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Fatty acid composition, antioxidant, antifungal activities, and functional group analysis of *Corylus jacquemontii* seeds grown in Kashmir

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ABSTRACT

Corylus jacquemontii (Decne.) is an important aromatic plant possessing nutritional and various therapeutic properties. This plant has got wide abundance in the Kashmir region with very low care cost. In this study, Soxhlet extraction was used to obtain different seed extracts. The highest yield observed was 32.25% and 30.27% in petroleum ether and acetone extracts, respectively. Gas chromatography coupled with a flame ionization detector was used to determine the fatty acid profile of petroleum ether extract. Unsaturated fatty acids were found in the dominant amount, notably 79.33% oleic acid. The antifungal activity against *Aspergillus niger*, *A. fumigates*, and *Penicillium marneffeii* and antioxidant assays such as CAT, APx, SOD, DPPH were observed in petroleum ether, ethyl acetate, acetone, and methanol extracts. The dominant inhibition against *A. niger* and *A. fumigates* was displayed by methanol extract with 16.78 mm and 19.23 mm inhibition zone, respectively, while *P. marneffeii* methanol (20.98 mm) acetone (20.27 mm) extracts were most effective. Moreover, all extracts displayed good antioxidant activities. These results increased the attention towards the importance of the present study.

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1. Introduction

Corylus jacquemontii (Decne.), commonly known as hazelnut, belongs to the Betulaceae family and is distributed worldwide, mostly in the coasts of the Black sea region of Turkey, Northern Europe (Italy, Spain, France, and Greece), and in some other parts of the world, especially Iran, Azerbaijan, China, and India. Hazelnut fruit is a nut connate with its tightly adherent fruit coat. This dry coat provides an average of 40% of a nut weight, and the remaining 60% constitutes a nut itself. These hazelnuts are mainly produced in Turkey (79.19%), Italy (11.18%), Spain (6.47%), and the United States (2.47%) (Demir and Beyhan, 2000). Traditionally, due to the

presence of a high quantity of fats, hazelnuts have been prescribed by the general public, but recent epidemiologic and clinical studies have concluded that frequent consumption of nuts has nutritional and health benefits, especially in the reduction of coronary heart diseases (Alphan et al., 1996; Durak et al., 1999; Elvevoll et al., 1990; Garcia et al., 1994). Various research has confirmed the benefits of introducing hazelnuts in human diets due to the presence of fat (around 60%), most of which are rich in monounsaturated fatty acids (MUFA) (primarily oleic acid), tocopherol (α -tocopherol), phytosterols (β -sitosterols), polyphenols, and squalene (Di Nunzio, 2019; Mercanligil et al., 2007). Besides the nutritional activities, these hazelnuts are also used as a unique and good flavor ingredient in various foods (Alasalvar et al., 2003, 2004).

The oxidants and free radicals play a dual role in toxic and beneficial compounds. They can be either useful or harmful to the human body. However, an overload of free radicals causes oxidative stress, which leads to various chronic and degenerative diseases. Certain

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synthetic antioxidants such as butylated hydroxytoluene (BHT) are used to prevent the oxidation of lipids in various foods. However, it has been found that the overuse of synthetic antioxidants leads to harmful human effects on human health (Valentão et al., 2002). Moreover, synthetic antioxidants are stable under particular conditions.

Pathogenic fungi are the main infectious agents for the plants, causing alterations during development as well as in the post-harvest stage. The genus *Alternaria* is the most common fungi on the phyllosphere (Lopes and Martins, 2005). It includes both plant-pathogenic and plant-saprophytic species that may damage the crop and causes post-harvest decay (Griffin and Chu, 1983), which ultimately decreases the economy of farmers and the food industry. These fungi also decrease the quality of fruits and seeds. In certain cases, due to the production of mycotoxins or allergens by fungi, these are indirectly related to toxic or allergic disorders among consumers. Synthetic fungicides can control fungi, but these are restricted to use by the cause of harmful effects of pesticides on human health and the environment (Harris et al., 2001; Hayes and Laws, 1991). The latest trend is searching for antimicrobial and antioxidant agents of plant origin because of their safe, eco-friendly, and cost-effective nature (Amadioha, 2000; Cheijinna, 2006; Tauchen et al., 2015). In this study, we took *C. jacquemontii* seeds from Kashmir because of their high abundance, low care cost, and tremendous traditional applications. Hence, in continuation of determination of the fatty acid composition of seed oils (Nengroo and Rauf, 2019, 2020). This work has investigated hazelnut seeds extracts for fatty acid composition, antioxidant activity, and functional group analysis. In addition, *in vitro* antifungal activity of seed extracts was also screened against *Aspergillus niger*, *A. fumigates*, and *Penicillium marneffeii*.

2. Materials and methods

2.1. Sampling

C. jacquemontii (locally known as Virin) seeds were collected from Lehenwan, Vailoo, and Lisser areas of Kokernag, Anantnag district,

South Kashmir (India). Sampling was made in different orchids; in October 2019, hazelnut samples were placed inside a polystyrene box. In their unshelled state, samples were kept at room temperature (25 °C) for two weeks until analyses were performed. A series of fatty acid methyl esters (FAMES), including *trans*-9C18:1, *trans*-10C18:1, and *trans*-11C18:1 isomers, were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA) and Sigma Aldrich (USA). All the reagents and internal standards used for the gas chromatography (GC) were of analytical grade and purchased from Fischer Scientific (UK).

2.2. Extraction of seed extracts

The nuts of seeds were manually cracked, and the kernels were ground under a dried air to a fine powder in a coffee grinder. A powder weight of 50 g was successively extracted by petroleum ether b.p. 40-60 °C (150 ml) followed by ethyl acetate, acetone, and methanol in a Soxhlet apparatus up to 6 h. The extraction percentages were determined as the difference in weight of dried samples before and after extraction. The extracts were dried over anhydrous sodium sulfate (Na₂SO₄) and were kept at 4 °C until further analyses.

2.3. Preparation of fatty acid methyl ester (FAME)

The hazelnut oil (200 mg) was treated with 3 ml of sodium methoxide in methanol (0.5 mol/l) at 100 °C in a water bath for 10 min. The solution was cooled at room temperature, and 2 ml of 12% (w/w) boron trifluoride (BF₃) in methanol was added. The mixture was heated in a water bath for 10 minutes and cooled to room temperature. After cooling, 1 ml of hexane was added, and the mixture was shaken vigorously. Then, 1 ml of 0.6% (w/v) sodium chloride was added. The organic layer was transferred into another screw-capped test tube with the help of a Pasteur pipette and finally dried by anhydrous sodium sulfate and filtered. The filtrate was concentrated under a gentle stream of nitrogen (Figure 1).

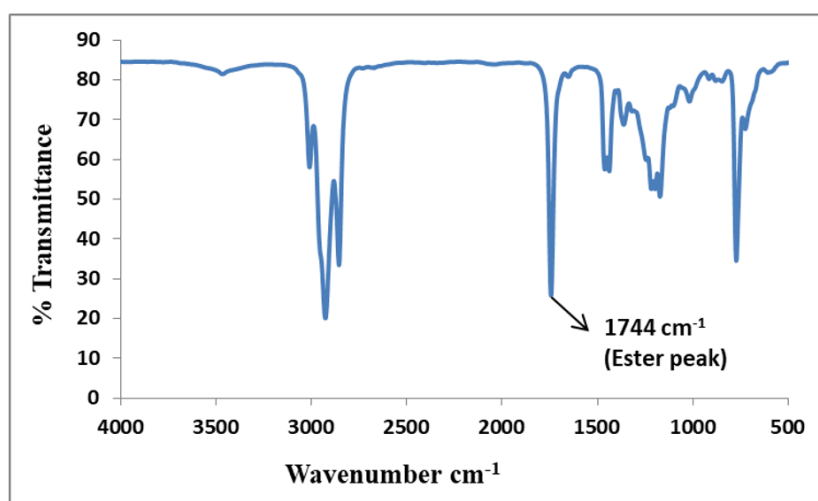


Figure 1. FT-IR analysis of fatty acid methyl ester (FAME) of *C. jacquemontii*

2.4. Gas chromatographic analysis

FAMES were analyzed using gas-liquid chromatography (GLC) with a flame ionization detector (FID). The sample (1 µl) was injected into

the GC, a Hewlett-Packard (HP) 5890 series 11 (Little Falls, Wilmington, DE, USA) equipped with a 60 m Supelcowax-10 capillary column (Supelco, Bellefonte, PA, USA) coated with poly-(ethylene glycol) (0.25 mm I.D., 0.25 µM film thickness). 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 kept there for 10 min. The carrier gas used for the analysis was helium at a 0.5 ml/min flow rate. The injector and detector temperatures were maintained at 200 and 250 °C, respectively. Samples were injected into the column inlet using a Hewlett-Packard 7673 automatic injector. FAME identification was based on retention times compared to those of standard FAMES.

2.5. Antifungal assay by disc diffusion technique

The petroleum ether, ethyl acetate, acetone, and methanol seed extracts of *C. jacquemontii* were screened for antifungal against *A. fumigatus*, *A. niger*, and *P. marneffi* by disc methods per the guidelines of the NCCLS on filamentous fungi diffusion (Bayer et al., 1966). The concentration of extracts, i.e., 10 µl, 15 µl, and 20 µl/disc, were used for analysis. The fungal cultures were grown on czapexdox broth (diffco). Twenty ml of agar media was poured into Petri dishes and allowed to solidify. The lawn of a particular fungal strain was made on the surface of agar media around the disc. The sterile discs (6mm diameter, Whatman filter paper no:42) were soaked in added concentrations (10 µl, 15 µl, and 20 µl) of extracts. A disc without extract was used as the negative control, while standard antibiotic nystatin was used as the positive control in this study as its efficacy for combating fungal infections has been proven in previous studies. The mycelia mat of *A. fumigatus*, *A. niger*, and *P. marneffi* of 7-day old culture were washed, suspended in normal saline solution, and then filtered through glass wool aseptically. The colony-forming units per ml of a suspension of the test fungi were determined, and inoculum was adjusted 1-5 X 100 ml. The appearance of conidia was used for *in vitro* antifungal assay tests. 0.1 ml of inocula were applied on the surface of the Czapek's dox agar (Diffco) plate and spread by using a sterile glass spreader. The test was performed in triplicate. These dishes were incubated for 48 h at 28 °C. The zone of inhibition in mm was determined after 48 h.

2.6. Antioxidant activity

2.6.1. Activity of catalase (CAT)

The activity of CAT was measured by the method of (Aebi, 1984) with slight modification by observing the disappearance of H₂O₂ at 240 nm through UV-vis spectrophotometer. The reaction mixture consists of 22.5 µl seed extracts, 100 mM phosphate buffer (pH 7.8), and 10 Mm H₂O₂. The activity was obtained by using the extinction coefficient of 0.03 mM⁻¹.cm⁻¹.

2.6.2. Activity of ascorbate peroxidase (APx)

The activity was done by Nakano and Asada (1981) procedure in a 3 ml reaction mixture containing phosphate buffer (50 mM, pH 7.0), 0.1 mM AsA, 0.1 mM H₂O₂, and seed extract. The AsA oxidation was observed by a decrease in absorbance at 290 nm (extinction coefficient 2.8 mM⁻¹.cm⁻¹). One unit of APx was defined as the amount of enzyme oxidizing 1 µmol of AsA per minute.

2.6.3. Activity of superoxide dismutase (SOD)

The SOD was determined according to the method described by Ramiro et al. (2006). Briefly the reaction mixture, containing 50 mM phosphate buffer (pH 7.8), 20 µM riboflavin, 75 µM nitroblue tetrazolium (NBT), 130 mM methionine, and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The reaction mixture was irradiated under fluorescent light tubes (40 µmol m⁻¹.s⁻¹) for about

10-15 min. The absorbance was measured at 560 nm by a UV-visible spectrophotometer. The samples for blanks and standards were run in accordance. The SOD activity, which gives half of the maximum inhibition of NBT reduction, was defined as one unit of SOD activity.

2.6.4. Radical scavenging activity (DPPH assay)

The radical scavenging activity of seed extracts of *C. jacquemontii* extracts was performed by following the procedure of Shimada et al. (1992) with minor modifications. Briefly, 200 µl of each extract (25-100 µg/ml) with 3.8 ml DPPH solution was kept in the dark for about 1 h at room temperature. The absorbance was monitored at 517 nm. The scavenging activity of each extract was obtained 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:

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

where, A standard is the absorbance of DPPH radical (without the test sample), and A sample is the absorbance of DPPH radical with the different extract samples of various concentrations. Synthetic antioxidant drug BHT was used as the positive control.

2.7. Functional group analysis

Absorbance spectra of hazelnut seed extracts were obtained using a Perkin-Elmer Spectrum One FT-IR spectrometer (UK) fitted with an Attenuated Total Reflectance (ATR) crystal of zinc selenide. The temperature of the ATR crystal was maintained at 65 °C. A very small amount (50-100 µl) of the sample was required to cover the surface area of the ATR crystal. All the samples were measured in duplicate. The spectra were collected continuously over a wavelength range of 500-4000 cm⁻¹ with a data resolution of 4 cm⁻¹, and the air was taken as a reference background material. The solvent taken was chloroform and acetone, depending on the polarities of the particular extract. After each scan, the ATR crystal was cleaned with tissue paper wet with ethanol, and then dried.

2.8. Statistical analysis

Estimation of treatment results was conducted in triplicate, and the values were reported as average along with their standard deviation. Duncan's multiple range tests were conducted at 5% significance using analysis of variance (ANOVA) while using the statistical software (IBM SPSS Statistics 20 New York, USA).

3. Results and discussion

3.1. Physicochemical properties of seed extracts

The yield of various extracts of *C. jacquemontii* after Soxhlet extraction is depicted in Table 1. The percentage yield ranges from 15 to 33%. However, extract obtained through petroleum ether showed a 32.25% yield followed by acetone 30.27% methanol 23.65%, while ethyl acetate extract showed an average yield (*p* < 0.05). Saponification value (SV) is an index of the molecular weight of triglycerols. Higher SV is related to the high proportion of shorter carbon chain length of the fatty acids (Kirk and Sawyer, 1991), and the SV is also used to check the adulteration of oils. Higher the SV better is the oil's soap-making ability (Nielsen, 1994). As given in Table 1, the SV of *C. jacquemontii* was found to be 177.32. This value was comparably less than its related species (*Corylus avellana*), which has 200.5 SV (Sharma et al., 2008). The iodine value

(IV) is an important tool to know the degree of unsaturation. As given in Table 1, the IV of *C. jacquemontii* was 92.98, which is comparably more than *Corylus avellana* (Sharma et al., 2008). This is mainly due to the presence of a high degree of unsaturated fatty acids compared to this species. However, it has been found that the

IV of various hazelnut species usually falls in the range of 90-95 (Crews et al., 2005), which supports our study on *C. jacquemontii*. Moreover, the SV above 90 helps us categorize *C. jacquemontii* in non-drying oils (Chang et al., 2019).

Table 1. Extracted yield (% w/w) of seed extracts, saponification, and iodine values of petroleum ether extracts of *C. jacquemontii*

Petroleum ether	Ethyl acetate	Acetone	Methanol	SV	IV
32.25 ± 0.55 ^a	15.62 ± 0.51 ^d	30.27 ± 0.43 ^b	23.65 ± 0.26 ^c	177.32 ± 1.68	92.98 ± 1.42

SV: saponification value, IV: iodine value

Values are arranged as mean ± S.D. (n = 3). In each row, different letters in superscript are significantly different at (p < 0.05) by Duncan's test.

3.2. Fatty acid composition

The total fat content was determined according to the Association of Official Analytical Chemists (Horwitz, 2010). The distribution of fatty acids (Table 2, Figure 2) showed that the *C. jacquemontii* is made of fatty acids with 14-22 carbons. The unsaturated fatty acid predominated (91.86%), mainly of oleic acid (C18:1) 79.33%, was the most dominant fatty acid found. The second, third, and fourth dominance was shown by linoleic acid (C18:2), palmitic acid (C16:0),

and stearic acid (C18:0) with the contribution of 12.21%, 4.95%, and 2.10% to the total fat, respectively (Table 1). Several other FAs were also detected but less in quantity (< 1%). Moreover, a very minute amount of 0.60% *trans*-fat (*t*-C18:1) was found. These results were in good agreement with the previous results reported on the FA profile of other species of hazelnuts (Savage et al., 1999; Xu et al., 2007; Xu and Hanna, 2010).

Table 2. Fatty acid composition of *C. jacquemontii*

S. No	Common and systematic names	Carbon numbers	Molecular formula	Area (%)
1	Myristic acid	C14:0	C ₁₄ H ₂₈ O ₂	0.04 ± 0.02
2	Pentadecanoic acid	C15:0	C ₁₅ H ₃₀ O ₂	0.02 ± 0.01
3	Palmitic acid	C16:0	C ₁₆ H ₃₂ O ₂	4.95 ± 0.12
4	Palmitoleic acid	C16:1	C ₁₆ H ₃₀ O ₂	0.17 ± 0.02
5	Heptadecanoic acid	C17:0	C ₁₇ H ₃₄ O ₂	0.06 ± 0.01
6	Heptadecenoic acid	C17:1	C ₁₇ H ₃₂ O ₂	0.03 ± 0.01
7	Stearic acid	C18:0	C ₁₈ H ₃₆ O ₂	2.10 ± 0.08
8	Oleic acid	C18:1	C ₁₈ H ₃₄ O ₂	79.33 ± 1.04
9	Elaidic acid	C18:1	C ₁₈ H ₃₄ O ₂	0.60 ± 0.03
10	Linoleic acid	C18:2	C ₁₈ H ₃₂ O ₂	12.21 ± 0.82
11	Linolenic acid	C18:3	C ₁₈ H ₃₀ O ₂	0.12 ± 0.01
12	Eicosanoic acid	C20:0	C ₂₀ H ₄₀ O ₂	0.05 ± 0.02
13	Eicosenoic acid	C20:1	C ₂₀ H ₃₈ O ₂	0.14 ± 0.03
14	Docosanoic acid	C22:0	C ₂₂ H ₄₄ O ₂	0.02 ± 0.06
15	^a Σ SFA			7.24 ± 0.62
16	^b Σ TUFA			91.86 ± 1.05

^aTotal saturated fatty acids (TSFAs)

^bTotal unsaturated fatty acids (TUFAs)

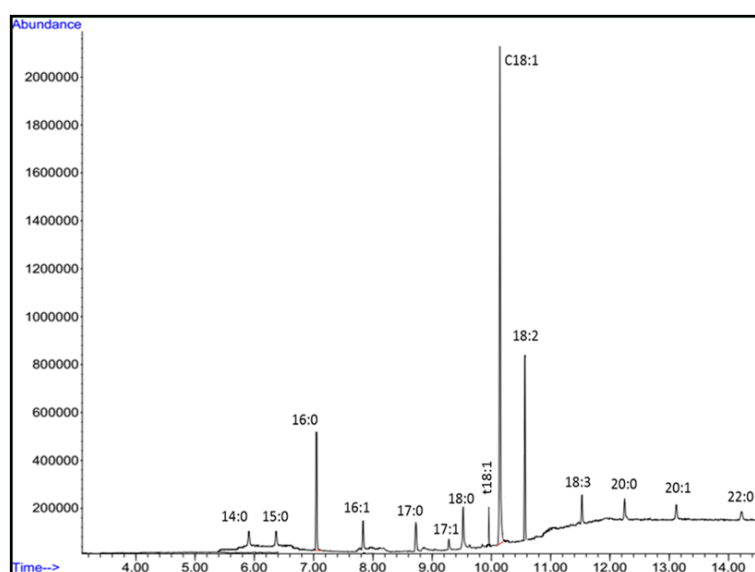


Figure 2. Gas chromatogram of FAME of *C. jacquemontii*

As mentioned, the distribution of fatty acids in *C. jacquemontii* oil is similar to widely consumed Almond used in the pharmaceutical and

cosmetic industry, which contains an average of 60-80% oleic acid, 7-30% linoleic acid, 0.1-1% linolenic acid, 4-9% palmitic acid, 2.5%

max stearic acid and 0.6% palmitoleic acid (Álvarez and Rodríguez, 2000). This fatty acid reveals that *C. jacquemontii* can be an excellent source of oleic acid, which performs nutritional benefits and affords oxidative stability to the oil. Moreover, its composition, similar to almond oil, could be used for cosmetic purposes (Álvarez and Rodríguez, 2000). The hazelnuts are used as an excellent food product besides various other applications, mainly due to their

diverse unsaturated fatty acids. The results were comparably similar on correlating the result of fatty acids to other *Corylus* species of the Betulaceae family (Chang et al., 2019). Hence, the nuts of this species could act as an excellent source of fatty acids and could be used as an alternative to some important nuts such as almonds, groundnuts, walnuts, etc.

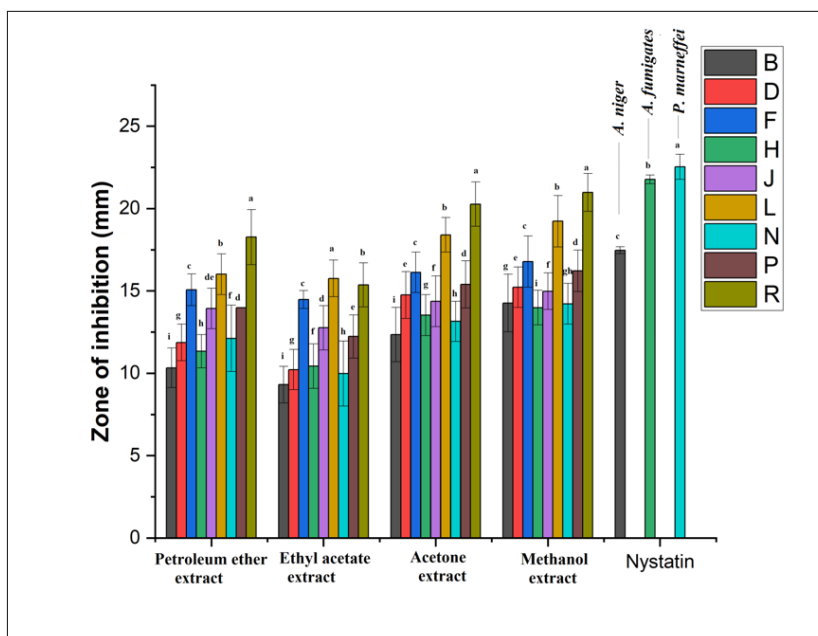


Figure 3. Antifungal activity of petroleum ether, ethyl acetate, acetone, and methanol extracts of *C. jacquemontii*. Values in different letters are significantly different at ($p < 0.05$). (Concentrations of the extracts: B, H, N: 10 L; D, J, P: 15 L; F, L, R: 20 L)

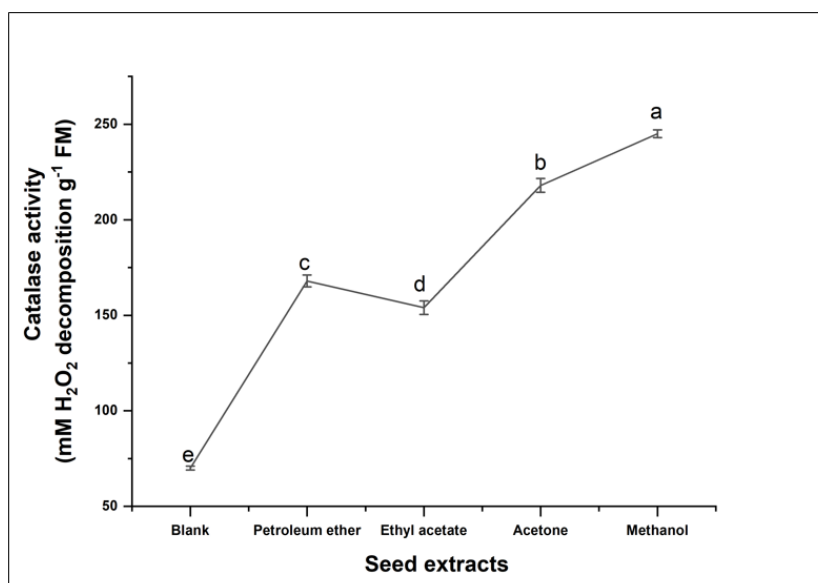


Figure 4. Catalase activity of petroleum ether, ethyl acetate, acetone, and methanol extracts of *C. jacquemontii*. Duncan's test shows that means with different letters are significant at ($p < 0.05$).

3.3. Evaluation of the antifungal activity of extracts

The control of infection caused by fungal has become a major problem due to resist of fungus against a series of commercially developed antibiotics. Thus the search for natural antifungal agents from natural sources has expanded (Webster et al., 2008; Nengroo

et al., 2020). This study assessed the antifungal activity of petroleum ether, ethyl acetate, acetone, and methanol seed extracts of *C. jacquemontii* against three fungal strains, including *A. fumigatus*, *A. niger*, and *P. marneffii*. All the extracts showed good antifungal activity (Figure 3). However, dominant inhibition was shown at a 20 μ l concentration of extracts. Against *A. niger*, the methanol extract

showed 16.78 mm (inhibition zone diameter) followed by acetone 16.12 mm concerning standard nystatin 17.47 mm at $p < 0.05$. In the case of *A. fumigates*, methanol extract showed predominant inhibition of 19.23 mm, followed by acetone extract 18.40 mm The petroleum ether extract showed 16.01 mm and ethyl acetate extract 15.76 concerning nystatin 21.77 mm. Against *P. marneffeii*, methanol and acetone extracts showed almost similar inhibition of fungi with 20.98 mm and 20.27 mm, respectively, followed by

petroleum ether extract 18.27 mm with nystatin showed 22.53 mm (Figure 3). These results were comparatively better compared to some of the plant extracts of related *Corylus* species against various microbes (Ceylan et al., 2013). Moreover, it has been found that the effect shown by the pure seed extracts of *C. jacquemontii* in this study was even better than silver nanoparticle extracts of *C. avellana* leaves (Eshghi et al., 2021).

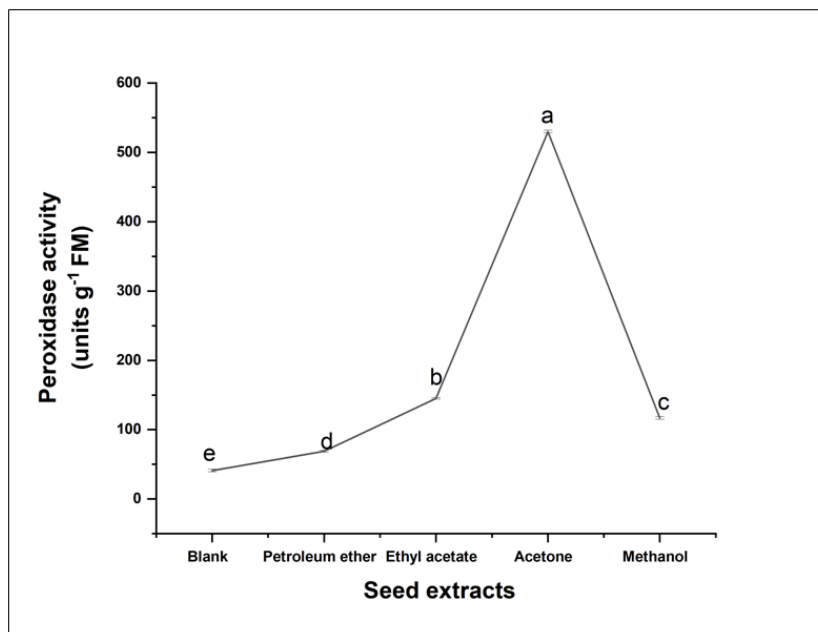


Figure 5. Peroxidase (Apx) activity of petroleum ether, ethyl acetate, acetone, and methanol extracts of *C. jacquemontii*. Means with different letters are significant at ($p < 0.05$).

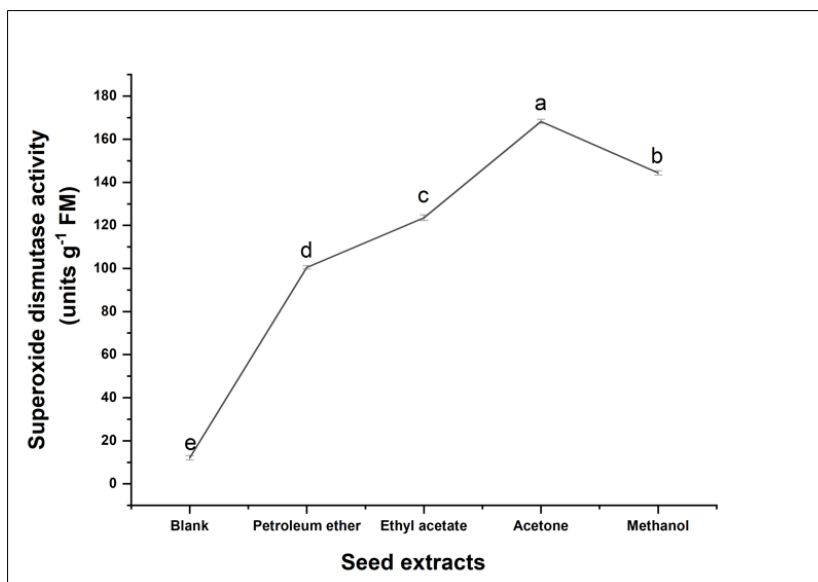


Figure 6. Superoxide dismutase activity of petroleum ether, ethyl acetate, acetone, and methanol extracts of *C. jacquemontii*. Means with different letters are significant at ($p < 0.05$).

3.4. Antioxidant activity

The CAT enzyme activity is wholly related to the decomposition of H₂O₂ radicals, in which the higher the decomposition of H₂O₂ radicals is, the higher the CAT activity is. As shown in (Figure 4), the data indicated the highest CAT activity was observed in the

situations of methanol and acetone extracts, followed by petroleum ether extract, while ethyl acetate extract showed moderate CAT activity concerning standard ($p < 0.05$).

The highest APx activity was shown by the acetone extract (530 U_g⁻¹ FM) as depicted in Figure 5, while the ethyl acetate (145 U_g⁻¹ FM)

and methanol (117 μg^{-1} FM) extracts of *C. jacquemontii* showed moderate activity at ($p < 0.05$). The lowest effect was shown in petroleum ether (69 μg^{-1} FM) to blank (41 μg^{-1} FM).

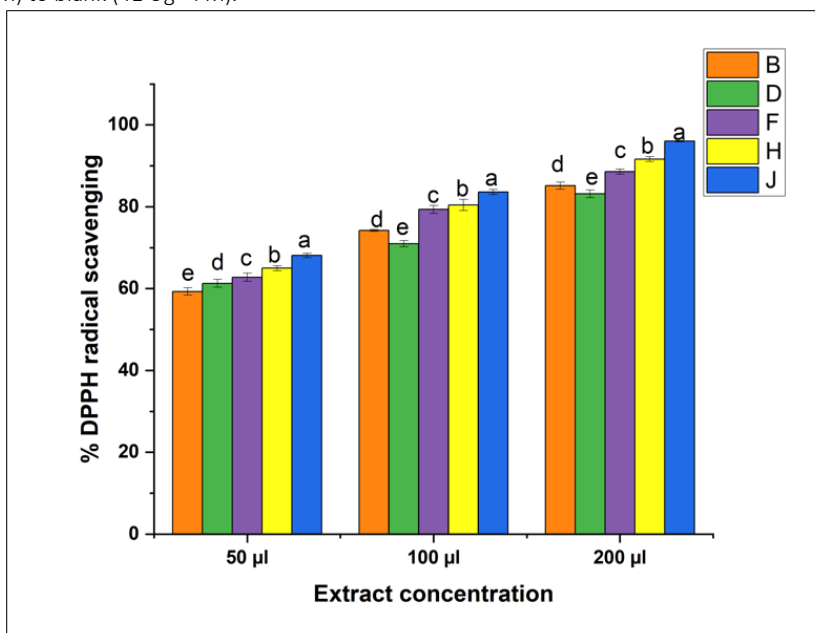


Figure 7. Scavenging activity (DPPH) of seed extract of B: petroleum ether; D: ethyl acetate; F: acetone; H: methanol of *C. jacquemontii*, and J: butylated hydroxytoluene (BHT) as standard. Different letters on each concentration are significantly different at ($p < 0.05$).

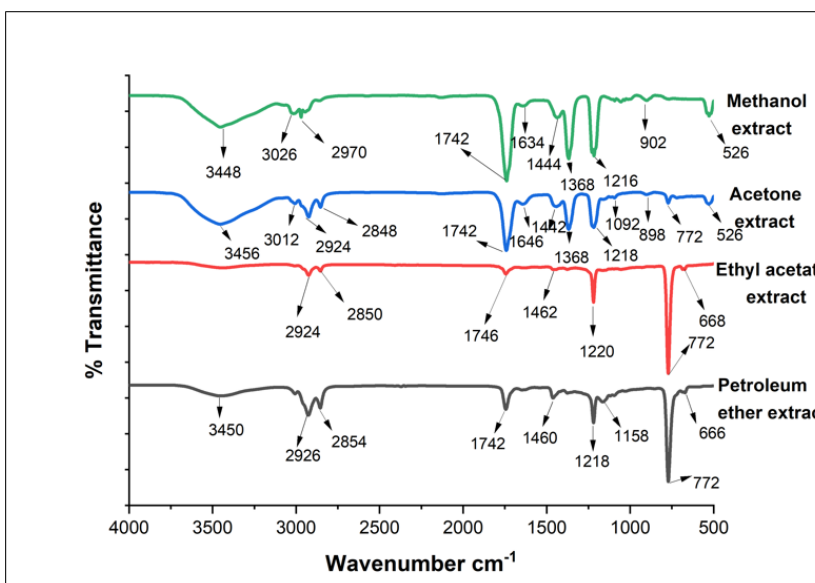


Figure 8. FT-IR analysis of petroleum ether, ethyl acetate, acetone, and methanol extracts of *C. jacquemontii*

As displayed in (Figure 6), the dominant SOD radical scavenging effect was shown by acetone (168.25 μg^{-1} FM) followed by methanol (144.34 μg^{-1} FM). Ethyl acetate (123.53 μg^{-1} FM) and petroleum ether (100.43 μg^{-1} FM) extracts showed average inhibition compared to other extracts.

The antioxidant potential of the petroleum ether, ethyl acetate, acetone, and methanol extracts was quantified as the concentration in ($\mu\text{g}/\text{ml}$) required to scavenge 50% of DPPH radicals. This method is commonly used to determine plants' antioxidants and their various extracts because it is simple, easy, and quick to be performed. Figure 7 shows the DPPH radical scavenging properties

of *C. jacquemontii* seed extracts. All the extracts show good radical scavenging activity. At 50 $\mu\text{g}/\text{ml}$, dominant inhibition was displayed in methanol extract (64.98%) followed by acetone (62.75%) and ethyl acetate (61.29%) extracts to BHT (68.09%). At 100 $\mu\text{g}/\text{ml}$, methanol extract showed the highest scavenging activity (80.44%) followed by acetone extract (79.36%), while the least was observed in ethyl acetate extract (70.98%) as compared to BHT (83.63%). However, the predominant effect was observed at 200 $\mu\text{g}/\text{ml}$ concentration of extracts with the order of decreasing radical scavenging methanol (91.66%) > acetone (88.58%) > petroleum ether (85.15%) > ethyl acetate (83.15%) extracts with BHT showed (96.04%). The inhibitions shown by seed extracts are comparatively

better than different extracts of bark, roots, and leaves of *Betula utilis* (Betulaceae family) from Kashmir (Wani et al., 2018). Moreover, the DPPH radical scavenging activity shown by *C. jacquemontii* seed extracts were comparatively similar with some important plant species from Kashmir viz., *Ulmus wallichiana*, *Celosia argentea*, *Sisymbrium irio*, *Aesculus indica*, and *Abies*

pindrow (Nengroo and Rauf, 2019) and some other plants in various environments (Tatari et al., 2012). These results could categorize this plant not only as a good food source but as an excellent source of the natural drug upon further modifications.

Table 3. FT-IR of petroleum ether, ethyl acetate, acetone, and methanol extracts of *C. jacquemontii*

Petroleum ether extract		Ethyl acetate extract		Acetone extract		Methanol extract	
Wavenumber (cm ⁻¹)	Functional group	Wavenumber (cm ⁻¹)	Functional group	Wavenumber (cm ⁻¹)	Functional group	Wavenumber (cm ⁻¹)	Functional group
3450	Alcohols	2924	-CH ₂ -	3456	Alcohols	3448	Alcohols
2926	-CH ₂ -	2850	-CH ₂ -	3012	<i>cis</i> -RHC=CHR	3026	<i>cis</i> -RHC=CHR
2854	-CH ₂ -	1746	-C=O (ester)	2924	-CH ₂ -	2970	-CH ₂ -
1742	-C=O (ester)	1462	-C-H (-CH ₂)	2848	-CH ₂ -	1742	-C=O (ester)
1218	-C-H (-CH ₂)	1220	-C-H (-CH ₂)	1742	-C=O(ester)	1634	C=C (<i>cis</i>)
1158	-C-O	668	Non-assigned	1646	C=C (<i>cis</i>)	1444	-C-H (-CH ₂)
666	Non-assigned			1442	-C-H (-CH ₂)	1368	-C-H (-CH ₂)
				1368	-C-H (-CH ₃)	1216	-C-H (-CH ₂)
				1218	-C-H (-CH ₂)	902	-HC=CH- (<i>cis</i>)
				1092	-C-O		
				898	-HC=CH- (<i>cis</i>)		
				772	Halides		

The table was constituted according to References (Guillen and Cabo, 1997; Silverstein et al., 2005; Vlachos et al., 2006).

3.5. FTIR analysis

FTIR spectroscopy is an important, sensitive, fast, non-destructive, and accurate technique in which a sample is needed in very little amount. This technique has found wide applications in grape oil as an adulterant (Nurrulhidayah et al., 2011), various edible oils used in frying (Zhang et al., 2012), and vegetable oils (Rohman et al., 2011). The FTIR transmission spectra of *C. jacquemontii* extracts (prepared in chloroform and acetone depending on particular extract's solubility) are given in (Figure 8). The data of peak values and probable functional groups are presented in Table 3. The peak in the range of 3500-3400 cm⁻¹ in the case of petroleum ether, acetone and methanol extracts is mainly of hydroxyl group, which could be due to the presence of any phenolic compounds as natural antioxidants or any antimicrobial agent (Robbins, 2003). The transmission spectra in the range of 3100-300 cm⁻¹ are mainly because of alkenes which may include naturally occurring compounds such as carotenoids, quinoline, etc. The sharp peak in the 1450-1440 cm⁻¹ is the ester functional group peak. This peak could depict the presence of ester bearing antimicrobial or antioxidant agents. The peak at 1630-1646 cm⁻¹ in the case of acetone and methanol extracts could be mainly the presence of *z*-stilbene, *cis*-beta-carotene, etc. In short, FTIR analysis gives general information regarding the functional group, which could be useful in isolating and purifying a particular bioactive compound responsible for various biological activities.

4. Conclusions

This work demonstrated that *C. jacquemontii* seed extracts could be a good source of oleic acid, as food ingredient with high antioxidant activities such as CAT, Pox, SOD, and DPPH and good antifungal activities against *A. niger*, *A. fumigates*, and *P. marneffeii*. The present study provides a base for further work, particularly the isolation and purification of individual bio-actives responsible for antioxidant and antifungal activities and the use of the *C. jacquemontii* seeds in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

CRedit authorship contribution statement

Zubair Rehman Nengroo: Conceptualization, Methodology, Antimicrobial activity, Software and writing
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Supplementary File

None.

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