

## **REPORT**

# **Antioxidant and nutraceutical value of wild medicinal *Rubus* berries**

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**Abstract:** Nutritional quality and antioxidant capacity of three edible wild berries (*Rubus ellipticus* Smith, *Rubus niveus* Thunb, *Rubus ulmifolius* L.) from Lesser Himalayan Range (LHR) were evaluated. Their edible portion was assayed for moisture, fats, ash, carbohydrates, proteins, fibers, essential minerals (Ca, P, Mg, K, Na, Cl, S, Mn, Zn, Fe, Cu, Se, Co, Ni) and DPPH free radical scavenging activity was applied to determine the antioxidant potential. The fruit of *Rubus ulmifolius* L. (blackberry) possessed the highest values of energy (403.29 Kcal), total protein (6.56g/100 g), Nitrogen (N) content (1500mg/100g), K (860.17mg/100g), Ca (620.56mg/100g), Zn (17.509mg/100g) and the strongest antioxidant activity (98.89% inhibition). While the raspberries (*Rubus ellipticus* Smith, *Rubus niveus* Thunb.) exhibited more significant contents of dietary fiber (5.90g/100g), carbohydrates (86.4 g/100 g) and Fe (4.249mg/100g). Significant variation was observed among the tested samples in all the investigated features. The combination of bio elements and active antioxidants clearly showed the applicability of these berries as a nutraceutical supplement.

**Keywords:** Antioxidant, Nutrition, *Rubus* berries, Mineral analysis.

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## **INTRODUCTION**

In present times, several wild edible species are considered as economically and nutritionally important because of their antioxidant potential and nutritional quality. The wild edibles with significant nutraceutical value are emerging as probable source of floral diversity conservation and advancement of rural communities (Maikhuri *et al.*, 2004). The utilization of wild fruit species is reported to be particularly more recurrent especially among food insecure areas of greater Himalayas, due to intensive exploration of their natural ingredients and bioactive compounds (Nahar *et al.*, 1990). Since these natural edibles can form a major component of the diet for local communities as they are a potential source of functional foods and nutraceuticals. Among the wild edible fruits of Himalayan range, few members of Genus *Rubus* (family Rosaceae) are considered as an important constituent of traditional diets of indigenous people. The *Rubus* species are collectively known as brambles (including blackberries and raspberries) and widely distributed across the globe from North Temperate Zone to the tropics (Southern hemisphere) (Hummer, 1996 & 2010; Kalkman, 2004). These berries are also consumed globally either because of good taste or as a source of natural pharmaceuticals like phenolics, tannins and flavonoids (Finn, 2008; Quideau, 2009; Vasco *et al.*, 2009; Rao and Snyder, 2010; Lee *et al.*, 2012). These

natural phytochemicals exhibit a wide range of biological effects including antioxidant, anti-carcinogenic, anti-inflammatory and antibacterial activities. Due to a high content and wide diversity of phenolics and flavonoids, raspberries are often regarded as natural health-promoting products (Nohynek *et al.*, 2006; Pantelidis *et al.*, 2007, Bobinaite *et al.*, 2012). Not only the fruit, but also the raspberry leaves and roots have been used traditionally as medicinal agents for muscle spasms, morning sickness, sour throats, diarrhea etc. The roots of *Rubus idaeus* L. are locally used for wound cleaning (Ryan *et al.*, 2011; Venskutonis *et al.*, 2007).

The phytochemical, antioxidant and medicinal attributes and health promoting constituents of cultivated *Rubus* berries are usually well recognized (Milivojevic *et al.*, 2011; Wang and Lin 2000; Kafkas *et al.*, 2008) however insufficient data is available about the nutritional quality and antioxidant capacity of wild *Rubus* species. Thus the main objectives of present study are to investigate and compare the nutritional status and antioxidant potential of three *Rubus* species (*Rubus ellipticus*, *Rubus niveus*, *Rubus ulmifolius*) that are most commonly consumed as edible fruit source among the tribal communities of Himalayas.

## **MATERIALS AND METHODS**

### ***Fruit materials***

Fresh and ripened berries of selected species viz. Raspberries (*Rubus ellipticus*, *Rubus niveus*) and

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Blackberry (*Rubus ulmifolius*) were collected randomly from various localities of Himalayan range during August 2012. After assemblage, the fruits were grouped by taxon and representative voucher specimens were authenticated and deposited at the Herbarium of Pakistan, Quaid-i-Azam University Islamabad, Pakistan. Healthy fruits were picked from the collected material and washed carefully to exterminate the impurities. These samples were air-dried or oven-dried (65°C, 48h) for further anti-oxidant and nutritional estimation.

#### Sample preparation

About 300g of dried berries were grounded to fine powder with a grinding-mill and passed through 24- mesh sieve. One portion from each of these composite samples was extracted with organic solvent methanol for anti-oxidant analysis and remaining dried powder (100g) was used for nutritional assessment.

#### In vitro anti-oxidant evaluation

Chemical assay like 2,2-diphenyl-1-picryl hydrazyl (DPPH) described by Kulisic *et al.* (2004), slightly modified by Obeid *et al.* (2005), was applied to evaluate the anti-oxidant potential of selected berries. The hydrogen atom donating ability of corresponding fruit extracts was measured from the bleaching of purple methanolic DPPH solution (Gulluce *et al.*, 2007). Aliquots (50-400µg/ml) of the tested samples were mixed with 950µl of 0.1mM DPPH in the absolute methanol. The reduction of DPPH radical was determined by measuring absorbance at 517nm after 30mins against a blank without DPPH. The results were expressed as the percent inhibition of DPPH radical and calculated as  $[(A_0 - A_s)/A_0 \times 100]$ , where  $A_0$ =Absorbance of DPPH without sample and  $A_s$ =Absorbance of DPPH with sample. Ascorbic acid was used as standard.

#### Nutritional quality assessment

##### Mineral composition

Wet digested samples of wild edible berries were used to determine the macro-elements (K, Ca, Mg and Na) and micro-elements (Fe, Zn, Cu, Pb, Mn, Cr) concentrations (AOAC, 1990). 1g of each sample powder was digested with HNO<sub>3</sub> and HClO<sub>4</sub> in ratio 4:1 for 15mins at 350°C till clear solution was obtained. Desired volume of double distilled water was added to the digested and cooled samples. Mineral contents in all the digested samples were analyzed through Atomic Absorption Spectrophotometer (Parkin Elmer Analyst, 200, USA). However, phosphorus was assessed by treating the same acid digests with ammonium molybdate and finally examined by UV-spectrophotometer (Shimazu, 1700 UV spectrophotometer) (AOAC, 1990).

##### Macronutrients estimation

The fruit samples were studied for their bio nutritive components (moisture, proteins, fats, carbohydrates, ash

and fibers) by using AOAC procedures (AOAC, 1990). The Ash values were resolute by weighing the incinerated residue acquired by Muffle Furnace (550°C) until it reached persistent weight. The moisture content was determined for all the fruits on the basis of fresh weight by oven drying (103±2°C for 12 h). Crude protein was quantified by nitrogen content (N× 6.25) using macro-Kjeldahl method (Horwitz and Latimer, 2005). The lipid content was estimated by soxhlet extraction of powdered samples with petroleum n- hexane. Crude fiber was obtained by extracting de-fatted berries with 1.25% NaOH and then incinerated in Muffle furnace (Neycraft JFF, 2000) at 600°C. Total carbohydrates were calculated as 100 - (% ash + % fats + % fiber + % protein) (Indrayan *et al.*, 2005). The food Energy value (kcal) was obtained by using following equation 4× (protein+ carbohydrates) + 9×fats (Nwabueze, 2006).

## RESULTS

Data regarding antioxidant potential, macro & micro mineral contents and proximate composition of *Rubus* berries are presented in Tables 1-4 respectively.

## DISCUSSION

#### DPPH Radical scavenging activity

Methanolic fruit extracts of *Rubus* species were tested for DPPH radical scavenging activity at a concentration of 1 mg crude extract/ml. Extracts showed adequate antioxidant activity (>50%). The highest (%) DPPH radical scavenging activity was shown by *Rubus ulmifolius* (98.89%) at the conc. of 400µg/ml (table 1) which is a little bit higher than that of the standard at the same concentration and at 50µg/ml it was 80.23% which is quite high even for small concentration. The antioxidant activity determined for blackberries by Moyer *et al.*, (2002) and Siriwoharn *et al.*, (2004) was lower than the antioxidant activity of blackberries in the present study. Values reported by Koca and Karadenizis (2009) for Wild blackberries of Turkish origin are comparable with the present study and higher than antioxidant activity calculated for cultivated blackberries by the same author. The results obtained in this investigation reveal that, even the minor fractions of *Rubus ulmifolius* fruits extract are free radical scavenger, which might be ascribed to their electron donating ability. *Rubus ellipticus* methanolic extract also exhibited relatively high antioxidant activity ranging from 45.97% to 84% (table 1). Report concerning the presence of strong antioxidant activity in methanolic extract of *Rubus ellipticus* fruits from Himalayan region is contemporary (Badhani *et al.*, 2011). High antioxidant activity in ethanolic, petroleum ether and aqueous extracts is also reported by Sharma and Kumar, (2011) for Indian samples. Flavonoids and phenolic compounds are also reported from *Rubus ellipticus* roots (Vadivelan *et al.*, 2009) which may be responsible for the antioxidant

activity. Slight differences were observed with literature reported on the studied fruits. Many factors such as plant habitat, maturity, growth condition, fruiting season, geographic location, soil type and amount of sunlight received, sample preparation and analytical procedures employed to perform the activity are responsible for difference in the antioxidant activities with literature (Al-Farsi *et al.*, 2007).

### Mineral content

#### Macro-minerals

Amount of N, K, Ca, Mg, Na and P in these fruits considerably are presented in table 2. Potassium was abundant in *Rubus ulmifolius* dried fruit powder (860.17 mg/100g DW). Potassium is a major mineral involved in diverse metabolism function and crucial for proper

activity of different cells, tissues and organs of human body. It is required by humans in high levels (> than 100 mg/day) (Ozcan, 2004). High amount of potassium increase iron utilization and resist hypertension (Adeyeye, 2002). All minor fruits contained high potassium level per 100 g dry weight than reported level in commonly consumed summer fruits including apple, apricot, jambulin, pear, mango etc. (Zahoor *et al.*, 2003) and many other fruits like banana (358 mg/100gm), orange (200 mg/100gm), guava (417 mg/100gm) and apple (90 mg/100gm) (Mahapatra *et al.*, 2012). The levels of Mg in fruits were in the range of 118.72±0.48 (*Rubus ellipticus*) to 179±0.89 (*Rubus niveus*) mg/100g DW (table 2). These levels were comparable to Mg level in berries consumed in Australia (Konczak and Rouille, 2011). Considering the Recommended daily requirement for human beings of

**Table 1:** Antioxidant activity of *Rubus* berries in methanolic extract (Absorbance of negative control=0.994)

Conc. (µg/ml)	% inhibition of <i>Rubus ellipticus</i>	% inhibition of <i>Rubus ulmifolius</i>	% inhibition of <i>Rubus niveus</i>	% inhibition of standard (AA)
50	45.97±0.002	80.28±0.00	68.30±0.002	53.73±0.001
100	54.82±0.001	87.62±0.001	74.54±0.001	67.55±0.001
200	72.23±0.002	94.46±0.002	80.38±0.002	81.98±0.001
400	84±0.001	98.89±0.002	91.64±0.001	98.08±0.001

**Table 2:** Macro minerals (mg/100 g on dry weight) contents in *Rubus* berries

Minerals (mg/100g)	<i>Rubus ellipticus</i>	<i>Rubus ulmifolius</i>	<i>Rubus niveus</i>
K	680.16±1.27	860.17±0.68	720±0.59
N	700±0.08	1500±0.07	500±0.06
P	1.26±0.001	1.22±0.002	1.48±0.02
Na	89.43±0.01	70.2±0.72	56.3±0.05
Ca	450.1±0.22	620.56±1.37	390±0.06
Mg	118.72±0.48	148.66±0.51	179±0.89

**Table 3:** Micro minerals (mg/100 g on dry weight) contents in *Rubus* berries

Micro mineral conc. (Mg/100gm)	<i>Rubus ellipticus</i>	<i>Rubus ulmifolius</i>	<i>Rubus niveus</i>
Fe	4.249±0.15	2.163±0.14	3.26±0.52
Zn	12.77±0.05	17.509±0.09	8.13±0.05
Cu	0.020±0.01	0.065±0.05	1.070±0.01
Pb	0.02±0.18	0.187±0.70	0.234±0.09
Mn	1.948±0.03	1.398±0.01	2.43±0.04
Cr	0.47±0.19	0.83±0.47	0.09±0.01

**Table 4:** Proximate composition of *Rubus* edible berries signifying (Moisture content in g/100 g of fresh weight while other nutrients in g/100 g of dry weight and energetic value in kcal/100 g of dry weight).

Parameters in terms of percentage	<i>Rubus ellipticus</i>	<i>Rubus ulmifolius</i>	<i>Rubus niveus</i>
Moisture	66.36±0.58	58.02±0.95	78.56±0.87
Ash	2.97±0.01	3.43±0.04	4.37±0.09
Crude proteins	4.37±0.52	6.56±0.44	3.28±0.87
Crude lipids	2.73±0.06	4.73±0.06	1.10±0.07
Crude fibers	3.53±0.17	1.66±0.06	5.90±0.25
Carbohydrates	86.4 ±0.38	83.62±0.35	85.35±0.25
Energy values in Kcal	374.0±1.56	403.29±0.69	364.42±0.56

Magnesium (2.0-5.0 mg/day) 2-5g of these fruits per day can meet the requirements of this important mineral. Mg together with Ca is essential cofactors in many enzymatic and cell division processes, for DNA synthesis and chromosomal segregation. Ca is chief constituent of bone and aids in teeth development process (Brody, 1994; Akpanabiatu *et al.*, 1998). Calcium contents of the studied fruits were also assessed to be considerably higher than known values of Ca in many commonly consumed fruits such as apple, apricot, jambulin, pear, mango etc. (Zahoor *et al.*, 2003) and other fruits including orange (11mg/100gm), strawberry (22 mg/100gm), guava (18mg/100gm) and pear (4 mg/100gm) as reported by Mahapatra *et al.*, (2012). Phosphorus was found to be the least abundant mineral elements in *Rubus* fruits. Phosphorous is required for various metabolic reaction in the body. The P levels in the fruits were comparable with the value reported for banana (Leterme *et al.*, 2006). As a conclusion of this study, it can be said the studied minor fruits are rich source of various macro minerals required for human body.

#### Trace elements

Among the studied micro-elements (Fe, Zn, Cu, Pb, Mn, Cr) Zn content were highest in all three fruits while lowest Zn content was observed in *Rubus niveus* dried fruit pulp (table 3). The Fe also content varied. This amount was nearly equivalent to the amount (5.70 g/100g edible portion) determined for *Rubus ideaus* by Plessi *et al.*, (1998). The Fe values are also comparable with recurrently consumed fruits including Apple, strawberry, Banana, guava, Grapes, pears and Pomegranates etc. (USDA, 2011) as well as certain fruits consumed in Mexico (1.1-4.1 mg/100g DW) and UK (0.4-4.4 mg/100g DW) (Sanchez-Castillo *et al.*, 1998). Zinc content of the minor fruits also varied which is the vital trace element is fundamental part of enzymes required for protein as well as nucleic acid formation and increase immunity against various ailments (Melaku, 2005). The deficiency symptoms include poor and infantile body development (Ihedioha *et al.*, 2011). Zn value obtained in the present study is higher as compared to value reported for cultivated raspberries fruits consumed as a best source of Zn in Finland state of Australia (Ekholm *et al.*, 2007). Copper and manganese contents are also found to be varied which are essential trace element assist to sustain blood glucose level, involved in formation of hemoglobin the immune system, in conjunction with vitamin K assist in blood clotting, in presence with B complex vitamins helps to resist anxiety conditions (Anhwange *et al.*, 2004). The Mn values are comparable with the value reported for frozen raspberries (0.21 mg%) (Spada *et al.*, 2010). Copper plays an important role in a number of biological processes (Zahir *et al.*, 2009). Deficiency of copper is major cause to nervous and cardiovascular diseases (Mielcarz *et al.*, 1997). The above metal was not detected in frozen fruit samples (Mielcarz *et al.*, 1997) however

minute quantity of this metal was determined in our study on shade dried fruits. The trace metals (Fe, Zn, Cu and Mn) have strong tendency of reduction due to presence of unpaired electrons and thus involved in scavenging free radicals formed during cellular processes (Rodriguez *et al.*, 2011). Pb and Cr appeared as the insignificant microelements, with lowest values were observed in these fruits. Pb level was low in *Rubus* fruits. Lead is a noxious metal, which can damage central and peripheral nervous systems (Lin-Fu, 1976; Muntean *et al.*, 1998; Kotas and Stasiscka, 2000). Regarding the mineral contents (table 3 & 4), the species *Rubus ulmifolius* had highest amount of most vital macro minerals and trace elements studied including Ca, Mg, N and Zn contents was the most outstanding one.

#### Proximate composition

The proximate composition of three wild brambles (*R. ellipticus*, *R. niveus*, *R. ulmifolius*) are presented in table 4. The moisture content was determined on fresh weight basis while all the other parameters (ash, protein, fat, fiber, carbohydrate) were based on dry weight. Overall, there was significant variation in the proximate composition among samples. The major components of the fruits were moisture and carbohydrates. The moisture content of the edible material is important as many of the physical properties including size, shape, viscosity, density, weight, volume and bulk density of fruit fluctuate with moisture percentage. Assessment of physical properties could be advantageous in fruits harvesting, transportation, storage and processing techniques (Fraser *et al.*, 1978; Omobuwajo *et al.*, 2003; Haciseferogullaria *et al.*, 2005) The moisture content of the fresh berries ranged from 58.02±0.32 (*R. ulmifolius*) to 78.08±0.24 (*R. niveus*) g/100 g. However, the amount of moisture in *Rubus ellipticus* fruit pulp was almost comparable to the reported range of Indian samples (Saklani *et al.*, 2011; Sundriyal and Sundriyal, 2001). This moisture range of the raspberries was similar to other wild berries like cranberries (*Vaccinium oxycoccus* L.) (Souci, *et al.*, 2008) and strawberries (*Arbutus unedo* L.) (Ozcan and Haciseferogullari, 2007; Barros *et al.*, 2010; Rodriguez *et al.*, 2011). Minute differences may be probably due to different environmental conditions such as water accessibility, wind and light exposure etc.

The percentage of ash oscillates between proteins and fat contents, being more abundant in *Rubus niveus* and minimum in *Rubus ellipticus*. These values are almost similar to those of Nigerian jujubes like *Z. mauritiana* reported by Nyanga *et al.*, (2012). However, the ash values in the current study were higher than the previously reported in *Rubus ellipticus* by Saklani *et al.*, 2011. These high ash values which signify the inorganic part of the plant are eloquent of high mineral values specifically the macro minerals (Adepoju, 2009; Momin and Kadam, 2011).

Blackberry contained significantly more crude protein than the raspberries (*Rubus niveus* and *Rubus ellipticus*). Other fruits such as orange, mango, grapes, banana and papaya have crude protein contents of 0.7, 0.6, 0.5, 1.2 and 0.6g/100g (Rathore, 2009). The crude protein values recorded for Yellow Himalayan raspberries were higher than as reported in *Rubus ellipticus* from other regions (Saklani et al., 2011; Sundriyal and Sundriyal, 2001).

Fiber content of fruits varied from 1.66% (*Rubus ulmifolius*) to 5.90% (*R. niveus*) DW (table 4). *Rubus ellipticus* fruits fiber content (1.66%) (table 4) was found to be similar to the earlier reported for the same fruit (Saklani et al., 2011). The distribution of fiber in *Rubus* species is similar to crude fiber content found in other wild berries e.g. *Crataegus monogyna* or *Vaccinium oxycoccus* berries and strawberries (Souci et al., 2008) and other wild fruits including *Arbutus unedo*, *Prunus spinosa*, *Rosa canina* (Barros et al., 2010).

Lipids are essential because they provide the body with maximum energy (Dreon et al., 1990). Nutritionally, fat is less abundant macronutrient being lower than 2% in most fruits analyzed by various scientists. The crude fat content ranged from 1.10±0.0 (*R. niveus*) to 4.73±0.0g/ 100g (*R. ulmifolius*). The present results showed relatively higher amount fats present in berries pulps except *R. niveus* as compared to most of the wild fruits (Demir and Ozcan, 2001; Ozcan and Haciseferogullari, 2007; Barros et al., 2010). The higher amount of lipids were also recorded in *R. ellipticus* fruit than the earlier report for the same fruit (Saklani et al., 2011).

Carbohydrates contents as calculated by difference were found to be major component and were higher than 80%. In fact, the fruits are considered to be richest source of carbohydrates, either monosaccharide or polysaccharides such as cellulose and starch (Demir and Ozcan, 2001; Ozcan and Haciseferogullari, 2007). Deficiency of carbohydrates can causes weakening of body tissues (Barker, 1996). Raspberries and blackberry showed high carbohydrate content, which feasibly elucidate sweet taste of these fruits (Adepoju, 2009). In view of their high carbohydrates contents of *Rubus* species, their consumption should be promoted for the specific purpose of satisfying starvation and hunger. On the basis of the proximate analysis, it can be intended that dry portion of 100 g fruits of *Rubus ulmifolius* assures, on average, 403 kcal of energy. High-energy values and carbohydrates content of *Rubus* fruit qualify them as a supplement of cereal grains.

## CONCLUSIONS

This study reported the nutraceutical contents of *Rubus* fruits and recommend as an alternative source of edible wild fruit at global perspectives.

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