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Assessment of phytochemical composition, antioxidant and antidiabetic potential of fruits of *Podophyllum hexandrum*

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Podophyllum hexandrum (family Berberidaceae) is an important endangered medicinal plant distributed in the temperate and subalpine areas of the Hindu-Kush Himalaya and its ripened fruits are consumed by local people of the region. Phytochemical composition (phenolic, flavonoid, flavonol, and anthocyanins), antioxidant activities through DPPH, FRAP, and ABTS assays, and antidiabetic property assessed through α-amylase enzyme inhibition, were evaluated in ripened fruits. The fruits of *P. hexandrum* appeared to be rich in total phenolics (3.13 mg GAE/g fw) and total flavonoid content (7.59 mg QE/g fw). The considerable amounts of total flavonols (7.98 mg QE/g fw) and anthocyanins (0.49 mg/g) were recorded in fruits. A substantial amount of riboflavin (0.17 mg/100 g), lycopene (0.11 mg/100 g), total carotene (2.07 mg/100 g) and β-carotene (0.31 mg/100 g) was also recorded in the fruits. *In vitro* antioxidant activity revealed that fruits were also rich in natural antioxidants (DPPH assay 1.42 mM; ABTS assay 1.83 mM and FRAP assay 2.74 mM AAE/100 g fw), which were comparable or higher than many widely consumed wild fruits of the region. Inhibition of α-amylase enzyme revealed significant antidiabetic activity (25.96 µg/mL extract) in fruits, which was slightly lower (18.29 µg/mL) in acarbose standard. The rich phytochemical, antioxidant, and antidiabetic potential of *P. hexandrum* fruits can be harnessed to improve health and nutritional status, enhance livelihoods among rural communities, and develop nutraceuticals.

Keywords: α-amylase, Berberidaceae, Himalaya, Mayapple, Nutrition security

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The Himalayan region represents a globally recognized biodiversity hotspot, supporting a rich heritage of unique edible, medicinal, and aromatic plant species. Despite this biological wealth, the region remains highly vulnerable to food insecurity due to harsh topography, fragmented landscapes, limited cultivable land, low agricultural productivity, climatic uncertainties, inadequate market access, and frequent natural hazards[1]. Currently, it has been estimated that around 30% of the population of the region is suffering from food insecurity and around 50% of the population is facing challenges of malnutrition[2]. In developing countries such as India, daily fruit and vegetable intake remains only 20-50% of the levels recommended by the World Health Organization[3]. The food security and livelihoods of Himalayan communities are largely dependent on the regional bioresources. Within the Himalaya, regional bioresources serve as critical livelihood and nutritional assets, particularly wild edible fruits enriched with metabolites and essential minerals, many of which also possess therapeutic properties[4,5]. However, numerous nutritionally and pharmacologically valuable species remain underexplored, necessitating systematic scientific investigation.

Podophyllum hexandrum Royle (family Berberidaceae) commonly known as Himalayan mayapple, is an endangered medicinal herb distributed across the Hindu-Kush Himalaya and southwestern China between 3000 and 4200 m elevation[6]. Podophyllotoxin, the major bioactive lignan present in the rhizomes and roots, is widely used as a precursor for several anticancer drugs and thus, has immense potential in the international market[7]. The plant bears oblong to ovoid berry fruits that are larger than those of other Berberidaceae members (4-5 cm long, 2-3 cm diameter), juicy but insipid, and consumed only when fully ripe. Traditionally, *P. hexandrum* fruits are used to treat fever in Indian medicinal systems[8]. Fruits of the species have been used in the treatment of renal and urinary disorders, gynecological and menstrual conditions[9,10]. Despite this long standing ethnomedicinal relevance for managing fever, renal

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and urinary disorders, and gynecological conditions, the nutritional composition, phytochemical constituents, antioxidant activity, and antidiabetic potential of *P. hexandrum* fruits have not been comprehensively evaluated. Therefore, the present study aims to scientifically validate these traditional uses through phytochemical and biological activity.

Exploration of wild edibles is necessary for harnessing their potential as wild edible fruits contribute substantially to food and nutritional security, rural economies, and cultural identity[11]. They also represent important genetic reservoirs for crop improvement, offering adaptive traits critical for climate resilience[5,12]. Throughout human history, wild bioresources have supported subsistence, especially in ecologically fragile regions. In the Himalaya, such fruits may play a strategic role in combating malnutrition and enhancing dietary diversity. Beyond basic nutrition, many wild fruits are rich in bioactive metabolites, particularly phenolics and flavonoids, which exhibit strong antioxidant and antidiabetic activities by scavenging free radicals and inhibiting metabolic enzymes associated with disorders such as diabetes, obesity, and hypertension[13]. These compounds support blood glucose regulation, lipid metabolism, oxidative defence, and prevention of non-communicable diseases.

Despite its ethnomedicinal relevance, broad geographic distribution, and documented pharmaceutical value of podophyllotoxin producing underground organs, the ripe fruits of *P. hexandrum* remain scientifically overlooked. Existing research has primarily focused on roots, rhizomes, or whole-plant chemistry, leaving substantial gaps regarding the nutritional composition, phenolic diversity, antioxidant mechanisms, and enzyme-modulating antidiabetic properties of the edible fruit portion. Moreover, no comprehensive assessment integrating phytochemical characterisation, multiple *in vitro* antioxidant assays, and α-amylase inhibition has been conducted, despite increasing recognition of wild Himalayan fruits as potential nutraceuticals. Therefore, the present study provides the first consolidated evaluation of nutritional, phytochemical, antioxidant, and antidiabetic attributes of *P. hexandrum* fruits. These findings are expected to clarify their functional food value. Additionally, the study will support evidence-based dietary and therapeutic use among Himalayan communities, which may support future nutraceutical and breeding initiatives. By scientifically validating a culturally consumed yet understudied wild fruit, this work addresses a critical knowledge gap and contributes to long-term food, health, and livelihood security in the Himalayan region.

Material and Methods

Plant material collection

Fully ripened and fresh fruits of *P. hexandrum* were collected from Thangu region of Sikkim, India (27.8997°N, 88.531°E; 3925 m asl). Taxonomic identification of the plant material was carried out with the help of Dr. Prakash Chettri (from SRC, NIHE), and a voucher specimen was deposited at the Herbarium of SRC, GBPNIHE. Infected or immature fruits (five to six fruits) were excluded and only healthy fruits were selected for analysis. Samples were carried to the laboratory in paper bags and preserved at -4°C prior to further analysis (Fig. 1). Seeds were carefully removed from fruit before analysis.

Reagent and instruments

Analytical-grade reagents were used for analysis throughout the study. ABTS salt [2,2′-azinobis-(3-

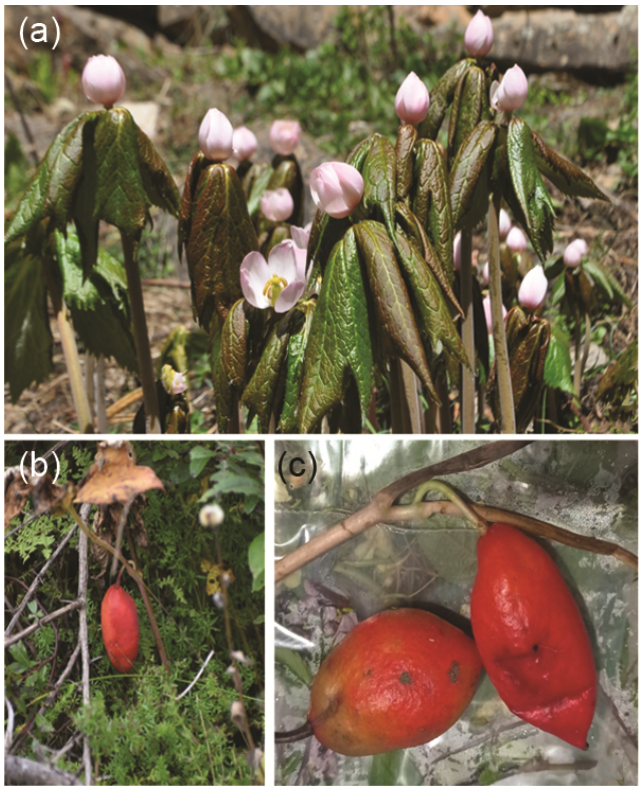

Fig. 1 — Photograph representing different plant parts of *P. hexandrum*, (a) Plant in flowering stage, (b) plant with mature fruit, (c) fruits of the species

ethylbenzothiazoline-6-sulphonilic acid)], DPPH (2,2-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tri-2-pyridyl-1,3,5-triazine), acarbose, gallic acid, ascorbic acid, catechin and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany). Other reagents including, methanol, acetone, ethanol, Folin-Ciocalteu's reagent, potassium permanganate, potassium per sulphate, potassium acetate, potassium chloride, ferric chloride, sodium acetate, sodium carbonate, sodium sulphate, glacial acetic acid, and hydrochloric acid were bought from Hi-Media (Mumbai, India).

Determination of total monomeric anthocyanins

For extraction of anthocyanins, 10 g of fruit samples were extracted in 100 mL of acidified ethanol prepared by mixing 95% ethanol with 5% 1.5N HCl. The homogenate was mixed thoroughly and kept in the dark at room temperature for inoculation (15 h). Thereafter, mixture was filtered. Total content of monomeric anthocyanins was estimated using the pH differential method[14]. Briefly, aliquots of the extract were diluted separately with equal volumes of two buffer solutions: pH 1.0 buffer prepared by 0.025 M potassium chloride and pH 4.5 buffer prepared by 0.4 M sodium acetate. Following incubation at room temperature for 15 min, absorbance readings were recorded at 520 and 700 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The absorbance value (A) was calculated as follows:

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$$

The content of monomeric anthocyanins was determined by using the following equation:

Monomeric anthocyanin pigment (mg/ml) = (A × MW × DF × 1000) / (ε × l);

Where, A: the absorbance difference between pH 4.5 and pH 4.5, MW: molecular weight of the reference anthocyanin, DF: dilution factor, ε: molar extinction coefficient. l:path length of cuvette.

Determination of riboflavin content

Riboflavin content was quantified following the spectrophotometric procedure recommended by the AOAC[15]. Briefly, 10 g of the fruits was homogenized in 100 mL of 50% ethanol (v/v) under continuous shaking for 1 h. An aliquot of 10 mL of the filtrate was mixed with 10 mL of 5% potassium permanganate followed by addition of 10 mL of 30% hydrogen peroxide. The mixture was kept for incubation in a hot water bath for 30 min. Subsequently, 20 mL of 40% sodium sulphate (w/v) was added to mixture solution to make the final volume of 50 mL. The absorbance of the resulting solution was recorded at 510 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). A calibration curve of different concentrations of riboflavin was prepared for quantification and the results were expressed as mg/100 g fresh weight.

Determination of β-carotene, lycopene and total carotenoids

β-Carotene, lycopene and total carotenoid contents of fruits were determined following solvent extraction and spectrophotometric analysis. Fresh fruit samples (10 g) were homogenised with 5 mL of acetone in a chilled mortar and pestle maintained in an ice bath. Subsequently, 1.0 g of anhydrous sodium sulphate was added to homogenate and mixed. The extract was elutriated using a paper filter, after continuous shaking for 1 h. The filtrate volume was adjusted to 10 mL using acetone and then centrifuged at 5000 rpm for 10 min. After centrifugation, the upper clear phase was carefully collected for analysis. Absorbance measurements were recorded at 662, 645, 505, 453, 470, 663 and 479 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan), with acetone serving as the blank. The content of lycopene, β-carotene, and total carotenoids were determined and expressed as mg/L using the equations described by the respective method[16]:

Chlorophyll a (Ca) = $11.75\ A_{662} - 2.350\ A_{645}$

Chlorophyll b (Cb) = $18.61\ A_{645} - 3.960\ A_{662}$

Total carotenoids = $1000\ A_{470} - 2.270$ Ca $- 81.4$ Cb/227

β-carotene = $0.854\ A_{479} - 0.312\ A_{645} + 0.039\ A_{663} - 0.005$.

Lycopene = $-0.0485A_{662} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}$

Extraction of phenolics and antioxidant activity

Fresh fruit samples (10 g) were used for the preparation of extracts intended for the determination of phenolic compounds (total phenolics, flavonoids, and flavonols) and antioxidant activity. Following manual removal of the seeds, the edible fruit part was homogenised using a grinder. The homogenate (10 g) was mixed with 100 mL of 80% aqueous methanol (v/v). The mixture was incubated in a water bath at 60°C for 1 h. After cooling to room temperature, the extract was subjected to continuous shaking for 15 h and resulting mixture was filtered. The filtrate was centrifuged at 8,000 rpm for 10 min and supernatant

was stored at 4°C until further analysis. The quantification of phenolics and antioxidant activity was carried out within two days of extraction. The dried extract, with a recovery yield of 115 mg (11.5%), was subsequently used for all antidiabetic assays.

Determination of total phenolic, flavonoid and flavonol content

Total phenolic content (TPC) of the fruit extracts was determined using the Folin–Ciocalteu colorimetric assay[17]. In 0.50 mL of methanolic extract, 4.50 mL of distilled water was added followed by addition of 0.50 mL of Folin–Ciocalteu reagent. After allowing the reaction to proceed for 5 min, the mixture was neutralized by the addition of 5.0 mL of 7% (w/v) sodium carbonate solution. The reaction mixture was then incubated in the dark at room temperature (approximately 20°C) for 90 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Total phenolic content was quantified using a gallic acid calibration curve prepared in 80% methanol and expressed as milligrams of gallic acid equivalents (GAE) per gram fresh weight.

Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method[17]. In brief, 1.0 mL of methanolic extract was combined with 3.0 mL distilled water. Furthermore, 1.0 mL of 10% (w/v) aluminium chloride solution, 0.20 mL of 1 M potassium acetate, and 5.60 mL distilled water was added to this reaction mixture. The reaction mixture was incubated at room temperature (approximately 20°C) for 30 min, after which absorbance was recorded at 415 nm. Flavonoid content was determined with the help of a standard curve of quercetin prepared in 80% methanol and results were expressed as milligrams of quercetin equivalents (QE) per gram fresh weight.

Total flavonol content was determined according to the method described by Dhyani *et al*.[18]. In brief, 2.0 mL of the extract was mixed with 2.0 mL of 2% (w/v) aluminium chloride solution and 3.0 mL of 5% sodium acetate solution. The mixture was incubated at 20-25°C for 2.5 h, and absorbance of the reaction mixture was measured at 440 nm. Quantification of total flavonol content was carried out using a standard curve of catechin prepared in 80% (v/v) methanol and the results were expressed as milligrams of quercetin equivalents (QE) per gram of fresh weight.

Antioxidant activity by free radical - scavenging ability of DPPH cation (DPPH assay)

The free radical scavenging activity of the fruit extracts was evaluated using the DPPH assay following the method described by Badhani *et al*.[17]. Briefly, DPPH reaction solution (0.1 mM) was prepared in 80% methanol. For the assay, 3.0 mL of the DPPH reaction solution was added to 1.0 mL of the sample extract and incubated in the dark at 20-25°C for 20 min to allow the reaction to occur. After incubation, the decrease in absorbance resulting from DPPH radical reduction was measured at 520 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Antioxidant activity was quantified using a calibration curve generated with different concentrations of ascorbic acid prepared in 80% (v/v) methanol. The results were expressed as millimoles of ascorbic acid equivalents (mM AAE) per 100 g fresh weight.

Antioxidant activity by free radical - scavenging ability of ABTS cation (ABTS assay)

Total antioxidant capacity of the fruit extracts was assessed using the ABTS radical cation decolorization assay described by Bhatt *et al*.[4]. To obtain the ABTS radical cation, 7.0 mM ABTS solution was mixed with 2.45 mM potassium per sulphate and the mixture was kept in the dark at 20°C for 16 h. Prior to analysis, the ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 0.700±0.05 at 734 nm. In the diluted ABTS radical cation working solution (3.9 mL), 0.10 mL of the methanolic extract of fruit was added and mixture was incubated in the dark at 20°C for 6 min. The absorbance was measured at 734 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). A blank containing 80% methanol in place of the sample extract was used for comparison. Antioxidant activity was quantified against a standard calibration curve prepared using different concentrations of ascorbic acid dissolved in 80% methanol. The antioxidant capacity of the samples was expressed as millimoles of ascorbic acid equivalents (mM AAE) per 100 g fresh weight.

Ferric reducing antioxidant power (FRAP) activity

Ferric reducing antioxidant power (FRAP) of the fruit extracts was evaluated according to the method described by Bhutia *et al*.[19]. The FRAP reagent mixture was freshly prepared by combining 10 volumes of 300 mM acetate buffer, 1 volume of 10 mM TPTZ dissolved in 40 mM HCl, and 1 volume of 20 mM ferric chloride solution. In 0.10 mL of methanolic extract, 3.0 mL of prewarmed (37°C) FRAP reagent mixture was added and allowed to react for 8 min at room temperature. The increase in

absorbance resulting from the reduction of the ferric–TPTZ complex was measured at 593 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The reducing antioxidant capacity of the extracts was quantified using a calibration curve prepared with varying concentrations of ascorbic acid in 80% methanol. Results were expressed as millimoles of ascorbic acid equivalents (mM AAE) per 100 g fresh weight (FW) of fruit.

Antidiabetic potential by inhibition of α-amylase assay

The α-amylase inhibitory activity of the dried fruit extract was evaluated according to the method of Xiao *et al*.[20]. Briefly, 0.5 mL of the extract solution (1 mg/mL dried extract) was combined with 0.5 mL of α-amylase enzyme solution (1 U/mL prepared in 20 mM phosphate buffer, pH 6.9, containing 6 mM NaCl). The reaction mixture was incubated at 37°C for 10 min. Thereafter, 0.5 mL of 1% soluble starch solution was added as the substrate, and the mixture was further incubated under the same conditions for an additional 10 min. The enzymatic reaction was stopped by the addition of 1.0 mL of 3,5-dinitrosalicylic acid (DNSA) reagent, followed by heating in a boiling water bath (100°C) for 5 min. Acarbose, at concentrations ranging from 10 to 50 µg/L, was used as the reference inhibitor. After cooling to room temperature, absorbance was recorded at 540 nm using a microplate reader. The percentage inhibition of α-amylase activity was calculated for each concentration, and the inhibitory concentration required to achieve 50% inhibition (IC_{50}) was subsequently determined.

Statistical analysis

All phytochemical and antioxidant analyses were performed using five independent replicates, each generated from a separate extraction. The results are presented as mean ± standard deviation (SD). Statistical analyses and descriptive data evaluation were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and PAST version 5.0 software[21].

Results and Discussion

Phytochemical composition

High-altitude plants are adapted to extreme UV radiation, freezing temperatures, and low oxygen by higher accumulation of antioxidant compounds[22]. Generally, phenolic compounds, which include phenolic acids, flavonoids and proanthocyanidins, are the largest source of antioxidants in fruits and vegetables[23]. Phenolic compounds including phenolic content, flavonoids and anthocyanins determine the sensory and nutritional quality of fresh fruits. Total phenolic content in fresh fruits (fw) of the *P. hexandrum* was recorded as 3.13 mg GAE/g (Table 1). While total flavonoid and total flavonol contents were recorded as 7.59 mg QE/g and 7.98 mg/g CE fw in the species. Here, higher values of total flavonoid content compared to total phenolic content can be explained by differences in calibration standards, (GAE vs QE), which represent equivalent concentrations rather than absolute values and are influenced by variations in their molar absorptivity and molecular weights. Furthermore, methodological and chemical factors contribute to this discrepancy. In the Folin–Ciocalteu reagent method, overall reducing capacity is estimated but it may underestimate due to variable reactivity towards specific phenolics and interfered by non-phenolic compounds. In contrast, the $AlCl_3$ colorimetric assay of total flavonoids selectively detects flavonoids through complex formation, often yielding stronger responses[16]. Liu *et al*.[24] quantified the total phenolic and total flavonoid content in different organs of the species and found that these parameters as 52.51 mM GAE/100 and 19.58 mM QE/100 g dry weight in fruits. These values were relatively lower than underground plant organs roots and rhizomes, but higher than aerial plant organs such as, stem, petiole, leaf and flowers. In another study, Li *et al*.[25] found that total phenolic content and total flavonoid content were found higher in peel part of the fruit of *P. hexandrum* of Weiyuan County Gansu Province of China. Furthermore, in altitudinal range between 2400 to 3000 m asl, the values of phenolic content and total flavonoid content were relatively higher in population located at low altitude.

In other wild edible fruits of family Berberidaceae, previous reports revealed that content of total

Table 1 — Major phytochemicals quantified in fresh fruits of *P. hexandrum*

Phytochemical	Content in fresh fruit
Total phenolics (mg gallic acid equivalent/g)	3.13±0.10
Total flavonoids (mg quercetin equivalent /g)	7.59±0.17
Total flavonol (mg quercetin equivalent /g)	7.98±0.64
Anthocyanin (mg cyanidin-3-glucoside equivalent/g)	0.49±0.04
Riboflavin (mg/100 g)	0.17±0.03
β-Carotene (mg/100 g)	0.31±0.05
Total carotene (mg/100 g)	2.07±0.02
Lycopene (mg/100 g)	0.11±0.00

phenolic was found as 17.24 mg GAE/g dry weight in cultivated *Mahonia aquifolium* fruits[14]; 65.8 mg GAE /100 g dw in *M. leschenailtii* fruits[26]. However, in Himalaya edible *Mahonia* fruits, it has been found as 0.72 mg/g GAE/g fw in *M. nepalensis*[27] and ranged between 1.56 to 1.80 mg/g GAE/g fw in populations of *M. jaunsarensis*[15]. Phenolic content has also been reported as 8.99 mg AE/g fw in *Berberis Canadensis* fruits, 9.232 mg GAE/g fw in *B. amurensis* fruits, 10.523 mg GAE/g fresh weight in *B. vulgaris* fruits, 12.432 mg GAE/g fw in *B. x declinata*, and 13.628 mg GAE/g fw in *B. koreana*[28]. Similarly, total flavonoids have been recorded as 95.5 mg QE/100 g in *Mahonia leschenaultii* collected from South India[26]. It has also been found between 1.49 to 1.73 mg/g GAE/g fresh weight in *M. jaunsarensis* collected from Uttarakhand Himalaya[15] and 0.70 mg/g GAE/g fresh weight in *M. nepalensis* collected from Sikkim Himalaya[27]. Total flavonoid content has been recorded as 10.1 mg/100 g in *Berberis goudotii*[29] and between 1.02 mg to 2.10 mg rutin equivalent/g in *Berberis canadensis*, *B. amurensis*, *B. vulgaris*, *B. x declinata*, and *B. koreana*[28]. Thus, these values of total flavonoid and total flavonols contents can be considered as comparable or higher than other edible fruits of the family Berberidaceae, however, in most cases comparisons were not possible due to different analytical method, extraction procedure, or standard used for quantification. Thus, *P. hexandrum* fruits can be used as a rich source of total phenolic and flavonoid content.

Anthocyanin content was found as 0.49 mg cyanidin-3-glucoside equivalent/g in *P. hexandrum* fruits. Other members of the family Berberidaceae are considered as rich source of anthocyanin content and in Himalayan member, it has been recorded as 3.99 mg/g in fresh weight in matured fruits of *M. nepalensis*[27], and between 18.93 to 22.12 mg/g among different populations of *M. jaunsarensis*[16]. High content of anthocyanin has been recorded in ethanol extract of domesticated *M. aquifolium* berries as 380.99 mg/100 g fw[14], and in *M. leschenailtii* fruits as 8.58 mg/100 g fw[26]. Comparatively, the *P. hexandrum* fruits represent relatively lower anthocyanin content than dark coloured small berry fruits of the family Berberidaceae. Generally, fruit anthocyanins are gaining more attention in modern diets transforming them into functional foods due to their strong antioxidant potential, and protective effect against lifestyle-related or chronic diseases such as diabetes, cardiovascular diseases, cancers and neurological disorders[27].

The phenolics (total phenolics, flavonoids and anthocyanins) present in fruits directly contributed to their antioxidant potential. Due to strong hydrogen donating and reducing properties, phenolics have strong free radical scavenging properties. Due to the presence of such nutraceutical agents, the fruits have many beneficial health properties, such as prevention or treatment of cardiovascular diseases, obesity, diabetes, aging related diseases, cancers, etc[4]. However, flavonoids determine sensory parameters of the fruits determining the colour and flavour. Also, flavonoids have health beneficial effects such as, anticancer, hypoglycemic, antioxidant and antibacterial properties. Flavonoids are sub-grouped into flavones, flavonones, flavanols, flavanonols, anthocyanins, proanthocyanidins, chalcones, condensed tannins and lignans. Specific groups of flavonoids identified in this species have diverse health beneficial effects[30].

Also, a considerable amount of riboflavin (0.17 mg/100 g), *β*-carotene (0.31 mg/100 g), lycopene (0.11 mg/100 g) and total carotene (2.07 mg/100 g) was recorded in fruits of *P. hexandrum*. Generally, 0.9 to 1.3 mg per day consumption of riboflavin has been recommended for adult human body[31]. However, dose in supplementation may be up to 50-60 mg daily and its higher dose might be recommended during specific medical conditions. *β*-carotene is considered as provitamin A and utilized during biosynthesis of vitamin A in the human body. *β*-Carotene, lycopene and total carotene can also help to reduce inflammation and oxidative stress, which can contribute to chronic conditions[32]. Lycopene has been reported to possess anticancer properties by inhibiting mutagenic processes and suppressing tumour progression and metastasis. Studies have demonstrated its potential efficacy against cancers of the respiratory system and the upper gastrointestinal tract[33], highlighting its significance as a bioactive compound with promising therapeutic applications in modern medicine.

Antioxidant activity

For rapid evaluation of antioxidant capacity of the food samples, *in vitro* assays (DPPH, FRAP and ABTS assays) are preferred. Here, free radical scavenging activity measured by DPPH assay was found as 1.42 mM AAE/100 g fw, and free radical scavenging activity measured by ABTS assay was found as 1.83 mM AAE/100 g fw. Ferric reducing antioxidant activity determined by FRAP assay was found as 2.74

mM AAE/100 g (Fig. 2). Previously, antioxidant activity by DPPH assay (IC_{50} values) was recorded as 36.44 µg/mL in fruits of species and these values were also relatively lower than underground plant organs roots and rhizomes, but higher than aerial plant organs such as, stem, petiole, leaves and flowers[25]. Antioxidant activity measured by FRAP assay was found higher in the peel part of the fruit than pulp and higher antioxidant potential was found in low altitude population (2400 m asl) than other populations[24]. All the major phytochemical quantified in the species including, phenolic content, flavonoids, anthocyanin, lycopene and *β*-carotene are strong antioxidants.

Antioxidant activity has been found as 35.26 mM, 49.95 mM and 136.34 mM Trolox equivalent/kg using DPPH, ABTS and FRAP assays, respectively in *Mahonia aquifolium* berries[18]. Similarly, antioxidant activity by ABTS, DPPH and FRAP assays have been recorded as 11.12 mM,5.58 mM and 9.01 mM ascorbic acid equivalent/100 g fw, respectively in *M. nepalensis*[27] and between 1.53-1.77 mM, 1.72-1.75 mM and 1.31-1.52 mM ascorbic acid equivalent/100 g fw, respectively in *M. jaunsarensis*[16]. The values were found higher than commercial apple fruits, which is known for high antioxidant potential[18]. Thus, based on current results, *P. hexandrum* fruits can be considered a rich source of natural antioxidants of plant origin.

Generally, intake of antioxidants by human diet can be assessed with the help of ORAC assay, and it has been recorded between 1.2 to 1.7 mM of ORAC/day in the developed countries, like USA[5]. However, the risk of free radical production and its mediated disease generation rate will be higher in the Himalayan region due to harsh environmental conditions and high radiation intensity than the lowland plain areas[1,22]. Thus, such fruits can be very important for enhancing the diet quality and disease prevention abilities in the Indian Himalayan region.

Antidiabetic through *in vitro* assays

Also, the fruit extract of *P. hexandrum* showed high α-amylase enzyme inhibitory activity (IC_{50} value: 25.96 µg/mL) and these values were comparable to acarbose (18.29 µg/mL) (Fig. 3). Inhibition of carbohydrate-hydrolysing enzymes such as α-amylase and α-glucosidase in the human digestive tract has emerged as an effective strategy for controlling blood glucose levels. Suppressing the activity of these enzymes helps reduce intestinal glucose absorption, thereby managing postprandial

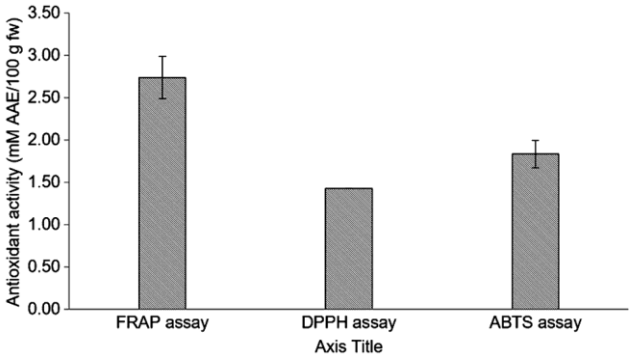

Fig. 2 — Antioxidant potential of fresh fruits of *P. hexandrum* determined by three *in vitro* assays

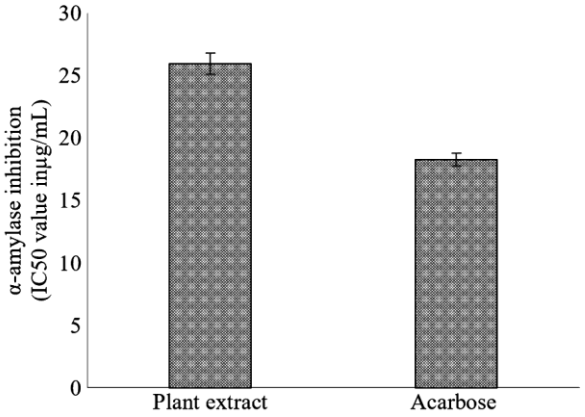

Fig. 3 — Antidiabetic potential of fresh fruits of *P. hexandrum* extract through the α-amylase inhibitory inhibition assay

hyperglycemia. Consequently, glucosidase inhibitors are widely used as therapeutic agents in diabetes management[34]. Numerous plant-derived compounds, including polyphenols, anthocyanins, and flavonoids, have demonstrated strong inhibitory effects on α-amylase and α-glucosidase. Since these enzymes play crucial roles in carbohydrate digestion, their inhibition contributes significantly to maintaining optimal blood glucose levels in diabetic individuals.

To manage complex metabolic disorders like Diabetes Mellitus, various approaches are used to regulate hyperglycemia, hyperlipidemia and other metabolic pathways, including inhibition of glucose metabolism and enhancement of glucose uptake and storage[34,35]. The enzyme α-amylase catalyses the hydrolysis of α-1,4-glycosidic linkages in starch to produce maltose and glucose, thereby contributing to postprandial hyperglycemia and its inhibition has shown great therapeutic importance in controlling postprandial glucose. Findings from other important plants reported in the literature demonstrate high α-amylase inhibitory potential of the *P. hexandrum*

fruits. According to reported studies, *Citrus macroptera* shows an IC_{50} value of 3.64±0.19 mg/mL of plant material[36], while *Carissa carandas* showed IC_{50} value of 29.66 mg/mL plant material[37]. Similarly, in edible fruit plants such as *Berberis integerrima* fruits exhibited inhibitory activity (IC_{50} = 1.14 mg/mL extract) comparable to acarbose (IC_{50} = 1.00±0.085 mg/mL, $p<0.05$)[38].

Conclusion

The present study demonstrates that the ripe fruits of *P. hexandrum* are a valuable source of nutritionally and pharmacologically relevant metabolites. High levels of phenolics, flavonoids, flavonols, anthocyanins, riboflavin, and carotenoids, together with strong antioxidant activity across DPPH, ABTS, and FRAP assays, confirm their capacity to effectively scavenge free radicals and mitigate oxidative stress. Additionally, notable α-amylase inhibitory activity highlights the potential role of these fruits in glycemic regulation and the management of metabolic disorders. Collectively, these findings validate the traditional consumption of *P. hexandrum* fruits and position fruits as a promising candidate for functional food development, nutraceutical research, and dietary diversification in Himalayan communities.

Given the endangered status and restricted high-altitude distribution of the species, sustainable harvesting practices, conservation efforts, and cultivation strategies are essential to prevent further population decline. Future research should focus on detailed compound identification, bioavailability and *in vivo* efficacy studies, product formulation, and evaluation of socio-economic adoption potential. By integrating scientific evidence with community knowledge and conservation planning, *P. hexandrum* fruits may contribute meaningfully to regional food security, public health, and livelihood enhancement in the Himalaya.

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Author Contribution

PA: Conceptualization; Data curation, formal analysis, & manuscript writing; MK: Data curation & formal analysis, SR: Conceptualization; experimental design; supervision; manuscript editing; review & editing.

Ethics Approval and Consent to Participate

Seeds of *P. hexandrum* were collected from non-protected areas natural habitats of Thangu area in Sikkim (following national guidelines, as the plants were not physically damaged and only ripe fruits were collected). All the necessary permissions were obtained from Department of Environment and Forest, Government of Sikkim, India. In this study, we have not been involved in any animal and human studies.

Declarations of Competing Interests

The authors have no competing interests to declare and confirm that no financial or personal affiliations influenced the conduct or outcomes of this research.

Declaration on the Use of Artificial Intelligence

The authors declare that artificial intelligence (AI)-assisted tools were not used in developing figures or writing manuscript, but used only to improve the language, grammar, and readability of the manuscript. All scientific content, data analysis, interpretation of results, and conclusions were developed and verified by the authors. The authors take full responsibility for the accuracy, originality, and integrity of the work presented in this manuscript.

Data Availability

All datasets produced as part of this study can be obtained from the corresponding author upon reasonable request.

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