoparius* and *R. hastatus* respectively. The PE extracts showed 15.62% for *C. scoparius* comparably similar with Alfalfa (Legume family) and 8.01% for *R. hastatus* which is quiet similar with other polygonaceae seed oils [30, 31]. Saponification value (S.V) is an indicator to determine average molecular weight indirectly chain length of any triglyceride [32]. Higher the S.V shorter the chain length of fatty acids. As shown in (Table 1), the S.V of PE extracts of both plants is comparably similar with 129.30 for *C. scoparius* and 125.96 for *R. hastatus*. Iodine value (I.V) gives an idea about degree of unsaturation, hence could be used to determine the oxidative stability of oils. As given in (Table 1), I.V of *C. scoparius* is 112.49 while as *R. hastatus* has 156.42. This difference may be mainly due to high content of polyunsaturated fatty acids (PUFAs) in *R. hastatus*.

### 3.2 Fatty Acid Composition

The fatty acid composition is an important parameter for determination of quality and stability of the oil. Therefore, its evaluation is necessary [33]. As given in GC (Fig. 2 and Fig. 3), and respective fatty acids were identified by mass spectra (Supplementary material), total number of fatty acids identified were 11 and 9 for *R. hastatus* and *C. scoparius* respectively. As displayed in (Table 2), the PE extracts were found rich in unsaturated fatty acids (UFAs) with 65.44% for *R. hastatus* and 68.23% *C. scoparius*. In *R. hastatus* dominant linolenic acid 53.53% was found, along with small amount of linoleic 3.62% and oleic 0.37% acids. In the previous study on fatty acid composition of *Rumex vesicarius* and *Rumex dentatus*, it was found that significant linoleic (21.1% and 13.8%) and linolenic (26.0 and 11.7%) acids respectively for *R. vesicarius* and *R. dentatus* were found [34]. However, in this study the chemical composition of the total methyl esters of fatty acids from the extracts of *R. hastatus* with dominant linolenic acid 53.53%. Moreover, according to our finding, *R. hastatus* petrol extract contain significant amount of palmitic acid whereas in *R. vesicarius* 32.0% and *R. dentatus* 33.8% were found in earlier study. This change in fatty acid composition may be due to different extraction technique and plant material taken, environmental and geographical variation of collected material as compared to earlier plants reported from similar families. Other fatty acids of *R. hastatus* were eicosenoic 3.62%, palmitoleic

| Name of plant | Different seed extracts | Saponification value | Iodine value |
|---------------|-------------------------|----------------------|--------------|
|               | PE | CF | EA | AT | MT | PE | MT |
| *R. hastatus* | 8.01 ± 1.21 | 29.28 ± 0.91 | 26.09 ± 0.32 | 48.81 ± 0.47 | 125.96 ± 0.91 | 156.42 ± 0.61 |
| *C. scoparius* | 15.62 ± 1.91 | 45.48 ± 1.44 | 122.4 ± 0.76 | 73.62 ± 0.45 | 66.93 ± 0.48 | 129.30 ± 1.40 | 112.49 ± 0.65 |

PE: petroleum ether extract; CF: chloroform extract; EA: ethyl acetate extract; AT: acetone extract; MT: methanol extract. Different letters in each row are significantly different (P < 0.05).
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Fig. 2  Gas chromatogram of fatty acid methyl ester of *R. hastatus*. 1: Myristic acid (C14:0); 2: Palmitoleic acid (C16:1); 3: Palmitic acid (C16:0); 4: Linolenic acid (C18:3); 5: Linoleic acid (C18:2); 6: Oleic acid (C18:1); 7: Eicosenoic acid (C20:1); 8: Eicosanoic acid (C20:0); 9: Docosenoic acid (C22:1); 10: Docosanoic acid (C22:0); 11: Tetracosanoic acid.

Fig. 3  Gas chromatogram of fatty acid methyl ester of *C. scoparius*. 1, 2, 3 are the same as fig2; 4: Linoleic acid (C18:2); 5: Oleic acid (C18:1); 6: Eicosenoic acid (C20:1); 7: Eicosanoic acid (C20:0); 8: Docosenoic acid (C22:1); 9: Docosanoic acid (C22:0).

Table 2  Saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids of petroleum ether (PE) extracts of *R. hastatus* and *C. scoparius*.

| Common and systematic names | Carbon numbers | Chemical formula | Area (%) | *R. hastatus* | *C. scoparius* |
|-----------------------------|----------------|------------------|----------|---------------|---------------|
| Myristic acid               | C14:0          | C\(_{14}\)H\(_{28}\)O\(_{2}\) | 1.72     | 1.89          |               |
| Palmitic acid               | C16:0          | C\(_{16}\)H\(_{32}\)O\(_{2}\) | 16.58    | 19.33         |               |
| Palmitoleic acid            | C16:1          | C\(_{16}\)H\(_{30}\)O\(_{2}\) | 2.60     | 1.91          |               |
| Oleic acid                  | C18:1          | C\(_{18}\)H\(_{32}\)O\(_{2}\) | 0.37     | 3.53          |               |
| Linoleic acid               | C18:2          | C\(_{18}\)H\(_{30}\)O\(_{2}\) | 3.62     | 59.42         |               |
| Linolenic acid              | C18:3          | C\(_{18}\)H\(_{30}\)O\(_{2}\) | 53.53    | 5.29          |               |
| Eicosanoic acid             | C20:0          | C\(_{20}\)H\(_{40}\)O\(_{2}\) | 1.82     | 0.96          |               |
| Eicosanoic acid             | C20:1          | C\(_{20}\)H\(_{38}\)O\(_{2}\) | 3.62     | 0.82          |               |
| Docosenoic acid             | C22:0          | C\(_{22}\)H\(_{42}\)O\(_{2}\) | 4.26     | 0.50          |               |
| Docosenoic acid             | C22:1          | C\(_{22}\)H\(_{40}\)O\(_{2}\) | 1.70     | 2.55          |               |
| Tetracosanoic acid          | C24:0          | C\(_{24}\)H\(_{48}\)O\(_{2}\) | 1.11     | -             |               |
| Unidentified acids          |                |                  | 1.03     | 1.00          |               |
| Total saturated acids       |                |                  | 25.49    | 22.68         |               |
| Total unsaturated acids     |                |                  | 65.44    | 68.23         |               |
2.60%, docosenoic 1.70% acids. Amongst sataturated fatty acids (SFAs) significant palmitic acid 16.58% was found along with smaller amounts of myristic 1.72% and tetracosanoic 1.11% acids. In case of C. scoparius belonging to leguminosae family the seed oils of this family found mainly consist of linoleic acid. In our study, the fatty acid composition consists of mainly of linoleic acid 59.42% which is found to be similar as compared to soyabean seed oils of leguminosae family [35]. Other UFAs includes small amounts of oleic 3.53%, palmitoleic 1.91%, eicosenoic 0.82% and docosenoic 2.55% acids. The saturated fatty acids include significant amount of palmitic acid 19.33% along with small amount of myristic 1.89%, eicosanoic 0.96% and docosenoic 0.50% acids.

3.3 Antioxidant Activity

The antioxidant activities of RCF, REA, RAT, RMT and CCF, CEA, CAT, CMT extracts of R. hastatus and C. scoparius respectively were determined using the DPPH radical scavenging, hydrogen peroxide, superoxide anion radical and NBT radical assay.

3.3.1 DPPH radical scavenging activity

DPPH radical scavenging assay is based on the fact that the seed extracts containing antioxidants react with DPPH radicals and convert it into yellow colored compound, di-phenyl hydrazine. Lower the absorbance of the reaction mixture, higher is the free radical scavenging activity. As depicted in (Fig. 4). All the extracts of R. hastatus showed better results of DPPH radical activity at all concentrations with respect to BHT but most dominant effect was shown at 100 µg/mL and 200 µg/mL concentration of seed extracts. At 100 µg/mL RMT 83.72% and RAT 83.41% showed dominant inhibition of DPPH radicals followed by RAT 82.94% whereas least effect was shown by RCF 79.64% (P < 0.05). At 200 µg/mL RAT 92.90% showed dominant effect followed by RMT 91.93%, REA 90.96% and RCF 88.58 with respect to BHT 95.59%. These results are comparably much better than the inhibition shown by various leaf extracts of this plant in previous study [36]. In case of C. scoparius dominant inhibition was shown at 200 µg/mL with CAT 93.07% followed by CMT 92.17%, CCF 90.93% and CEA 89.83% with BHT 95.59% (P < 0.05), which is relatively better as compared to Cytisus villosus [37]. At other concentrations the seed extracts CAT, CCF and CEA of C. scoparius showed relatively good results with respect to BHT (Fig. 4).

Fig. 4  DPPH radical scavenging activity of seed extracts of R. hastatus and C. scoparius. RCF and CCF: chloroform extract; REA and CEA: ethyl acetate extract; RAT and CAT: acetone extract; RMT and CMT: methanol extract of R. hastatus and C. scoparius respectively; BHT: Butylated hydroxytoluene. Data are expressed as means ± S.D (n = 3). Different letters in each concentration are significantly different at (P < 0.05) according to Duncan’s test.
3.3.2 NBT radical scavenging assay

Superoxide scavenging activity is determined as NBT assay (Fig. 5). At 25 µg/ml and 50 µg/ml the extracts RAT, RMT and RCF of *R. hastatus* showed relatively good NBT scavenging comparably less than the inhibition shown by its ethyl acetate extract of leaves in previous study at 40 µg/ml [36]. However, dominant effect was shown by RAT followed by RCF and RMT extracts at 200 µg/mL while at 100 µg/mL the order of increasing magnitude of scavenging was RMT > RCF > RAT > REA (P < 0.05). In *C. scoparius* the extracts CEA, CCF and CMT showed relatively good inhibition of radical at all concentrations with respect to BHT while the least effect was shown by AT (P < 0.05).

3.3.3 H$_2$O$_2$ radical scavenging assay

In general the extracts of *R. hastatus* shows relatively better H$_2$O$_2$ radical scavenging activity than *C. scoparius*. As shown in (Fig. 6), Extracts RCF, REA and RMT of *R. hastatus* at 25 µg/ml showed good results while at 50 µg/mL and 100 µg/mL the order of increasing H$_2$O$_2$ radical scavenging was RAT > REA > RCF > RMT which is relatively good as compared to inhibition shown by different root extracts of this plant [36]. The most dominant effect was shown at 200 µg/mL with REA 89.14% found to be dominant followed by RAT 87.66% and RCF 83.90% while RMT showed least inhibition 74.41% with respect to BHT 92.44% (P < 0.05). In case of *C. scoparius* the dominant effect was shown by CEA and CAT followed by CMT and CCF at 100 µg/mL and 200 µg/mL concentration. At 25 µg/mL CAT and CMT showed good results while at 50 µg/mL CMT, CEA and CAT showed reasonable good radical inhibition with respect to BHT. The inhibition shown by various *C. scoparius* extracts are comparatively better as compared to various methanol seed extracts of *Lathyrus sativus* belongs to same leguminous family [38].

3.3.4 ABTS radical scavenging activity

All the extracts of *R. hastatus* and *C. scoparius* showed powerful ABTS radical scavenging activity. As presented in (Fig. 7) the extracts RMT, RAT and REA of *R. hastatus* showed good results of inhibition at 25 µg/mL, while at 50 µg/mL all its extracts showed better results with respect to BHT. The dominant effect was shown at 100 µg/mL with the order of inhibition of radicals was shown by RAT > REA > RMT > RCF and at 200 µg/mL RMT and RAT showed dominant effect followed by REA and
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![Graph 1](image1.png)

**Fig. 6** H$_2$O$_2$ radical scavenging activity of defatted seed extracts of R. hastatus and C. scoparius. RCF and CCF: chloroform extract; REA and CEA: ethyl acetate extract; RAT and CAT: acetone extract; RMT and CMT: methanol extract of R. hastatus and C. scoparius respectively; BHT: Butylated hydroxytoluene. Data are expressed as means ± S.D (n = 3). Different letters in each concentration are significantly different at (P < 0.05) according to Duncan’s test.

![Graph 2](image2.png)

**Fig. 7** ABTS radical scavenging activity of defatted seed extracts of R. hastatus and C. scoparius. RCF and CCF: chloroform extract; REA and CEA: ethyl acetate extract; RAT and CAT: acetone extract; RMT and CMT: methanol extract of R. hastatus and C. scoparius respectively; BHT: Butylated hydroxytoluene. Data are expressed as means ± S.D (n = 3). Different letters in each concentration are significantly different at (P < 0.05) according to Duncan’s test.

RCF (P < 0.05). These results are much better as compared to inhibition shown by various root extracts of this plant even at much higher concentrations [36]. In C. scoparius at 25 µg/mL and 50 µg/mL the order of magnitude of ABTS radical scavenging was CCF > CEA > CAT > CMT. At 100 µg/mL CCF 82.07% and CAT 81.77% showed dominant effect followed by CEA 79.08% while CMT showed least effect with BHT 83.57 (P < 0.05). The order of increasing radical scavenging of extracts at 200 µg/mL was CCF > CMT > CEA > CAT (Fig. 7).

### 3.4 Antibacterial Activity

The antibacterial activity of extracts is shown in (Fig. 8 and Fig. 9). All the extracts showed good antibacterial activity against P. aeruginosa, however
dominant zone of inhibition was shown by *C. scoparius* extracts, CAT (22.28 mm) followed by CMT (18.27 mm) with respect to streptomycin (25.96 mm). The extracts RAT (16.44 mm), REA (15.16 mm) and RMT (14.29 mm) of *R. hastatus* showed moderate inhibition, lowest inhibition was shown by RCF, CEA and CCF extracts against *P. aeruginosa* at (P < 0.05). Against *S. aureus* the most dominant effect was shown by CAT (24.22 mm) and RAT (20.42 mm) with respect to streptomycin which showed 27.88 mm (P < 0.05). The decreasing order of inhibition shown by other extracts follows CMT > RMT > REA > RCF > CCF > CEA. Against *E. coli* (Fig. 8 and Fig. 9) RAT (23.14 mm) showed almost similar inhibition as displayed by streptomycin (23.95 mm) at (P < 0.05) which is comparably similar as shown by acetone extract of *Rumex vesicarius* [39]. Moreover, the extracts CAT (21.97 mm), CMT (20.32 mm), REA (19.33 mm) showed dominant effect against *E. coli*, while as RMT, CCF and CEA displayed moderate effect. The extracts of *C. scoparius* in this study showed comparably similar inhibition as displayed by *Caesalpinia pulcherrima* (Legume family) extracts against same strains of bacteria [40].

### 3.5 Functional Group Analysis by FTIR

FTIR is an important tool for determination of various functional groups. This technique is based on peak values which gives idea about functional groups. In this study ATR-FTIR was used to determine various functional groups in extracts of *R. hastatus* and *C. scoparius*. As given in (Fig. 10 and Fig. 11) the IR peaks of RCF, REA, RAT, RMT and CCF, CEA, CAT, CMT extracts are pointed out and respective peak values are functionalized in accordance with (Table 3). The wide IR in the range of 3410-3470 cm\(^{-1}\) shown by all extracts of two plants is mainly of hydroxy group which may be due to the presence of polyphenols, tocotrienols, vitamin E and Gallic acid or some other hydroxyl group containing antioxidants and antibacterial agent e.g. catechol and eugenol. The extracts of *C. scoparius* such as CCF (3008 cm\(^{-1}\)), CAT (3016 cm\(^{-1}\)) and CMT (3012 cm\(^{-1}\)), which could be due to presence of carotenoids (alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin) or presence of any antibacterial agent which may contain unsaturated functioning such as piperine, tannins, cocaine and Estragole etc [41]. All extracts

![Antibacterial activity of seed extracts against *P. aeruginosa*, *S. aureus* and *E. coli*. Values marked by the different small case letters are significantly different at P < 0.05 (Duncan test). RCF: *R. hastatus* chloroform extract; REA: *R. hastatus* ethyl acetate extract; RAT: *R. hastatus* acetone extract; RMT: *R. hastatus* methanol extract; CCF: *C. scoparius* chloroform extract; CEA: *C. scoparius* ethyl acetate extract; CAT: *C. scoparius* acetone extract; CMT: *C. scoparius* methanol extract; Control: streptomycin.](image-url)
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Fig. 9 Antibacterial activity of RCF: *R. hastatus* chloroform extract; REA: *R. hastatus* ethyl acetate extract; RAT: *R. hastatus* acetone extract; RMT: *R. hastatus* methanol extract; CCF: *C. scoparius* chloroform extract; CEA: *C. scoparius* ethyl acetate extract; CAT: *C. scoparius* acetone extract; CMT: *C. scoparius* methanol extract; C: streptomycin against *P. aeruginosa*, *S. aureus* and *E. coli*.

Fig. 10 Fourier transform infrared spectroscopic analysis of RCF: chloroform extract; REA: ethyl acetate extract; RAT: acetone extract; RMT: methanol extract of *R. hastatus*. 
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Fig. 11  Fourier transform infrared spectroscopic analysis of CCF: chloroform extract; CEA: ethyl acetate extract; CAT: acetone extract; CMT: methanol extract of *C. scoparius*.

Table 3  Evaluation of FT-IR spectrum of various seed extracts of *R. hastatus* and *C. scoparius*.

### R. hastatus

|     | RCF | REA | RAT | RMT |
|-----|-----|-----|-----|-----|
| PV  | FG  | PV  | FG  | FG  | FG  |
| 3456| Alcohol | 3464 | Alcohol | 3454 | Alcohol | 3452 | Alcohol |
| 2922| –CH₂– | 2992 | –CH₂– | 3002 | RHC=CHR (Cis) | 2964 | –CH₂– |
| 2852| –CH₂– | 2932 | –CH₂– | 1754 | C=O (ester) | 2072 | –CH₂– |
| 1748| –C=O (ester) | 2848 | –CH₂– | 1612 | NI | 1734 | –C=O |
| 1636| C=C cis-olefins | 1768 | –C=O | 1454 | –C–H (–CH₃) | 1638 | C=C cisolefins |
| 1464| –C–H (–CH₃) | 1452 | –C–H (–CH₃) | 1370 | –C–H (–CH₃) | 1442 | C–H (–CH₂) |
| 1374| –C–H (–CH₃) | 1374 | –C–H (–CH₃) | 1238 | C–H (–CH₂) | 1368 | –C–H (–CH₃) |
| 1224| –C–H (–CH₂) | 1242 | C–H (–CH₂) | 1054 | –C–O | 1216 | –C–H (–CH₂) |
| 1056| –C–O | 904 | –HC=CH– (cis) | 1052 | –C–O |
| 934 | HC=CH– (trans) | 772 | –C–H | 1012 | –C–O |
| 852 | =CH₃ | 616 | NI | 638 | NI |
| 616 | NI | 512 | NI | 532 | NI |

### C. scoparius

|     | CCF | CEA | CAT | CMT |
|-----|-----|-----|-----|-----|
| PV  | FG  | PV  | FG  | FG  | FG  |
| 3410| Alcohol | 3458 | Alcohol | 3444 | Alcohols | 3942 | Alcohol |
| 3008| RHC=CHR (Cis) | 2916 | –CH₂– | 3016 | RHC=CHR (Cis) | 3012 | RHC=CHR (Cis) |
| 2922| –CH₂– | 1630 | C=C cis-olefins | 2922 | –CH₂– | 2970 | –CH₂– |
Table 3 to be continued

| PV  | Functional Group | Peak Value (cm⁻¹) |
|-----|------------------|------------------|
| 2856| –CH₂–            | 1428             |
| 1740| –C=O (ester)     | 1224             |
| 1648| C=C (cis)        | 672              |
| 1548| NI               | 1646             |
| 1458| –C–H (–CH₃)      | 1542             |
| 1364| –C–H (–CH₃)      | 1460             |
| 1218| C–H (–CH₃)       | 1364             |
| 1056| –C–O             | 1214             |
| 966 | HC=CH– (trans)   | 1150             |
| 644 | NI               | 1090             |
| 890 | –HC=CH– (cis)    |                  |
| 766 | –C–H             |                  |

RCF: chloroform extract; REA: ethyl acetate extract; RAT: acetone extract; RMT: methanol extract of \( R. \) hastatus. CCF: chloroform extract; CEA: ethyl acetate extract; CAT: acetone extract; CMT: methanol extract of \( C. \) scoparius. PV: peak value (cm⁻¹); FG: functional groups; NI: not identified. [43-45].

except CEA showed IR peaks at 1734-1768 cm⁻¹ which could be due to ester bearing functional group such as methionine or antibacterial agent such as fatty esters, pectins, etc [42]. The peak at 1630-1648 cm⁻¹ could be due to the presence of cis beta carotene, \( \alpha \)-stilbene etc. The peaks in between 1200-1020 cm⁻¹ could be due to presence of ether group bearing compounds such as vitamin C and E, flavonoids, anthocyanins etc. or could be due to presence of antibacterial agents such as flavones, flavonols and flavonols, etc. In general the FTIR analysis gives valuable information about general functional groups present in extracts of worked plants, which could be very helpful for further work regarding isolation and characterization of particular bioactive substance.

4. Conclusions

The results obtained in present study clearly demonstrate that Soxhlet extraction produces high yields of seed extracts. Dominant linolenic and linoleic acid are found in \( R. \) hastatus and \( C. \) scoparius respectively, apart from significant palmitic acid. Moreover chloroform, ethyl acetate, acetone and methanol extracts of these plants showed good DPPH, NBT, \( H₂O₂ \), ABTS radical scavenging and antibacterial activities. These findings clearly show that the extracts of these plants can be used in nutraceutical and cosmetic industries or functional food ingredients upon further modifications.

Acknowledgement

We are thankful to UGC for the award of Non-Net fellowship, MANF (3rd. author) and Department of Chemistry, PHET, Agricultural Microbiology and Instrumentation Facility (USIF), Aligarh Muslim University for providing the requisite facilities to carry out this work. Also, Prof. Sajad Gangoo is duly acknowledged for collection, authentication of seeds.

Conflict of interest

No conflict of interest.

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