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Nutritional value and antioxidant properties of *Viburnum mullaha* Buch.-Ham. ex D. Don fruit from central Nepal

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Abstract: *Viburnum mullaha* is a popular wild edible fruit widely distributed in Nepal's mid-hills. Locals consume the fruits of the plants raw and use them to treat a variety of diseases. High value of fat (15%) and protein (3.94%) was evaluated in the seed compared to the pulp with fat 2.1% and protein 1.17%. Both the pulp and the seed exhibited high values of total phenolic content (3346.1 1 ± 0.05 and 2331.03 ± 0.02 mg QAEs/100 g respectively). The samples showed weak antioxidant activity when evaluated by diphenylpicrylhydrazyl (DPPH) antioxidant assay as 50% of radical scavenging activity were 1350 ± 0.01 µg/mL and 1340 ± 0.77 µg/mL, respectively. The presence of minerals (iron, copper, manganese, and zinc) was evaluated by absorption atomic spectroscopy (AAS). The pulp contained iron and zinc whereas the seed contained iron, manganese, and zinc. The pulp methanolic extract at selected concentrations (125, 62.5, 31.25, and 15.63 mg/mL) did not show any inhibitory effect against the tested bacterial strains. These findings suggest the fruit to be rich in fats, total phenolic content, weak antioxidants, possesses micronutrients in trace amounts, and does not hold antibacterial properties at the selected concentrations.

Key words: *Viburnum mullaha*, pulp, seed, nutrients, phytochemicals

1. Introduction

Foraging wild plants for food and medicinal purposes is an ancient practice that was formerly thought to be obsolete by most modern consumers; however, it is currently gaining popularity (Niveditha, 2017; Lila & Dunlap, 2021). Local populations benefit from the vegetation because it provides a range of staple and supplement foods, and economic revenue, and also promotes food security (Uprety et al., 2012; Islam et al., 2019; Mishra et al., 2021). Wild fruits have shown to be nutritionally rich with an abundance of phytochemicals especially antioxidant activity (Bvenura & Sivakumar, 2017; Lila & Dunlap, 2021). In Nepal, the use of native wild edible fruits indicates the wide knowledge of the local communities (Uprety et al., 2012). The genus *Viburnum* is one of the vital forest wild fruit species found in subtropical-temperate ecotone forest and the Himalayas region in Nepal (Adhikari et al., 2017) and provide an ornamental, pharmaceutical, and dietary contribution to consumers (Adhikari et al., 2019; Gautam et al., 2020; Gurung et al., 2020).

The genus *Viburnum* is the evergreen, semievergreen or deciduous shrubs and small trees belonging to Adoxaceae (Lobstein et al., 1999; Adhikari et al., 2019; Sharifi-Rad et al., 2021). The plant comprises more than 230 species distributed primarily within temperate forest regions of the northern hemisphere and in mountain parts of Central and South America, South East Asia, South Eastern Australia, and Tasmania (Lobstein et al., 1999; McNeill & Malécot, 2002; Maikhuri et al., 2012; Sharifi-Rad et al., 2021). In Nepal, altogether 4 species of *Viburnum* have been reported (*V. cotinifolium*, *V. cylindricum*, *V. erubescens,* and *V. mullaha*) (Gautam et al., 2020).

Viburnum mullaha Buch.-Ham. ex D. Don, locally called "Molo"', "Mallah" and "Herah" is one of the unexplored potential underutilized wild edible fruits reported from various hilly areas of Nepal (Acharya & Mukherjee, 2014; Gautam et al., 2020, Bhatt, 2021). *V. mullaha* especially holds fuelwood, ethnomedicinal, and food value (Adhikari et al., 2019; Gurung et al., 2020). The plant is used for treating ailments by traditional healers (Kunwar, 2017; Gurung et al., 2020), especially the fruit juice attribute potential to cure poisoning (Adhikari et al., 2019). However, in the recent past, efforts were made to add value to its fruits while making juice and squash

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(Maikhuri et al., 2012). Therefore, this unexplored plant has a huge potential in the pharmaceutical and food industry if research is directed toward the downstream applications for drug and product manufacturing.

Limited studies have been conducted regarding biochemical estimations of *V. mullaha*. Maikhuri and coworkers (2012) evaluated vitamins, minerals, nutritional contents, and energy while Singh et al., (2017) found the pulp of the plant as the potential source of polyphenol with rich antioxidants, antielastase, anticollagenase, and antityrosinase activity.

Although, both the pulp and seeds of the plant are consumed throughout the country, no information is available on their nutrients, phytochemicals, minerals, and antibacterial properties in Nepal. Interestingly, a few studies on pulp have been reported elsewhere (Maikhuri et al., 2012; Singh et al., 2017), but there are no such reports regarding the seeds of *V. mullaha*. Therefore, in addition to the pulp, we herein reported the nutritional and phytochemical composition of the seeds of this plant.

2. Materials and methods

This study has explored the nutritional and phytochemical composition of *V. mullaha* as well as its antimicrobial properties against multi-drug resistant gram-positive and gram-negative bacteria. Association of official analytical chemists' (AOAC) protocols was used for analyzing nutrients and phytochemicals, and standard (AOAC, 1995) guidelines were used to test the antimicrobial properties of plant extract.

2.1. Plant material and sampling

In October 2020, the ripened berries of *V. mullaha* were collected from the locality of Phulchoki, Lalitpur (27.5711° N, 85.4056° E), a central hilly region of Nepal, using garden pruner. The berries were matured and ripened, with fleshy exocarp and mesocarp with enhanced sourness, from a single harvest. The berries were put into zipper bags and delivered to the Biological Resources Laboratory at Nepal Academy of Science and Technology (NAST) within 2 h. After washing the samples with sterile water, healthy and disease-free fruit berries were separated and dried under a shade for 48 h followed by oven drying at 40 °C for 72 h. Bacterial and fungal contamination were closely monitored in the samples. The seeds were separated carefully from pulp and both the samples were ground to a fine powder using mortar and pestle. The samples were stored in an airtight container at 4 °C for further analysis.

2.2. Nutritional composition

2.2.1. Protein content

Protein was determined using a modified Bradford assay (Bradford, 1976). In a hybridization shaking oven-MO-AOR (Innovative Life Science Tools, USA), 200 mg of dried powdered sample was mixed with 20 mL 18.2 MΩ water (Millipore, Milli-Q) and incubated for 24 h at 50 °C and 100 RPM. After 24 h of standing time, the solution was filtered using Whatman no. 1 filter paper. The filtrate was used for the determination of protein content. In a 1:10 ratio, the sample filtrate and a freshly produced Bradford reagent were added to the microfuge tube. The test was carried out three times. All of the tubes were adequately vortexed and incubated for at least 5 min at room temperature. While absorbance might rise over time, samples should be incubated at room temperature for no more than 1 h (Stoscheck, 1990). The absorbance of each 200 µL mixture was measured at 595 nm against a blank in a Multiskan Sky/Microtitre spectrophotometer (ThermoFisher Scientific, USA) equipped with SkanIt software version 5.0. Bovine serum albumin (BSA) was used to calculate the standard curve (0–400 µg/mL; $y = 0.001x + 0.257$; $R^2 = 0.974$) and the results were expressed in g/100 g of sample.

2.2.2. Carbohydrate content

A colorimetric method using a modified anthrone reagent was used for this analysis (Osborne and Voogt, 1978). Pretreatment of 0.5 g samples with 15 mL of 52% (v/v) $HClO_4$ and 10 mL of Milli-Q water was carried out and stored in the dark for 18 h (García-Herrera et al., 2014). After this period, samples were filtered, and the volume of the filtrate was diluted ten times. The volume of 5 mL of 0.1% (w/v) anthrone solution in 70% (v/v) H_2SO_4 was added to 1 mL of sample extract. The mixture was kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green color. Then, a 200 µL cooled mixture was added to the 96 well-plates, and the absorbance was measured at 630 nm in the Agilent Technology Cary UV-Vis spectrophotometer. Glucose was used to calculate the standard curve (0–100 µg/mL; $y = 0.008x - 1.06$; $R^2 = 0.979$) and the results were expressed in g/100 g of sample.

2.2.3. Fat content

The dry sample (5 g) was weighed on a glazed paper and transferred into an extraction thimble. The thimble was introduced into Soxhlet extractor (SM Scientific Instruments, India) over a pad of cotton wool so that the top of the thimble was well above the top of the siphon. A clean, dry, round bottom flask was weighed and fitted with the extractor. Petroleum ether (100 mL) was poured along the side of the extractor until it began to siphon off (Xiao et al., 2012). The equipment was assembled with the flask at 40 °C–60 °C, and the extractor was connected with the condenser. The cool water circulation was started in the condenser, and the extraction was allowed for 6 h. The thimble was removed with the material from the extractor. The ether was evaporated at 40 °C to dryness using rotary evaporator-HS-2005 V-N (Hahnshin scientific co., South Korea). The receiver flask was dried in a hot air oven at 100

°C for 1 h, cool, and weighed. The result was expressed as g/100 g of sample for fat content (Chew et al., 2011)

2.2.4. Ash content

Ash content was estimated using the dry ashing method (Chew et al., 2011; Onivogui et al., 2014). After weighing 0.5 g of sample in a silica crucible, the sample was incinerated in a cold muffle furnace set at 550 °C until whitish/grayish ash was obtained. The silica crucible was cooled, and the ash formed was weighed. The result was expressed as g/100 g of sample for ash content.

2.2.5. Mineral analysis using atomic absorption spectrophotometer

The digestion procedure followed was dry ashing in which the dried sample (0.5 g) was first placed onto a porcelain crucible. The furnace temperature was slowly increased from room temperature to 450 °C and 500 °C for the sample respectively, in 1 h. The sample was washed for about 4 h until a white or grey ash residue was obtained. The residue was then dissolved in 1 mL concentrated HNO_3, to prepare ash solution and left it for 24 h. Again, it was digested with 1 mL 25% HNO_3 which was then filtered with ashless paper and the resulting solution was transferred to a 50 mL volumetric flask and made up to a volume with Milli-Q water. Preparation of the blank control, in the absence of analytes, was also carried out in a similar manner (Paul et al., 2014; Gebrelibanos et al., 2016). Cu, Fe, Zn, and Mn concentrations were measured in the samples using Atomic Absorption Spectrometry (AAS) coupled with hydride generation (AAS Agilent Technologies, 240 FSI flame).

3.3. Phytochemical analysis

3.3.1. Plant extracts preparation

The sample was extracted using the method described previously (Al-Harrasi et al., 2014), with minor modifications. Briefly, 1 g of each dried and powdered *V. mullaha* seed and pulp were weighed separately, and 20 mL of methanol was added to both samples. The mixture was placed in a shaking incubator at 100 revolutions per min (RPM) at 37 °C for 24 h. The mixture was filtered through Whatman no. 1 filter paper and the filtrates were stored at 4 °C. With 20 mL methanol addition to the residue, the mixture was placed again in a shaking incubator at 100 RPM and 37 °C for 24 h. Then, the mixtures were filtered through Whatman no. 1 filter paper. Finally, all the filtrates from the 1st and 2nd filtration were mixed. The samples were evaporated to dryness in a rotary evaporator at 40 °C. The pulp and the seed extracts were stored in a refrigerator until further analysis and they were redissolved in methanol accordingly when tests were performed.

3.3.2. Phenolic content

Total phenolic content was estimated using a modified Folin-Ciocalteu assay (Singleton & Rossi, 1965). To 50 µL of plant extract, 150 µL of Folin and Ciocalteu's phenol reagent was added. After 3 min, 150 µL of a saturated sodium carbonate solution was added to the mixture, and the volume was adjusted to 1500 µL with milli-Q water. The reaction was kept in the dark for 90 min. Then, 200 µL of the reaction was added to the 96 well plates, and absorbance was read at 725 nm in the spectrophotometer. Gallic acid was used to calculate the standard curve (25–500 µg/mL; $y = 0.0005x + 0.063$; $R^2 = 0.950$). The result was expressed as mg of gallic acid equivalents (GAEs) per 100 g of extract.

3.3.3. Flavonoid content

Total flavonoid content was estimated using the modified aluminum chloride method (Chang et al., 2002). To 100 µL of plant extract, 100 µL of 2% $AlCl_3$. H_2O solution was added. The mixture was incubated in the dark for 1 h, and absorbance was read in the spectrophotometer. All tests were performed in triplicate. Quercetin was used to calculate the standard curve (25– 250 µg/mL; $y = 0.018x + 0.055$; $R^2 = 0.986$). The result was expressed as mg of quercetin equivalents (QAEs) per 100 g of extract.

3.3.4. Vitamin C content

To determine vitamin C content, the modified Klein and Perry (1982) method was used. For this, 50 mg dry methanolic extract of *V. mullaha* was extracted in 5 mL of 1% meta-phosphoric acid for 45 min at room temperature. The solution was filtered through Whatman No. 4 filter paper. To 100 µL of each sample, 900 µL of 2, 6-dichlorophenolindophenol (DCPIP) was added and mixed properly. Two-hundred µL of reaction was added to 96 well plates, and the absorbance was taken within 30 min against a blank was measured in the spectrophotometer at 515 nm. All tests were performed in triplicate. Ascorbic acid was used to calculate the standard curve (25–100 µg/mL; $y = -0.002x + 0.692$; $R^2 = 0.981$). The result was expressed as mg of ascorbic acid/100 g of extract.

3.3.5. β-carotene and lycopene content

β-carotene and lycopene content of the sample was determined based on Nagata and Yamashita (1992). After weighing 100 mg of dried methanolic extract, 10 mL of the acetone-hexane mixture (4:6) was added for 1 min. The mixtures were shaken vigorously and filtered through Whatman no. 4 filter paper. After that, 200 µL of the sample was added to the plate, and absorbance was measured at 453, 505, and 663 nm respectively in the spectrophotometer. All samples were assayed in triplicate. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/ 100 mL) = $-0.0458\ A663 + 0.372\ A505 - 0.0806\ A453$ and β-carotene (mg/100 mL) = $0.216\ A663 - 0.304\ A505 + 0.452\ A453$. The results were expressed as µg of carotenoid/g of the sample.

3.3.6. DPPH radical-scavenging activity (DPPH assay)

DPPH Radical-Scavenging activity was determined with the modified Blois method (Blois, 1958). Various *V. mullaha* extracts (100 µL) concentrations were mixed with 900 µL of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). Then, 200 µL of each reaction mixture was added to the enzyme-linked immunoassay (ELISA) plate reader (ThermoFisher Scientific, USA), and the reduction of the DPPH radical was determined by measuring the absorption at 517 nm in the spectrophotometer. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of RSA (EC_{50}) was calculated from the RSA percentage graph against extract concentration. Ascorbic acid was used as standard.

3.4. Antimicrobial assay

3.4.1. Crude extract preparation

Five gram of the powdered pulp was dissolved in 25 mL of ethanol for three days and filtered through Whatman filter paper no. 1. The filtrate was evaporated to dryness in a rotary evaporator at 40 °C. The extract was kept at 30 °C for a week to obtain a completely dry sample and stored at 4 °C for further analysis.

3.4.2. Working sample preparation

Different concentrations (125, 62.5, 31.25, and 15.63 mg/mL) of working samples were prepared by dissolving dried pulp extract in 5% dimethyl sulfoxide (DMSO).

3.4.3. Agar well diffusion test

The bacterial strains for this study were originally obtained from the American Type Culture Collection (ATCC). *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70062, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, and *Staphylococcus aureus* ATCC 25923 were routinely cultured at 37 °C in nutrient agar. Gentamicin was used as the positive control while 5% DMSO was employed as negative control.

3.5. Statistical analysis

The results were the average of three measurements. Means and standard deviation were computed using Microsoft Excel 2007. Graphical illustrations were prepared using GraphPad Prism 8.4.3.

4. Results and discussion

4.1. Nutritional analysis

The nutritional content of *V. mullaha* is shown in Table 1. Protein, carbohydrate, fat, and ash were present in both pulp and seed. The protein and fat contents were higher in the seed than in the pulp and the carbohydrate content was higher in the pulp (Figure). Maikhuri et al., (2012) first reported the nutritional composition of *V. mullaha* pulp and revealed significantly higher levels (~10 times) of protein, carbohydrate, and fat (Figure) than our study. Another species of this genus, *V. dilatatum* showed 0.1 g/100 g of protein content and 0.2 g/100 g of fat (Kim et al., 2005) and the values were considerably lesser (~ 10 times) than the contents in this study. Fruits are a minor protein source and a good source of carbohydrates (Grusak & Dellapenna, 1999). The carbohydrate value in *V. mullaha* pulp was 17.3%. Akbulut et al., (2008) reported *V. opulus* L. fruit with very lower carbohydrate value (6.34%).

Table 1. Nutritional composition and mineral contents of *V. mullaha* and its comparison with first report.

Parameters	**Present study**		**Maikhuri et al. (2012)**
Macronutrients	**Pulp (g/100 g)**	**Seed (g/100 g)**	**Pulp (g/100 g)**
Protein	1.17 ± 0.007	3.94 ± 0.004	11.3
Carbohydrate	17.3 ± 0.0	6.3 ± 0.01	18.4
Fat	2.1	15	18.4
Ash	3.02 ± 0.12	4 ± 0.006	-
Micronutrients	**Pulp (mg/L)**	**Seed (mg/L)**	**Pulp (mg/L)**
Fe	0.0239 ± 0.13	0.0218 ± 0.108	0.68 ± 0.019
Cu	ND	ND	0.05 ± 0.03
Mn	ND	0.0204 ± 0.017	0.08 ± 0.03
Zinc	0.0323 ± 0.013	0.00143 ± 0.019	0.05 ± 0.003

ND means not determined
- means test not performed

Nutritional composition of plants is highly dependent on intrinsic and extrinsic factors like soil, climate, weather, harvesting time, ripening time, altitude, and fertilizer usage (Hornick, 1992; Haque et al., 2009; Dawadi et al., 2022).

To the best of our knowledge, this is the first report on the nutritional composition of *V. mullaha* seeds. The results showed the seeds to be a good source of protein. Previously, 5% of the protein was reported in *V. opulus* seed (Karimova et al., 2004). The results also revealed high-fat content in the seeds. As of a previous report, the fat extracted from *V. opulus* seed in petroleum ether revealed triacylglycerol as a predominating neutral lipid and the total fat content accounted for 11.7% (Yunusova et al., 1998). A complete lipid profile of the *V. mullaha* seed is needed to understand the class and significance of the lipid. Ash content of *V. mullaha* pulp and seed did not show much difference (Table 1). A previous study suggested a low value (0.4%) of ash content in *V. dilatatum* (Kim et al., 2005). Ash content may help in predicting the total amount of macro minerals like Na, K, Ca, and Cl and micro minerals like Fe, Cu, Mn, and Zn in plants (Rupérez, 2002; Díaz et al., 2020). AAS analysis of the ash revealed the presence of micronutrients in the fruit. Although Fe and Zn were present in both the pulp and seed, Mn was found only in the seed. Maikhuri et al., (2012) reported several minerals; P, K, Ca, Mg, Fe, and Cu in *V. mullaha* pulp and according to them the level of Fe and Cu were extremely high (Table 1). Minerals play an important role in human health, and their concentrations can vary in plants, even within the same species, depending on a variety of factors. Their content in plants depends on soil composition and the availability of minerals in the plant environment (Grusak & Dellapenna, 1999).

4.2. Phytochemical analysis

The phytochemicals analysis of *V. mullaha* extract showed the pulp was rich in phenols (Table 2). Singh (2016) first reported the TPC (1257 ± 40 mg GAE/100 g) and TFC (3503 ± 203 mg CE/100 g) in *V. mullaha* fruit. Cam and Kuscu (2007) observed 355.59 ± 3.72 mg GAE/100 g of TPC and 151.70 ± 1.64 mg CE/100 g of TFC in *V. opulus* fruit flesh. The TPC and TFC values are highly affected by environmental factors like soil and crop conditions, climate and weather conditions and the stage of fruit during sampling (Díaz et al., 2020). Table 2 showed a low level of vitamin C in pulp. Maikhuri et al., (2012) found high levels (122.27 mg/g) of vitamin C in *V. mullaha* pulp. Previously, the vitamin C level of *V. opulus* L. fruit was reported as 595.24 mg/kg (Akbulut et al., 2008). The β-carotene was present in a trace amount and lycopene was not detected in *V. mullaha* pulp (Table 2). Fruits have a varying concentration of carotenoids and lycopene, depending upon cultivar and climatic conditions (Lee et al., 2005; Brandt et al., 2006). Regarding the pulp Bhusal and colleagues (2020) found similar content in other wild edible fruits from Nepal, including *Berberis angulosa* (0.05 mg/g) and *Eriobotyrya dubia* (0.07 mg/g) (Bhusal et al., 2020). In the present context, seeds have been also investigated for pigment. The seed contained pigment more than the pulp in this fruit. It is reported that seeds

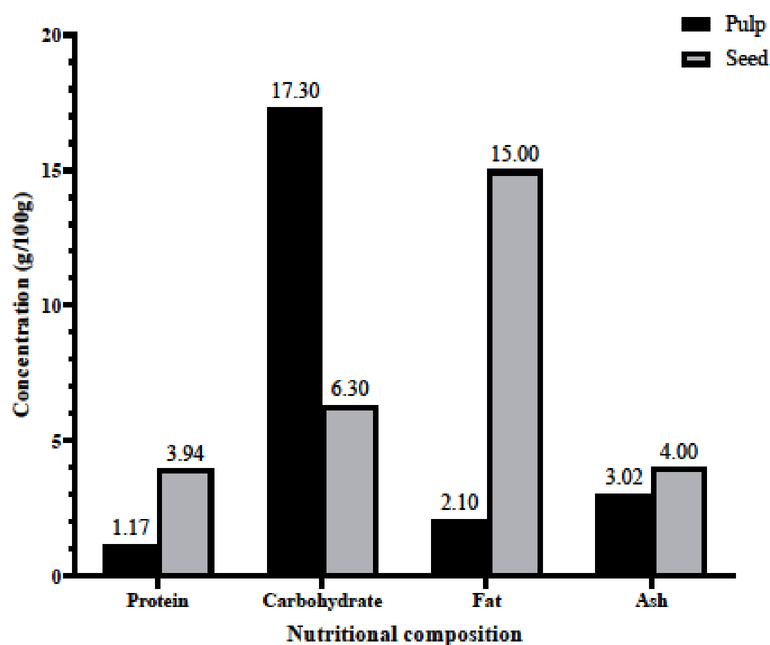

Figure. Nutritional composition of pulp and seed samples of *V. mullaha*.

Table 2. Phenols, flavonoids, ascorbic acid, β-carotene and lycopene contents (mean ± SD) in methanolic extract.

Parameter	Dry methanolic extract	
	Pulp	Seed
Total phenolic content (mg GAEs /100 g)	3346.11 ± 0.05	2331.03 ± 0.02
Total flavonoid content (mg QAEs/100 g)	233.45 ± 0.035	197.09 ± 0.14
Vitamin C (mg AA/100 g)	351 ± 0.003	304.62 ± 0.17
β-carotene (mg carotenoids/g)	0.083	1.46
Lycopene (mg carotenoids /g)	ND	ND

of the family Fabaceae contain all of the major carotenoids found in photosynthetic tissues (Fernández-Marín et al., 2017).

Lycopene has a number of biological properties that protect against oxidation (Chen et al., 2013), mutagenesis (Weisburger, 1999), and carcinogenic activity (Weisburger, 1999; Bhuvaneswari & Nagini, 2005). Moreover, macrophages supplied with β-carotene or lycopene decrease cellular cholesterol synthesis while increasing macrophage low-density lipoprotein (LDL) receptor activity, resulting in LDL clearance from the plasma (Fuhrman et al., 1997). The study demonstrated that the pulp of *V. mullaha* is a moderate source of phytochemicals.

The *V. mullaha* seed contained various phytochemicals such as phenol, flavonoid, vitamin C as well as β-carotene (Table 2). The findings of the present study showed that the seeds are good sources of phytochemicals. Earlier study reported TPC (1231.03 mg GAE/100 g) and TFC (1032.39 mg CE/100 g) but no vitamin C in the seed of *V. opulus* (Cam et al., 2007). Another study found 0.054 mg/g of carotenoids in *V. opulus* seeds (Yunusova et al., 1998). Several environmental factors including variation in altitude influence the composition and quantity of bioactive compounds in plant parts of *V. opulus* (Yurteri, 2020).

4.3. DPPH radical-scavenging activity

The DPPH radical scavenging activity of standard ascorbic acid and *V. mullaha* were analyzed. The 50% of RSA (EC_{50}) value was determined using DPPH radical scavenging activity. EC_{50} of standard ascorbic acid, pulp and seed were 54.9 ± 0.013 µg/mL, 1350 ± 0.01 and 1340 ± 0.77 µg/mL respectively. The EC_{50} value of pulp and seed was similar but quite higher than ascorbic acid. The antioxidant properties of both pulp and seed were weak in nature. According to Phongpaichit et al. (2007), IC_{50} values between 10–50 µg/mL represented strong antioxidant properties and >100 µg/mL represented weak antioxidant properties (Phongpaichit et al., 2007). A previous report found DPPH radical scavenging activity of *V. mullaha* at 1428.3 ± 0.5 mg AAE/100 g FW (Singh et al., 2017). However, Cam et al. (2007) found a lower EC_{50} value of *V. opulus* seed (2.35 ± 0.56 mg sample/mg DPPH) than its pulp (24.56 ± 2.38 mg sample/mg DPPH).

It was found that flavonoids like quercetin and epigallocatechin were better antioxidants than phenolic acids like gallic acid (Rice-Evans et al., 1996). Pereira et al., (2018) reported strong antioxidant activities of calabura fruits even in a lower amount of flavonoids like catechin, gallocatechin, epigallocatechin, naringenin, and quercetin. Blueberries with high levels of flavonoids like proanthocyanidins and anthocyanins also showed high antioxidant activity (Huang et al., 2012). Our study found a low flavonoid value of *V. mullaha* seed and pulp, and this could contribute to its lower antioxidant activity. A complete profile analysis of phenol and flavonoid could help to better correlate them with antioxidant activity.

Overall, the *V. mullaha* seeds and pulp showed good nutritional value, however, both seeds and pulp were poor antioxidants. The seeds of *V. mullaha* are not consumed by humans in north India (Maikhuri et al., 2012) but consumed in Nepal. Yunusova et al., (2004) reported the seeds of *V. opulus* were a food-industry waste, however, it could be used as a feed additive in a ground form together with the principal feed in many communities where it is even eaten by the people. In Nepal, where both the pulp and seeds of *V. mullaha* have been consumed by the people, the results of the present study showed the application potential of the fruit as an additional source of nutrients.

4.4. Antibacterial assay against ATCC cultures

The pulp extract of *V. mullaha* with concentrations of 125, 62.5, 31.25, and 15.63 mg/mL was subjected to five ATCC cultures. None of the pulp extracts showed a zone of inhibition for the bacterial strains used. However, the antimicrobial activities of some species of *Viburnum* were reported by previous studies (Sagdic et al., 2006; Bibi et al., 2010; Česonienė et al., 2012). However, at tested concentrations, the pulp extracts of *V. mullaha* did not show antibacterial activity against tested microorganisms in this study.

5. Conclusion

Evaluation of nutritional and phytochemical properties of the fruits of *V. mullaha* indicated that both the seeds and the pulp contained various nutrients and phytochemicals. The seed was rich in protein and fat while the pulp was rich in carbohydrate and phenolics. The current findings indicate that the fruit is an important source of nutrients and phytochemicals, as well as having the potential for value addition.

6. Author's contribution

LRB designed the study and under his supervision PD conducted the investigation with laboratory experiments and data analysis. SB assisted in the Environment and Climate Study laboratory while SM assisted in the Biological Resources Laboratory. PD, RS, SM, and SB drafted the manuscript and it was revised by LRB, TPJ, and JKR. All the authors read the final draft and approved the manuscript.

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Conflict of interests

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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