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Phytochemical analysis and therapeutic applications of some wild edible fruits growing in Uttarakhand Himalayas

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ABSTRACT

The purpose of the investigation was to evaluate the phytochemical composition and biological properties of indigenous wild edible fruits that grow in the Uttarakhand Himalayas in India. Plant extracts were prepared employing the cold percolation method in both nonpolar and polar solvents, *i.e.*, hexane and methanol. Subsequent GC-MS analysis of the hexane extracts, namely *Pyracantha crenulata* hexane extract 01 (PCHE01), *Berberis asiatica* hexane extract 02 (BAHE02), *Rubus ellipticus* hexane extract 03 (REHE03), *Ficus palmata* Forsk hexane extract 04 (FPHE04), and *Myrica esculenta* hexane extract 05 (MEHE05), revealed the identification of more than 32, 40, 44, 53, and 48 constituents, which accounted for 74.4, 83.4, 78.9, 70.0, and 73.2% of the overall composition, respectively. The nutritional elements of *Pyracantha crenulata* (PC), *Rubus ellipticus* (RE), *Myrica esculenta* (ME), *Ficus palmata* Forsk (FP) and *Berberis asiatica* (BA) were also studied. The results indicated that the boron content was highest in all samples. Hexane and methanol fruit extracts were studied for their total phenolic and flavonoid content, which revealed variations. Both extracts were examined for different biological activities. The antioxidant activity was evaluated using three different methods. In vitro evaluation of anti-inflammatory activity was performed by measuring the denaturation of egg albumin protein. In the methanolic extract, the lowest IC₅₀ value was recorded for REME3 at 7.50±0.03 µg/mL. Likewise, in the hexane extract, BAHE02 exhibited a minimum IC₅₀ value of 4.47±0.87 µg/mL. The evaluation of antidiabetic activity of hexane and methanol extracts was carried out through an α-amylase inhibition assay. The comprehensive biological activity assays and elemental analyzes underscored the significant nutraceutical value of these plants. It was evident that these plants have the potential to serve as effective nutrient supplements and could be of considerable industrial importance in the field of the nutraceutical sector. This research is important not only from an academic perspective, but also for establishing a valuable database that can guide the sustainable use of wild edible plants.

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1. Introduction

Plants form the basis of sophisticated traditional medical systems, which have been practiced for thousands of years and continue to offer human race cutting-edge remedies. Most people still receive everyday medical care from their traditional media (medical plants and other items) on this planet. To improve their survival chances, plants have evolved complex mechanisms, such as the ability to produce different secondary metabolites [1]. According to the World Health Organization

(WHO), 60% of the population regularly employs medicinal plants to treat a variety of diseases, while approximately 40% of the population uses such plants in pharmaceutical pursuits [2]. Medicinal plants are used to combat diseases and promote both the physical and mental well-being of people [3]. Aromatic botanicals have found diverse applications in the realms of nutraceuticals and pharmaceuticals, serving as growth enhancers, antimicrobial agents, immune stimulants, antioxidants, flavor enhancers, natural colorants, and preservatives for food products, all while having synthetic chemical equivalents [1].

Table 1. Taxonomical details of plant material.

Plant name	Site of collection	Latitude (N), Longitude (E)	Time of collection	Amount of powder taken	Yield (g)		Herbarium number
					In hexane	In methanol	
<i>P. crenulata</i>	Lohaghat, Champawat, Uttarakhand	29.4042°N, 80.0842°E	May 2022	0.295 Kg	5.0	15.0	GBHUP 1555
<i>B. asiatica</i>	Lohaghat, Champawat, Uttarakhand	29.4042°N, 80.0842°E	May 2022	0.082 Kg	3.0	21.0	GBPUH 1556
<i>R. ellipticus</i>	Bhimal, Nainital, Uttarakhand	79.551914°N, 29.346082°E	May 2022	0.099 Kg	8.0	19.6	GBPUH 1557
<i>F. palmata</i>	Lohaghat, Champawat, Uttarakhand	29.4042°N, 80.0842°E	May 2022	0.140 Kg	13.0	12.3	GBPUH 1558
<i>M. esculenta</i>	Lohaghat, Champawat, Uttarakhand	29.4042°N, 80.0842°E	May 2022	0.125 Kg	7.0	13.0	GBPUH 1999

Berberis asiatica Roxb. ex. DC. (Berberidaceae) is frequently utilized as an alternative to "Daruharidra" also known as *B. aristata* DC., in Ayurvedic medicine. Given its importance as a medicinal plant, it is widely used in the treatment of various conditions, including eye disorders, skin ailments, jaundice, and rheumatism [4]. In addition, different types of *B. asiatica* species are utilized in medicinal, nutraceutical, and cosmetic formulations. [5]. *Rubus ellipticus* Sm., a member of the Rosaceae family, is commonly referred to as the yellow Himalayan raspberry and is predominantly found along the edges of forests. It thrives in extensive regions of mountains and lowlands in India and Sri Lanka. As a wild raspberry, *R. ellipticus* bears edible fruit with medicinal properties, including astringent, febrifuge, kidney, miscellaneous, and stomachic attributes. Fruit juice is used to treat fever, colic, cough, and sore throat [6]. *Ficus palmata* Forssk. from the Moraceae family has the majority of members within tall trees or shrubs, with herbs being less common and often containing milky juice. *F. palmata* is important as a widely used medicinal plant. It is consumed primarily as a dietary component to treat constipation, lung ailments, and bladder problems. Additionally, its sap is applied in wart treatment. It is also used in the treatment of a variety of conditions, such as gastrointestinal disorders, hypoglycemia, antitumor effects, ulcer prevention, diabetes management, lipid reduction, and fungal inhibition [7]. *Pyracantha crenulata* (D.Don) M. Roem., also known as *Crataegus crenulata* Roxb. (Rosaceae) and locally called Ghingaaru, thrives in the Himalayas spanning from Sutlaj to Bhutan, typically at altitudes ranging from 800 to 2500 meters. Within Ayurvedic medicine, it is recognized for its therapeutic benefits in the treatment of various conditions such as liver, stomach, and skin diseases. This is attributed to its diuretic, depurative, tonic, antirheumatic, cardio tonic, hypoglycemic, hypotensive, anti-inflammatory, and lithontripic properties [8]. *Myrica esculenta* Buch.-Ham. ex D.Don, which belongs to the Myricaceae family, is commonly known as Kaphal, Boxberry, and Kathphala. It is renowned for its wild edible fruits, which flourish at altitudes ranging from 900 to 2100 meters above sea level (asl). This species is found to be distributed from the Ravi region eastward to Assam, Khasi, Jaintia, Naga, Lushi hills and extends to regions including Malaya, Singapore, China and Japan. Local residents are highly appreciated for their delicious fruits and processed derivatives [9].

Work on phytochemical composition and some biological activities in wild edible fruits such as *Syzygium cumini* (L.) Skeels and *M. esculenta* has already been reported in our laboratory [10]. Furthermore, we continue our research to analyze the phytochemical content and explore various biological activities, including pesticide effects, as well as therapeutic applications such as antioxidant, anti-inflammatory, anti-diabetic, and pharmacological activities. In our current study, we have focused on five fruits, commonly found in the Indian Himalayan region, with the aim of determining their phytochemical composition and potential therapeutic uses.

2. Experimental

2.1. Plant material and collection site

The fruits of the fresh plant were gathered from various altitudes within the Kumaun region of Uttarakhand in India. The fruits of plants collected were identified by Dr. Dharmendra Singh Rawat, a Plant Taxonomist, of the Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar, Uttarakhand, India along with the herbarium number Table 1.

2.2. Preparation of plant extracts

To ensure the quality of the plant fruits, a careful selection process was used. The fruits were meticulously cleaned using tap water to remove any dust, dirt, or contaminants. Subsequently, the fruits were air-dried in the shade until all moisture was eliminated. After the drying process, the material was ground finely. To prepare the extracts of the powdered material, a standard protocol was followed that incorporated a cold percolation method, commonly practiced [11]. Various quantities of powdered plant material were immersed in both methanol and hexane within a sealed container and left at room temperature for a minimum of seven days. During this time, regular agitation was carried out at specified intervals. This method aimed to soften the walls of plant cells and release soluble phytochemicals. After the seven-day period, the supernatant was filtered through Whatman No. 1 filter paper, while the remaining residues were used for the second and third extractions. Following the third extraction, the filtrates were removed by solvent removal through steam distillation. This extraction process was repeated until a colorless extract was obtained. The yields of the extracts were quantified using Equation 1, and subsequently, the extracts were preserved at a temperature of 4 °C for future analysis.

$$\text{Percent yield} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100 \quad (1)$$

2.3. GC-MS analysis

The analysis of the phytochemical composition of the extract was carried out using a GC-MS system, which featured a DB-5 silica capillary column with specifications including a 0.25 µm film thickness and internal dimensions of 30 m × 0.25 mm. Helium was used as a carrier gas at a flow rate of 1.21 mL/min, with a split ratio of 1:10. The oven temperature was controlled within the range of 50-280 °C. An aliquot of 1 µL of the essential oil sample in hexane was injected in a ratio of 1:10 (v/v). The injector was set to a temperature of 260 °C, with a pressure of 69.0 k Pa and a total flow rate of 16.3 mL/min. Mass spectra were recorded by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40-450. The identification of the chemical constituents of essential oils was performed by comparing their mass spectral fragmentation patterns and their KI (Kovats Index) with those of the MS library (NIST14.lib, FFNSC2.lib,

WILEY8.lib) and comparing the spectra with data from the literature [12].

2.4. Elemental analysis

Standard procedures were adhered for the identification of micro and macro elements such as N, P, K, Ca, Mg, Zn, Fe, Mn, Cu, and S using atomic absorption spectroscopy (AAS) (Advance Scientific Instrument Service, model- AAS4141).

2.4.1. Detection of nitrogen

The quantification of nitrogen in plant material was carried out using the Kjeldahl method. This involved the digestion of one gram of plant sample in digestion flasks with the aid of sulfuric acid, in conjunction with a catalytic mixture containing copper sulfate and potassium sulfate. The Kjeldahl macro unit used for this purpose was the KEL PLUS KES 12LR TS model manufactured by Pelican Equipment in India. Following a thorough digestion process, the digested material was distilled under strong alkaline conditions. The liberated ammonia was captured in a 4% boric acid solution containing a mixed indicator within the macro Kjeldahl distillation apparatus (ELITE EX VA, manufactured by Pelican Equipment in India). The trapped ammonia was then titrated using standard sulfuric acid [13]. The nitrogen content was determined by calculating the percentage based on the obtained titration value.

2.4.2. Digestion of plant samples for estimation of nutrients

Initially, a plant sample weighing one gram was subjected to pre-digestion with 5 mL of nitric acid overnight. Subsequently, the digestion process was carried out using a diacid mixture, composed of nitric acid and per chloric acid in a 10:4 ratio [14] of 100 mL with double distilled water and preserved in a designated PVC container intended for the analysis of all elements except nitrogen.

2.4.3. Detection of phosphorus

The phosphorus content within the digested plant sample was determined using the vanadomolybdophosphoric yellow color method, carried out in a nitric acid medium. The color intensity was then measured at a wavelength of 420 nm using a spectrometer (Model 2203, Systronics, India), following the procedure described in the reference [13].

2.4.4. Detection of potassium

Potassium in the digested plant sample was estimated by atomizing the diluted diacid extract in a flame photometer (Model 128, Systronics, India) as described by reference [13].

2.4.5. Detection of calcium and magnesium

The determination of calcium and magnesium in the digested plant sample involved titration against a standard versenate solution, with murexide and Eriochrome black T indicators used for calcium and calcium plus magnesium, respectively. The magnesium content was then calculated as the difference between the concentration of calcium plus magnesium and calcium [13].

2.4.6. Detection of sulphur

The sulfur content in the plant sample was determined using a portion of the digested plant extract by the turbidimetric method. This analysis was carried out using a spectrometer (Model 2203, manufactured by Systronics, India)

and involved the use of barium chloride, following the procedure described in the reference [15].

2.4.7. Detection of micronutrient cations (Fe, Zn, Mn, and Cu)

Following an appropriate dilution of the diacid extract, the samples were introduced into the atomic absorption spectrophotometer (AAS PLUS; manufactured by Motras Scientific Instruments Pvt. Ltd.), employing a suitable hollow cathode lamp. The analysis was carried out to determine the content of iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) within the plant sample [16].

2.4.8. Detection of boron

The boron content of the plant sample was estimated using dry ashing of the plant sample followed by the azomethine H method (Spectrometer Model 2203, Systronics, India) as described in the reference [17].

2.5. Total phenolic content

To assess total phenolics, the Folin-Ciocalteu technique was followed [18-20]. The reagents used for the analysis included: 1 N Folin-Ciocalteu reagent (FCR), a saturated solution of sodium carbonate, and gallic acid. In this procedure, 0.5 mL of various concentrations of extracts were mixed with 1 N Folin-Ciocalteu reagent and 1.0 mL of a 20% Na₂CO₃ aqueous solution. Subsequently, 5.0 mL of distilled water was added to the mixture, which was thoroughly combined. After incubation at room temperature for 30 minutes, the absorbance was measured at 765 nm. A standard curve was generated using gallic acid and the results were expressed as milligrams of gallic acid per gram of dry weight sample.

2.6. Total flavonoid content

To evaluate the total flavonoid content, the aluminum chloride colorimetric assay method outlined in references [21,22] was used. To prepare the stock solution, 10 mg of various extracts were dissolved in 10 mL of 80% methanol and in another sample 80% hexane. Then 1 mL of the stock solution was combined with 1.25 mL of water, followed by the addition of 0.75 mL of a 5% NaNO₂ solution in a test tube. The mixture was incubated at room temperature for 5 minutes. Subsequently, 0.15 mL of 10% AlCl₃ was introduced and after a 6-minute interval, 0.5 mL of 1 N NaOH and 275 μ L of distilled water were added. The solution was thoroughly mixed, resulting in the development of a pink color, which was measured using a photo spectrometer at 510 nm. The standard curve for the flavonoid content was validated using various concentrations of catechin and the concentrations of the samples were determined using the calibration curve. The total flavonoid content in the sample extracts was expressed as milligrams of catechin equivalents per 100 grams of dry material. All samples were analyzed in triplicate.

2.7. Antioxidant activity

The antioxidant activity potential was measured in terms of an extract's ability to scavenge DPPH radicals. Various methods were used to gauge the antioxidant characteristics of these extracts, which included DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, hydroxyl radical scavenging activity, and metal chelating activity.

2.7.1. DPPH free radical scavenging activity

The radical scavenging activity of the methanolic and hexane extracts was evaluated using a slightly modified version of the method given in references [23-25]. In summary, this method relies on the extract's capacity to counteract the DPPH radical. The reagents used included 0.4 mM DPPH dissolved in methanol, gallic acid, and BHT. DPPH is a stable free radical that can undergo electron or hydrogen radical acceptance to become a stable diamagnetic molecule. The activity was measured at 517 nm spectrophotometrically (Thermo Scientific Evolution-201 series). These measurements were taken after mixing the extracts with varying amounts (5 to 25 µg/mL) of freshly prepared MeOH solution containing 0.004% DPPH. BHT served as the reference antioxidant, and each measurement was performed in triplicate. The control consisted of the reaction mixture without the test solution (DPPH radical solution). The IC₅₀ values for all samples were determined by regression analysis. The percent inhibition (%IC) of DPPH's free radical inhibition was calculated using Equation 2.

$$\% \text{ IC} = (A_0 - A_t) / A_0 \times 100 \quad (2)$$

where A₀ = Absorbance value of control sample, A_t = Absorbance value of test sample, IC = Inhibitory concentration.

2.7.2. Metal chelating activity

The metal chelating activity of the extracts was assessed following the procedures practiced [26-24]. Briefly, this method relies on measuring the formation of the ferrous iron-ferrozine complex at 562 nm to gauge the extracts' capability to chelate Fe²⁺ from antioxidants. The following reagents were used: 5 mM ferrozine, 2 mM FeCl₂·4H₂O, and sodium of ethylenediamine tetraacetate (Na₂EDTA). The evaluation of the metal chelating activity of Fe²⁺ by components of solvent extracts was carried out using a spectrophotometric method. This approach is founded on the Fe²⁺ chelating capacity of antioxidants, assessed by measuring the absorbance of the ferrous ion-ferrozine complex formed at 562 nm [28]. For each test, 0.1 mL of 2 mM FeCl₂·4H₂O, 0.2 mL of 5 mM ferrozine, and 4.7 mL of methanol were mixed with different concentrations of the samples tested (5, 10, 15, 20, 25 µg/mL). The solution was thoroughly mixed and then allowed to incubate for 10 minutes. Subsequently, the absorbance of the test sample was measured at 562 nm. All readings were taken in triplicate, with Na₂EDTA (0.01 mM) serving as the standard. The percent of chelating activity was plotted against concentration and the standard curve was drawn using a standard chelating agent (Na₂EDTA) to calculate the IC₅₀ values for standard and different samples of solvent extracts. The metal-chelating activity of the tested samples was calculated using Equation 3.

$$\% \text{ IC} = (A_0 - A_t) / A_0 \times 100 \quad (3)$$

where A₀ = Absorbance value of control sample, A_t = Absorbance value of test sample, IC = Inhibitory concentration.

2.7.3. Hydroxyl radical scavenging activity

A standard protocol was followed to assess the hydroxyl radical scavenging activity of extracts [24,29,30]. Reagents used were 0.17 M hydrogen peroxide, 1 mM 1,10-phenanthroline, 0.1 mM ferrous sulfate, 0.2 M sodium phosphate buffer (pH = 7.8) and ascorbic acid. The experiment involved varying amounts (5 to 25 µg/mL) of different extracts, combined with 0.6 mL of a 40 mM H₂O₂ solution prepared in phosphate buffer with a pH of 7.4. Subsequently, the absorbance of the resulting reaction mixture was compared to that of a blank solution, which consisted of the extract in phosphate buffer without H₂O₂. As a

positive control, BHT was used. IC₅₀ values of all samples were calculated with regression method analysis. The hydroxyl radical scavenging percentages for the different extracts (in both hexane and methanol) and the standard antioxidant were then calculated (Equation 4).

$$\% \text{ Scavenging} = (A_0 - A_t) / A_0 \times 100 \quad (4)$$

where A₀ = Absorbance value of control sample, A_t = Absorbance value of test sample, IC = Inhibitory concentration.

2.8. Anti-diabetic activity

The standard method [31,32] was used to determine the antidiabetic activity of methanol and hexane extracts with a slight modification. The reagents used were 0.02 M sodium phosphate buffer (pH = 6.9), 1% starch solution, DNS reagent, acarbose, and α-amylase. We tested various amounts (5 to 25 µL) of the standard medication, acarbose, as well as different strengths of the extracts in hexane and methanol. The reaction mixtures were allowed to react with 200 µL of α-amylase enzyme and 100 µL of 2 mM phosphate buffer (pH = 6.9). The reaction mixture was incubated for 20 minutes before adding 100 µL of a 1.0% starch solution. After a 5-minute incubation period, 500 µL of dinitrosalicylic acid reagent was mixed with the test solution, followed by boiling for 5 minutes in a water bath. The absorbance at 540 nm was measured using a Thermo Scientific EVOLUTION-201 series spectrophotometer. As for the control, the same treatment was applied, but instead of 200 µL of enzyme, buffer was used as a substitute for the positive control. The percentage of inhibition of the alpha-amylase enzyme was determined using Equation 5.

$$(\%) \text{ Inhibition} = (A_0 - A_t) / A_0 \times 100 \quad (5)$$

where A₀ = Absorbance value of control sample, A_t = Absorbance value of test sample, IC = Inhibitory concentration.

2.9. In-vitro anti-inflammatory activity

We used a slightly modified version of the methodology described to assess the anti-inflammatory activity of methanol and hexane extracts [33,34]. Reagents used were 0.02 M phosphate buffer and sodium diclofenac. The reaction mixture consisted of 200 mL of fresh albumin protein and 2.0 mL of hexane and methanol extracts at various amounts (5 to 25 µL). To this mixture, 2.8 mL of phosphate-buffered saline (pH = 6.4) was added to achieve a final volume of 5.0 mL. The reaction mixture was initially incubated at 37 °C for 15 minutes and then further heated at 70 °C for 5 minutes. After cooling, the absorbance at 660 nm was measured. Diclofenac sodium was used as a positive control, and double-distilled water served as a standard treatment to measure absorbance. Equation 6 was used to calculate the percentage of inhibition of protein denaturation.

$$(\%) \text{ Inhibition} = (A_0 - A_t) / A_0 \times 100 \quad (6)$$

where A₀ = Absorbance value of control sample, A_t = Absorbance value of test sample, IC = Inhibitory concentration.

3. Results and discussion

3.1. GC-MS analysis

GC-MS analysis of *P. crenulata* has been reported to exhibit β-carotene, lycopene, carbohydrates, proteins, saponins, flavonoids, terpenoids, alkaloids, and tannins [35].

Table 2. Chemical composition of some of the main compounds present in five different fruit extracts *.

Compound	%Contribution	KI	Molecular formula	Mass fragmentation, m/z
[A] Chemical composition of PCHE01				
Hexatriacontane	29.9	3600	C ₃₆ H ₇₄	M ⁺ = 338: 43, 57 (100%), 71, 85, 99
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	9.2	3350	C ₃₀ H ₅₂ O ₂	M ⁺ = 444: 41, 55, 67, 81 (100%), 95
Tetracontane	7.3	3997	C ₄₀ H ₈₂	M ⁺ = 562: 43, 57 (100%), 71, 85, 99
Octadeca-9,12-dienoyl chloride	4.9	2139	C ₁₈ H ₃₁ ClO	M ⁺ = 298: 41, 55, 67(100%), 81, 95
Octadecenoic acid, methyl ester	3.9	2093	C ₁₉ H ₃₄ O ₂	M ⁺ = 294: 41, 55, 67 (100%), 81, 95
B-Sitosterol	3.6	2731	C ₂₉ H ₅₀ O	M ⁺ = 414: 41, 43, 57, 81, 95, 414 (100%)
Tridecanedial	3.2	6190	C ₁₃ H ₂₄ O ₂	M ⁺ = 252: 41, 55(100%), 81, 95, 109
α-Tochopherol	2.0	3149	C ₂₉ H ₅₀ O ₂	M ⁺ = 430: 41, 43, 57, 165 (100%), 430
[B] Chemical composition of BAHE02				
10-Nonadecanol	20.0	2072	C ₁₉ H ₄₀ O	M ⁺ = 284: 41, 55, 69, 83 (100%), 97
Nonanoic acid	17.3	1907	C ₉ H ₁₈ O ₂	M ⁺ = 352: 41, 55, 67, 79 (100%)
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	12.1	3350	C ₃₀ H ₅₂ O ₂	M ⁺ = 444: 41, 55 (100%), 67, 81, 95
Tetracontane	5.2	3997	C ₄₀ H ₈₂	M ⁺ = 562: 43, 57(100%), 71, 85
Tridecanedial	3.9	6190	C ₁₃ H ₂₄ O ₂	M ⁺ = 212: 27, 41, 55 (100%), 67, 95
1,3-Dicyclopentyl-2-n-dodecylcyclopentane	2.9	-	C ₂₇ H ₅₀	M ⁺ = 308: 41, 55, 67 (100%), 81, 95
1-Heptadec-1-ynyl-cyclohexanol	2.4	2713	C ₂₃ H ₄₂ O	M ⁺ = 278: 41, 55, 68 (100%), 82, 95
cis,cis-7,10,-Hexadecadienal	2.3	1816	C ₁₆ H ₂₈ O	M ⁺ = 236: 41, 55, 67 (100%), 81
[C] Chemical composition of REHE03				
Tetracontane	14.6	3997	C ₄₀ H ₈₂	M ⁺ = 506: 41, 43, 57, 71, 85(100%)
Heneicosane	6.7	2109	C ₂₁ H ₄₄	M ⁺ = 296: 41, 57 (100%), 71, 85
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	6.6	3350	C ₃₀ H ₅₂ O ₂	M ⁺ = 444: 41, 55 (100%), 76, 81, 95
3R,4S-Epoxy-6Z,9Z-nonadecadiene	5.6	1907	C ₁₉ H ₃₄ O	M ⁺ = 278: 41, 55, 67, 81, 79 (100%)
α-Tochopherol	5.5	3149	C ₂₉ H ₅₀ O ₂	M ⁺ = 416: 41, 57, 71, 151 (100%), 191, 416
14-Methyl-8-hexadecyn-1-ol	2.3	-	C ₁₇ H ₃₄ O	M ⁺ = 252: 41, 55, 67, 81 (100%), 95, 109
[D] Chemical composition of FPHE04				
Lupenyl acetate	9.8	3380	C ₃₂ H ₅₂ O ₂	M ⁺ = 468: 43 (100%), 69, 81, 95
Linoleic acid	8.7	2183	C ₁₉ H ₃₄ O ₂	M ⁺ = 280: 41, 55, 67 (100%), 81
1-Bromotetracosane	5.5	2703	C ₂₄ H ₄₈ Br	M ⁺ = 416: 41, 43, 57 (100%), 82
Lanosterol acetate	3.2	3022	C ₃₂ H ₅₂ O ₂	M ⁺ = 468: 41, 55, 69 (100%), 81, 95, 39
Linolenic acid	3.0	2101	C ₁₉ H ₃₂ O ₂	M ⁺ = 294: 41, 55, 67 (100%), 81
Palmitic acid	2.1	3350	C ₁₇ H ₃₄ O ₂	M ⁺ = 444: 41, 55 (100%), 67, 81
Hexatriacontane	2.1	3600	C ₃₆ H ₇₄	M ⁺ = 506: 43, 57 (100%), 71, 85
Methyl commate D	2.0	2923	C ₃₁ H ₅₀ O ₄	M ⁺ = 486: 49, 55, 69, 95, 107, 119, 133, 218 (100%)
[E] Chemical composition of MEHE05				
Linoleic acid	16.2	2183	C ₁₉ H ₃₄ O ₂	M ⁺ = 280: 41, 55, 67 (100%), 81
Palmitic acid	7.6	3350	C ₁₇ H ₃₄ O ₂	M ⁺ = 256: 41, 55, 60, 73 (100%)
13,27-Cyclours-11-en-3-ol	4.1	3022	C ₃₂ H ₅₀ O ₂	M ⁺ = 468: 41, 43, 69 (100%), 81
Hexatriacontane	3.8	3600	C ₃₆ H ₇₄	M ⁺ = 506: 41, 43, 57 (100%), 71, 85, 99
Acetic acid, 17-(1,5-dimethylhex-4-enyl)-4,4,8,10,14-pentamethyl-2,3,4,5,6,7,8,9,10,11,12,14,15,16-tetradecahydro-1H-cyclopenta[a]phenanthrene	2.7	-	C ₃₂ H ₅₂ O ₂	M ⁺ = 468: 41, 69 (100%), 81, 95, 109
Lup-20(29)-en-3-ol	2.1	-	C ₃₀ H ₅₀ O	M ⁺ = 318: 41, 55, 67, 79 (100%)
β-Amyrin acetate	2.0	2976	C ₃₂ H ₅₂ O ₂	M ⁺ = 424: 218 (100%), 203, 95, 55

* PCHE01 = *P. crenulata* hexane extract 01, BAHE02 = *B. asiatica* extract, REHE03 = *R. ellipticus*, FPHE04 = *F. palmate* extract, MEHE05 = *M. esculenta* extract, KI = Kovate index.

The current study reveals the first report on *P. crenulata* oleoresin. It is noteworthy that this is the first report presenting the GC-MS analysis of BAHE02 (Table 2, Figure 1). Previous studies on *B. asiatica* have reported the presence of various compounds such as berberine, steroids, flavonoids, coumarins, terpenoids, and phenols. Furthermore, alkaloids constitute the primary class of phytoconstituents found in *B. aristata* such as karachine, oxyberberine, oxycanthine, berbamine and taxilamine, as documented by references [7,36,37]. Previous studies on *R. ellipticus* have reported the presence of secondary metabolites such as phenols, carbohydrates, flavonoids, glycosides, alkaloids, saponins, tannins, resin, and protein fibers [38,39]. A phytochemical study was conducted on the aerial components of *F. palmata* led to the discovery of six compounds: germanicol acetate, psoralene, bergapten (5-methoxy-psoralene), vanillic acid, flavone glycoside, and rutin. Alkaloids, steroids, flavonoids, tannins, sitosterols, carbohydrates, and polyphenols such as psoralene, bergapten, gallic acid, vanillic acid, glaucol acetate, as well as steroidal teroidal glycosides, have been reported in *F. palmata* [40,41]. It is noteworthy that earlier investigations on *Myrica esculenta* have documented a variety of compounds, including alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, tannins, amino acids, 1-ethyl-4-methylcyclohexane, myo-inositol, methyl-d-lyxofuranoside, 2-furancarboxyaldehyde, 2,5-furandionedi-hydro-3-methylene, furfural, oxirane, quercetin along with gallic acid, myricanol, myricanone,

epigallocatechin 3-O-gallate, two prodelphinidin dimers: epigallocatechin-(4β→8)-epigallocatechin 3-O-gallate and 3-O-galloyl-epigallocatechin-(4β→8)-epigallocatechin 3-O-gallate [42,43].

3.2. Biochemical assay

3.2.1. Total phenolic content

Significant variations in the total phenolic content were observed in both the hexane and methanol extracts of all five fruits. The order of the phenolic content of all plant fruit extract was quantitatively found in the order of FPME4 (66.92±0.096 mg/g GAE) > BAHE02 (66.62±0.423 mg/g GAE) > FPHE04 (58.94±0.134 mg/g GAE) > BAME2 (47.14±0.092 mg/g GAE) > MEHE05 (45.74±0.167 mg/g GAE) > REHE03 (40.02±0.661 mg/g GAE) > REME3 (37.17±0.096 mg/g GAE) > PCHE01 (26.50±0.536 mg/g GAE) > MEME5 (23.24±0.880 mg/g GAE) > PCME1 (22.13±0.435 mg/g GAE). The calculation of the total phenolic content was performed using a standard calibration curve generated with different dilutions of gallic acid. The variations observed in the total phenolic content across different extracts are likely attributed to differences in the solubility of phenolic compounds in solvents with varying polarities. It is important to note that the total phenolic content has been reported in previous studies for the individual fruits: *P. crenulata* [35], *B. asiatica* [44], *R. ellipticus* [39], *F. palmata*

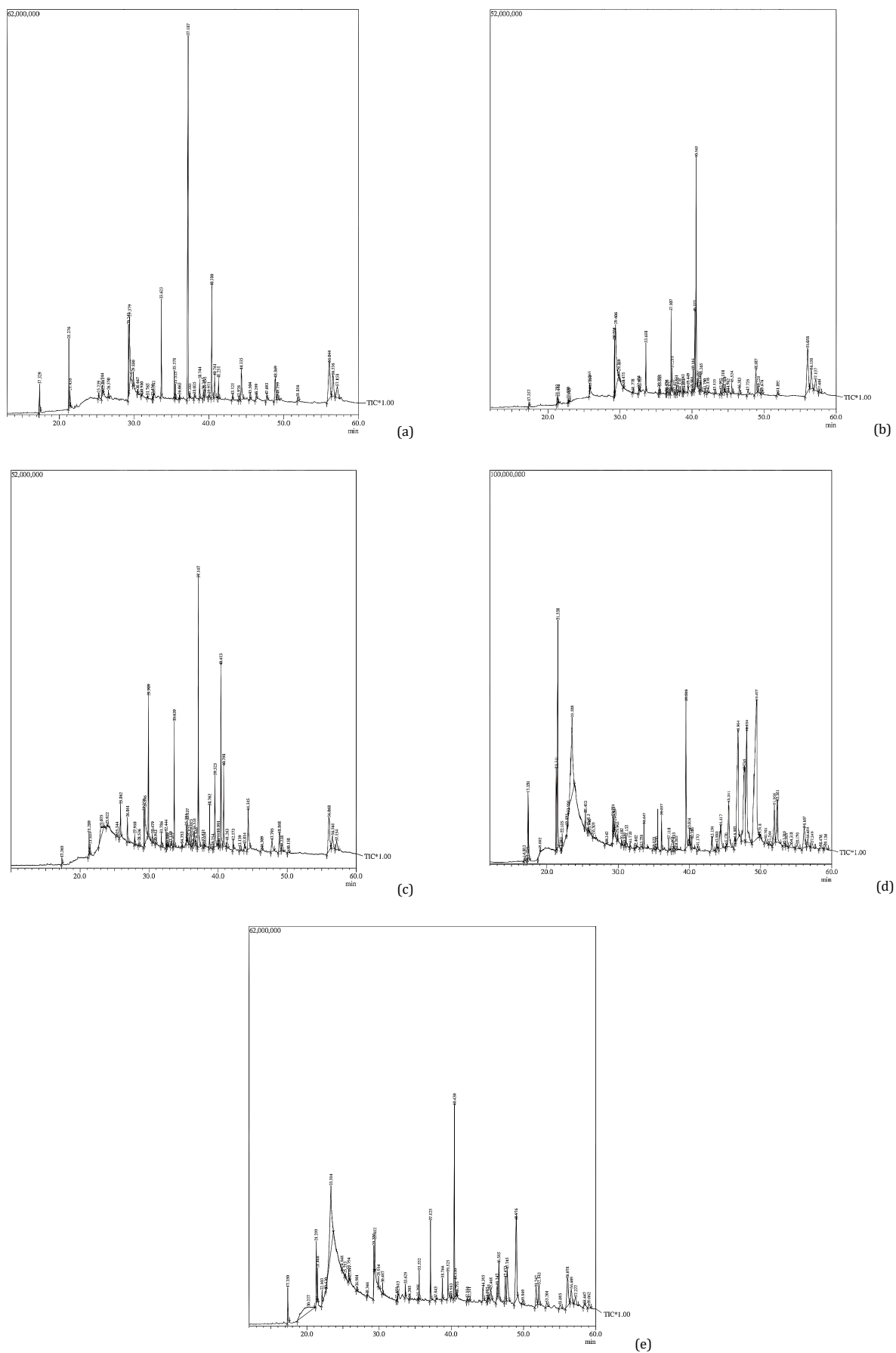


Figure 1. Gas chromatograms of PCHE01 (a), BAHE02 (b), REHE03 (c), FPHE04 (d), and MEHE05 (e).

Table 3. Total phenolic and flavonoid content in extracts *.

No	Sample	Total phenolic content (mg/g GAE)	Total flavonoid content (mg/g CNE)
1	PCHE01	26.50±0.536	31.43±0.075
2	BAHE02	66.62±0.423	42.03±0.223
3	REHE03	40.02±0.661	41.39±0.230
4	FPHE04	58.94±0.134	36.35±0.223
5	MEHE05	45.74±0.167	21.52±0.271
6	PCME1	22.13±0.435	25.91±0.434
7	BAME2	47.14±0.092	44.14±0.125
8	REME3	37.17±0.096	28.48±0.534
9	FPME4	66.92±0.096	69.54±0.227
10	MEME5	23.24±0.880	23.87±0.374

* PCHE01 = *P. crenulata* (Hexane), BAHE02 = *B. asiatica* (Hexane), REHE03 = *R. ellipticus* (Hexane), FPHE04 = *F. palmata* (Hexane), MEHE05 = *M. esculenta* (Hexane) PCME1 = *P. crenulata* (Methanol), BAME2 = *B. asiatica* (Methanol), REME3 = *R. ellipticus* (Methanol), FPME4 = *F. palmata* (Methanol), MEME5 = *M. esculenta* (Methanol).

Table 4. Antioxidant activity of hexane and methanol extracts.

Sample/Standard	Antioxidant activity in terms of IC ₅₀ (µg/mL±SD)					
	DPPH free radical scavenging activity		Metal chelating activity		Hydroxyl scavenging activity	
	Hexane extract	Methanol extract	Hexane extract	Methanol extract	Hexane extract	Methanol extract
PCHE01/PCME1	7.53±0.03	11.58±0.01	11.81±0.03	7.96±0.01	0.04±0.05	12.09±0.19
BAHE02/BAME2	7.74±0.07	7.84±0.02	9.35±0.03	24.29±0.12	5.27±0.07	18.52±0.22
REHE03/REME3	5.16±0.02	2.85±0.06	11.15±0.01	16.02±0.06	17.17±0.45	6.76±0.03
FPHE04/FPME4	7.13±0.05	3.50±0.03	26.99±0.08	7.52±0.05	8.16±0.03	9.67±0.34
MEHE05/MEME5	4.82±0.07	6.60±0.01	13.54±0.07	11.48±0.04	5.52±0.05	6.38±0.11

* PCHE01 = *P. crenulata* (Hexane), BAHE02 = *B. asiatica* (Hexane), REHE03 = *R. ellipticus* (Hexane), FPHE04 = *F. palmata* (HEXANE), MEHE05 = *M. esculenta* (Hexane), PCME1 = *P. crenulata* (Methanol), BAME2 = *B. asiatica* (Methanol), REME3 = *R. ellipticus* (Methanol), FPME4 = *F. palmata* (Methanol) MEME5 = *M. esculenta* (Methanol).

[45], and *M. esculenta* [43]. However, this study provides the first report of the phenolic content in these fruits using different solvents. The quantitative composition of the total phenolic content is presented in Table 3.

3.2.2. Total flavonoid content

It was achieved by measuring the acid hydrolysates of the various extracts in 1.2N HCL. The order of the flavonoid content with all the fruit extract was in the order FPME4 (69.54±0.227 mg/g CNE) > BAME2 (44.14±0.125 mg/g CNE) > BAHE02 (42.03±0.223 mg/g CNE) > REHE03 (41.39±0.230 mg/g CNE) > FPHE04 (36.35±0.223 mg/g CNE) > PCHE01 (31.43±0.075 mg/g CNE) > REME3 (28.48±0.534 mg/g CNE) > PCME1 (25.91±0.434 mg/g CNE) > MEME5 (23.87±0.374 mg/g CNE) > MEHE05 (21.52±0.271 mg/g CNE). Total flavonoid has also been reported in *P. crenulata* [35], *B. asiatica* [44], *R. ellipticus* [39], *F. palmata* [45], *M. esculenta* [43]. However, the phenolic content in different solvents is being reported for the first time. The quantitative composition of total flavonoids in different extracts is represented in Table 3.

3.3. Antioxidant activity

3.3.1. Hydroxyl radical scavenging activity

The extracts of hexane and methanol showed a dose-dependent ability to scavenge hydroxyl radicals at various concentrations (5, 10, 15, 20, and 25 µg/mL). As a reference, ascorbic acid was used as a standard antioxidant. In methanol extract, the hydrogen peroxide radical scavenging activity was found to be highest in MEME5 (6.38±0.11 µg/mL) and lowest in BAME2 (18.52±0.22 µg/mL). In terms of IC₅₀ values, the order of hydrogen peroxide radical scavenging activity was as follows: BAME2 (18.52±0.22 µg/mL) > PCME1 (12.09±0.19 µg/mL) > FPME4 (9.67±0.34 µg/mL) > REME3 (6.76±0.03 µg/mL) > MEME5 (6.38±0.11 µg/mL). In the methanol extract, the most effective antioxidant, with the lowest IC₅₀ value, was MEME5 (6.38±0.11 µg/mL), indicating its potential as a strong natural antioxidant. Similarly, in the hexane extract, PCHE01 demonstrated the highest activity (0.04±0.05 µg/mL), while REHE03 exhibited the minimum activity (17.17±0.45 µg/mL). In the hexane extract, PCHE01 shows the lowest IC₅₀ value (0.04±0.05 µg/mL), so it could act as a good natural antioxidant.

3.3.2. DPPH free radical scavenging activity

Dose-dependent antioxidant activity was evaluated by evaluating the DPPH radical scavenging capacity of fruit extracts. The tested concentrations (5 to 25 µg/mL) of the extract showed an effective inhibition of DPPH free radicals. A noticeable reduction in absorbance values was observed as a function of concentration. The extracts were compared with standard antioxidant agents, butylated hydroxyl toluene (BHT), and ascorbic acid, to evaluate their capacity to neutralize free radicals. REME3 displayed the highest activity, with an IC₅₀ value of 2.85±0.06 µg/mL, while PCME1 exhibited the lowest activity, with an IC₅₀ value of 11.58±0.01 µg/mL. The order of DPPH scavenging activity, based on IC₅₀ values, was as follows: REME3 (2.85±0.06 µg/mL) < FPME4 (3.50±0.03 µg/mL) < MEME5 (6.60±0.01 µg/mL) < BAME2 (7.84±0.02 µg/mL) < PCME1 (11.58±0.01 µg/mL). The methanol extract showed the lowest IC₅₀ value for REME3 (2.85±0.06 µg/mL), indicating its potential as a powerful natural antioxidant. In the hexane extract, the highest DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was observed in MEHE05, with an IC₅₀ value of 4.28±0.07 µg/mL, and the lowest activity was recorded for BAHE02, with an IC₅₀ value of 7.74±0.07 µg/mL. The results, including the IC₅₀ values, are provided in Table 4.

3.3.3. Metal chelating activity

In the methanol extract, metal chelating activity was observed in relation to concentration. The order of metal chelating activity, based on IC₅₀ values, was observed as follows: FPME4 (7.52±0.05) < PCME1 (7.96±0.01) < MEME5 (11.48±0.04) < REME3 (16.02±0.06) < BAME2 (24.29±0.12). The highest activity was observed in FPME4, with an IC₅₀ value of 7.52±0.05, indicating its potential as a strong antioxidant. The lowest activity was found in BAME2 (24.29±0.12). Similarly, in the hexane extract, the highest metal chelating activity was observed in BAHE02 (9.35±0.03), and the lowest activity was observed in FPHE04 (26.99±0.08). The order of metal chelating activity in the hexane extract, based on IC₅₀ values, was as follows: BAHE02 (9.35±0.03) < REHE03 (11.15±0.01) < PCHE01 (11.81±0.03) < MEHE05 (13.54±0.07) < FPHE04 (26.99±0.08). Among these, BAHE02 exhibited the lowest IC₅₀ value (9.35±0.03), suggesting its potential as a strong natural antioxidant. The results, including the IC₅₀ values, are provided in Table 4.

Table 5. *In-vitro* anti-inflammatory activity of hexane and methanol extracts *.

Sample/Standard	<i>In vitro</i> anti-inflammatory activity in terms of IC ₅₀ (µg/mL±SD)	
	Hexane extract	Methanol extract
PCHE01/PCME1	6.79±0.03	10.07±0.04
BAHE02/BAME2	4.47±0.87	8.06±0.02
REHE03/REME3	7.16±0.03	7.50±0.03
FPHE04/FPME4	5.14±0.06	10.08±0.04
MEHE05/MEME5	10.07±0.23	16.46±0.02

* PCHE01 = *P. crenulata* (Hexane), BAHE02 = *B. asiatica* (Hexane), REHE03 = *R. ellipticus* (Hexane), FPHE04 = *F. palmata* (HEXANE), MEHE05 = *M. esculenta* (Hexane), PCME1 = *P. crenulata* (Methanol), BAME2 = *B. asiatica* (Methanol), REME3 = *R. ellipticus* (Methanol), FPME4 = *F. palmata* (Methanol) MEME5 = *M. esculenta* (Methanol).

3.4. *In-vitro* anti-inflammatory activity

The *in vitro* anti-inflammatory activities of the extracts were evaluated using the protein denaturation method with fresh hen's egg albumin. The level of protection for albumin increased as the concentration of the extracts increased. As the dose amount increased, the absorbance values decreased, indicating a concentration-dependent inhibition of protein denaturation. Sodium diclofenac concentration range of 50 to 250 µg/mL was used as a reference drug and exhibited a concentration-dependent inhibition of protein denaturation. In the methanol extract, the *in vitro* anti-inflammatory activity was examined concerning concentration. BAME2 showed the highest activity with an IC₅₀ (8.6±0.02 µg/mL), while MEME5 showed the lowest activity with an IC₅₀ (16.46±0.02 µg/mL). The order of *in vitro* anti-inflammatory activity, based on IC₅₀ values, was as: REME3 (7.50±0.03 µg/mL) < BAME2 (8.06±0.02 µg/mL) < PCME1 (10.07±0.04 µg/mL) < FPME4 (10.08±0.04 µg/mL) < MEME5 (16.46±0.02 µg/mL). Among these, REME3 exhibited the lowest IC₅₀ (7.50±0.03 µg/mL), suggesting its potential as a strong natural anti-inflammatory agent. Similarly, in the hexane extract, the activity was observed to be highest in BAHE02 (4.47±0.87 µg/mL) and lowest in MEHE05 (10.07±0.23 µg/mL). In terms of the IC₅₀ value, the order of *in vitro* anti-inflammatory activity observed was: BAHE02 (4.47±0.87 µg/mL) < FPHE04 (5.14±0.06 µg/mL) < PCHE01 (6.79±0.03 µg/mL) < REHE03 (7.16±0.03 µg/mL) < MEHE05 (10.07±0.23 µg/mL). In hexane extract, BAHE02 showed the lowest IC₅₀ value (4.47±0.87 µg/mL), so might act as a good natural anti-inflammatory. The anti-inflammatory activity of *P. crenulata* [46,47], *R. ellipticus* [6], and *M. esculenta* [48] has also been previously reported. For *Berberis asiatica* and *F. palmata* extract (hexane and methanol) extract, the anti-inflammatory activities have been reported for the first time in the current investigation. The results observed with the IC₅₀ values are depicted in Table 5.

3.5. Antidiabetic activity

The purpose of this investigation was to assess the potential antidiabetic properties of hexane and methanol extracts from five Himalayan plants that inhibit the enzyme amylase. The absorbance readings of the samples showed a decreasing trend with increasing extract concentration, while the percentage of amylase inhibition increased. Among the extracts, the methanol extract demonstrated the highest α-amylase inhibition activity, while the hexane extract exhibited slightly lower α-amylase inhibition activity. The α-amylase inhibition activity of the methanol extract was found to be concentration dependent, with the highest activity observed in PCME1 (97.061±0.056 µg/mL) and the lowest in REME3 (75.339±0.032 µg/mL). In terms of IC₅₀ values, the order of α-amylase inhibition activity was as follows for the methanol extract: PCME1 (4.02±0.05 µg/mL) < FPME4 (5.26±0.02 µg/mL) < MEME5 (7.52±0.02 µg/mL) < BAME2 (8.81±0.02 µg/mL) < REME3 (15.16±0.02 µg/mL). The methanolic extract of PCME1 exhibited the lowest IC₅₀ value (4.02±0.05 µg/mL), suggesting its potential as a natural antidiabetic agent. Similarly, in the hexane extract, the highest activity was observed in PCHE01 (5.37±0.01 µg/mL),

and the lowest in MEHE05 (11.7±0.05 µg/mL). The order of α-amylase inhibition activity for the hexane extract, based on IC₅₀ values, was: PCHE01 (5.37±0.01 µg/mL) < BAHE02 (6.42±0.01 µg/mL) < FPHE04 (7.63±0.03 µg/mL) < REHE03 (7.69±0.01 µg/mL) < MEHE05 (11.7±0.05 µg/mL). Although the antidiabetic properties of *R. ellipticus* [49], *F. palmata* [46], and *M. esculenta* [50] have been documented in prior research, it is important to note that the antidiabetic activities of the extracts of *P. crenulata* and *B. asiatica* fruit hexane and methanol are reported for the first time in the current investigation. The results observed with the IC₅₀ values are depicted in Table 6.

Table 6. Antidiabetic activity of hexane and methanol extracts.

Sample/Standard	Antidiabetic activity in terms of IC ₅₀ (µg/mL±SD)	
	Hexane extract	Methanol extract
PCHE01/PCME1	5.37±0.01	4.02±0.05
BAHE02/BAME2	6.42±0.01	8.81±0.02
REHE03/REME3	7.69±0.01	15.16±0.02
FPHE04/FPME4	7.63±0.03	5.26±0.02
MEHE05/MEME5	11.7±0.05	7.52±0.02

* PCHE01 = *P. crenulata* (Hexane), BAHE02 = *B. asiatica* (Hexane), REHE03 = *R. ellipticus* (Hexane), FPHE04 = *F. palmata* (HEXANE), MEHE05 = *M. esculenta* (Hexane), PCME1 = *P. crenulata* (Methanol), BAME2 = *B. asiatica* (Methanol), REME3 = *R. ellipticus* (Methanol), FPME4 = *F. palmata* (Methanol) MEME5 = *M. esculenta* (Methanol).

3.6. Elemental analysis

The nutritional elemental analysis using atomic absorption spectroscopy was conducted on plants, namely, *P. crenulata* (PC), *R. ellipticus* (RE), *M. esculenta* (ME), *F. palmata* (FP), and *B. asiatica* (BA). The findings showed that the boron content was the highest in all samples, with values of 282.3, 389.0, 360.8, 382.7, and 432.9 mg/kg, respectively. The detailed quantitative composition of all these nutritional elements can be found in Table 7. The result indicates that the nutritional values and mineral contents of these wild fruits under investigation were richer than those of the commercial fruits and very much comparable with the various wild fruits reported earlier. The elemental analysis of *M. esculenta* indicated a notably high iron concentration, while sodium concentration was found to be minimal, as reported by [51]. The findings of this study highlight the diverse composition of extracts from five wild fruits in the Himalayan region of Uttarakhand.

Biological activity assays and elemental analyses underscore the significant nutraceutical potential of these plants. They could serve as effective dietary supplements and hold industrial significance in the field of nutraceuticals. Fruit extracts require a comprehensive examination of their nutritional potential through *in vivo* studies. This research is of significance not only from an academic perspective but also for the establishment of a valuable database that can guide the sustainable use of edible wild plants.

4. Conclusions

The findings of this research highlighted the diverse composition of five wild fruit extracts, namely *P. crenulata*, *R. ellipticus*, *M. esculenta*, *F. palmata*, and *B. asiatica*, from the Uttarakhand Himalayan region.

Table 7. Elemental analysis of some wild fruits growing in the Himalayan region*.

No	Nutritional elements	<i>P. crenulata</i>	<i>B. asiatica</i>	<i>R. ellipticus</i>	<i>F. palmata</i>	<i>M. esculenta</i>
1	Total nitrogen (%)	1.26	2.10	1.43	1.57	1.48
2	Total phosphorus (%)	0.092	0.21	0.16	0.27	0.16
3	Total potassium (%)	0.74	0.77	0.76	1.91	0.64
4	Total sulfur (%)	0.14	0.16	0.16	0.18	0.20
5	Total iron (mg/kg)	139.80	80.60	42.60	99.60	80.60
6	Total manganese (mg/kg)	-	-	-	-	-
7	Total zinc (mg/kg)	1.80	2.40	0.40	9.40	-
8	Total copper (mg/kg)	-	15.20	17.40	-	-
9	Total boron (mg/kg)	282.30	389.00	360.80	382.70	432.90
10	Total calcium (%)	4.00	4.00	2.40	2.40	3.84
11	Total magnesium (%)	3.40	2.40	2.90	1.60	1.05

* "-" = Not detected.

The dried fruits were processed using a cold percolation technique for extraction, employing both hexane and methanol solvents. The resultant hexane extracts were analyzed through GC-MS, while elemental analysis was conducted using AAS. Furthermore, more extensive exploration of fruit extracts is warranted to fully understand their nutritional potential, necessitating in vivo studies. This step is crucial not only for academic purposes but also for establishing a comprehensive database for the sustainable and responsible exploitation of the rich diversity of wild edible flora. Such an approach would enable the cultivation and use of these plants in a manner that balances human needs with the preservation of the natural ecosystem.

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Ethical approval: All ethical guidelines have been adhered to.

Sample availability: Samples of the compound are available from the author.

Data availability: Data will be available upon request.

CRedit authorship contribution statement

Conceptualization: Bhawana Verma, Stuti Arya; Methodology: Bhawana Verma, Om Prakash; Software: Tanuja Kabdal, Stuti Arya; Validation: Om Prakash, Ravendra Kumar; Formal Analysis: Dharmendra Singh Rawat, Om Prakash; Investigation: Ravendra Kumar, Shiv Kumar Dubey; Resources: Dharmendra Singh Rawat, Om Prakash; Data Curation: Bhawana Verma, Stuti Arya; Writing - Original Draft: Bhawana Verma; Writing - Review and Editing: Stuti Arya, Tanuja Kabdal; Visualization: Sonal Tripathi, Vandana Arya; Funding acquisition: Om Prakash, Ravindra Kumar; Supervision: Om Prakash; Project Administration: Om Prakash, Shiv Kumar Dubey.

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