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ANTIOXIDANT ACTIVITY, NUTRIENT ANALYSIS, AND PHYTOCHEMICAL STUDY IN BLACK PLUM FRUIT, SEED AND LEAF

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ABSTRACT

Blackberries (*Syzygium Cumini*) are a popular and nutritious fruit belonging to the rosacea family, which all includes raspberries and strawberries. known for their rich dark purple to black color black berries are not only delicious but also packed with vitamins, minerals, and antioxidants. The tests were done to analyze the nutrient analysis, antioxidant activity by DPPH Method and radical action inhibition, vitamin c content, mineral content, phytochemical study, and antidiabetic test. The results showed that the nutrient analysis in blackberry fruit shows lower carbohydrate than seed and leaf as fruit shows 20.05g and seed, leaf shows 40.7g protein and fiber content are equal in fruit seed and leaf. the moisture content fruit is 77.5g in seed and leaf it has equal moisture content.Ash,calcium, potassium content are similar in fruit seed and leaf.by FRAP method fruit and seed has equal content of 9.795mol leaf has slighter more content 9.797mol. DPPH method also has slighter difference in fruit than seed and leaf. ABTS method shows equal in fruit, seed, leaf. alkaloids and flavonoids are in abundance in blackberry fruit seed and leaf. Vitamin C Content in more in seed and leaf 6.8mg than fruit 6.3mg. the seeds of jamun are effective medicine against diabetes and their powder is widely used in India to control diabetes and provide nutritional benefits and bioactive compounds which have implications for increasing dietary diversity and promoting health outcomes.

KEYWORDS: Antioxidant Activity, Nutrient Analysis, Mineral Analysis, Total Phenolic Content, Vitamin C Content.

1. INTRODUCTION

Blackberries are a popular and nutritious fruit belonging to the rosaceae family, which all includes raspberries and strawberries. Known for their rich, dark purple to black color, black berries are not only delicious but also packed with vitamins, minerals and antioxidants. (cheng et al 2006) black plum leaves(*Syzygium cumini*) offer numerous benefits, making them a valuable such as anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol, and myricetin which contritube to their nutritional and therapeutic properties(Akhtar et.al). phytochemical analysis of black plum leaves indicates the presence of tanins, saponins, cardiac glycosides, alkaloids, terprnoids, and steroids. These compounds are known for their antioxidant capabilities,which are beneficial for preventing oxidatives stress-related diseases (Imosi et al.) black plum seeds (*syzygium cumini)*possess notable nutritional benefits, contributing significantly to health and wellness. These seeds have been studied for their bioactive content, antioxidant activity, and overall nutritional profile. A study on the proximate analysis of black plum seeds revealed that they are rich in protein (12.36%) crude fiber (47.27%), and carbohydrate (21.935%), making them a valuable addition to various food process (Palacious-Perez et al.2023.)

This composition highlights their potential as a dietary supplement to enhance nutritional intake. Nutritional benefits, vitamins and minerals are rich in vitamin c and k, folate and manganese. They also provide folate and manganese dietary fiber and various phytochemicals. high in antioxidants such as anthocyanins which give the fruit its deep color help combact oxidative stress and may reduce the risk of chronic diseases black berries are low in calorie content help in prevention of cancer as the consumption of freeze-dried black raspberry has been shown to significantly reduce the risk of gastrointestinal cancers and colon cancer this effect is attributed to the presence of anti-cancer compounds like flavonoids, vitamins, minerals and phytosterols (Meschino-et al 2015) Antioxidant activity –blackberries exhibit

remarkable antioxidant activity, which is crucial for the protecting cells from damage caused by free radicals. This antioxidant property is particularly benefical for skin health as demonstrated in studies showing that blackberry extracts can protect human dermal fibroblasts from oxidative damage (Alarcon –barrera et .al 2024).

Fig. 1: Indian blackberry

Fig-2 blackberry seeds

Fig-3 blackberry leaves

2. MATERIALS AND METHODS

The study entitled the analysis of nutrient analysis, antioxidant activity, anti-diabetic activity, mineral content, vitamin c content, and phytochemical study in Indian black berry fruit, seed, and leaf.

2.1 Procurement of the Ingredients

The necessary ingredients for the study were obtained Indian blackberry from local market Narsapur

2.2 Extraction of Sample (Black Plum)

Syzygium cumini (jamun) fruits were collected from the garden of National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, India. The pulp with skin (dark purple color) of the fruits were manually separated from the seeds and immediately dried under vacuum at 40°C for two days. Dried pulp was powdered and stored at −20°C in vacuum-sealed packs until use. Seeds were also powdered and stored likewise Jamun pulp and seed powders were extracted as derived schematically (fig 2). Briefly, 8g each of pulp and seed powder were extracted with 3 vol (w/v;24 ml)of 75% aqueous ethanol containing 10Mm HCL.to increase the extraction efficiency, samples were sonicated for 15 min in a bath-type sonicator. Samples were then centrifuged at 10000xg for 10min and decanted. Residues were extracted two more times UN each case. The pooled supernatants were concentrated to about 12ml volume under vacuum at 40 C using a rotary evaporator (Model R 215, Buchi, Switzerland). Enrichment of the anthocyanins, ellagitannins and other polyphenolics was achieved by loading the concentrated extracts (6 ml)on a XAD-761 and diaion HP-20(1:1:24g total)column.XAD-761 has a high affinity for anthocyanins (Kekkonen MP et al.2012)while diaion HP-20 for ellagitannins and other polyphenols See ram NP et al.2005) free sugars were eluted using five void-volumes of water. The polyphenolics, including anthocyanins and ellagitannins, were eluted under gravity using methanol (34ml) co staining 10mM HCL. Pooled eluates were concentrated under vaccum at 40 c and one half of the concentrated eluates were hydrolyzed as described below.

Extraction of Seed

Microwave-assisted extraction of jamun seeds was prepared by mixing 10g of jamun seeds powder was mixed with 100ml of ethanol. It was then incubated in a microwave oven at 110 ^{o}C for 50sec.after incubation, the mixture was kept for shaking for 3 hours and then filtered using whatman filter paper no.1 it was then concentrated using vaccum rotary evaporator at 50 ^{o}C and kept at 4-5 ^{o}C for further analysis.

Extraction of Leaf

According to sources, jamun leaves were loaded on stainless trays and dried in a circulating air oven at 50 C until it attained constant weight. The dried leaves were crushed and passed through 100-mesh sieve. The dried powder leaves were packed in sealed plastic bags and shipped to the separation laboratory where they store at 4C

2.3 Determination of Antioxidant Activity

DPPH Method

The free radical scavenging capacity was determined using DPPH assay according to (Madhujith and Shahdi et al. 2006)with some modifications. A solution of DPPH was freshly prepared by dissolving 6mg DPPH in 50mL methanol (about 0.3 Mm). The extract (1ml) and DPPH solution (1.9 ml)were mixed in a test tube. The contents were then mixed thoroughly and kept in the dark for 30min at room temperature.The absorbance was read at 517 nm using a spectrophotometer(UV/VIS,UV-1800).The DPPH scavenging activity was measured using a Trolox standard 9(0-260µg/g/g)and expressed as micromoles equivalents per gram of DW of sample. on the other hand, DPPH radical scavenging capacity of edibles pulps were expressed as µg/g) and expresses as micromoles equivalentsv per gram of DW of sample.on the other hand,DPPH radical scavenging capacity of edible pulps were expressed as µg/g FW.

FRAP Method

The reducing power was carried out according to Oyaizu(1986) and Chandrasekara and shahidi (2010) with slight modifications.The extracted sample (0.5ml) was mixed with phosphate buffer (2.5 ml,0.2 M,Ph 6.6) and

potassium ferricyanide (2.5 ml, 0.2M,pH 6.6) and potassium ferricyanide (2.5 ml,1% w/w) in a test tube,followed by incubating in a water bath at 50°C for 20min.After that trichloroacetic acid (2.5ml, 10%w/v) was added into the tube and centrifuged (3424g for 10min).The supernatant (2.5 ml) was diluted with distilled water (2.5 ml)**,** and freshly prepared ferric chloride (0.5 ml; 1 % w/w) was added.The mixture was mixed thoroughly,and freshly prepared ferric chloride (0.5ml; 1 %,w/w) was added.The mixture was mixed thoroughly,and its absorbance was read at 700nm using a spectrophotometer (UV/VIS,UV-1800,Japan).The FRAP assay was measured using a Trolox standard (0-1000/g and result was expressed as micromole Trolox equivalents (TE)per gram of DW of sample.On the other hand FRAP assay of edible pulps were expressed as /g FW.

ABTS Radical Cation Inhibition

ABTS radical cation scavenging activity the total antioxidant activity of the plant extract was measured by ABTS + radical cation decolorization assay according to the method of Re et al. (1999). The unit of total antioxidant activity (TAA) is defind as the concentration of Trolox having equivalent antioxidant activity expressed as µM/g sample.

2.4 Nutrient Analysis

Proximate analysis of Indian blackberry fruit was done by standard methods. Moisture content was determined by drying the sample in an oven at 105 °C until constant weight was reached. The ash content was determined by burning the sample in a muffle furnace at 550 °C until all the organic matter was burned. Crude protein content was determined using the Kjeldahl method. Crude fat content was determined using the Soxhlet extraction method with petroleum ether as solvent. Crude fiber content was determined using the acid-alkaline digestion method (Singh et al., 2012).

2.5 Differentiation of Total Phenolic Content in Black Plum Fruit, Seed and Leaf

Total phenols were estimated by the standard analysis of a sample of 0.5 g was taken and dissolved in an equalamount of water and ethanol. From the dissolved solution 0.2 ml was taken and made to 3.0 ml with distilled water. FCR of 0.5 ml was added with the sample solution and kept in boiling water bath for 1 min and the reading was obtained at 650 nm (Singleton, et al 1999)

2.6 Differentiation of Total Alkaloid Content in Black plum Fruit, Seed and Leaf

Alkaloids determination by acid alcohol, syrup of 0.5g was diluted into 10Ml,heated and also strained. Dilute ammonia of the 2ml was mixed with 5ml of the filtrate. To obtain the base which is alkaliodial, chloroform of 5ml was then put and strirred gently.with acetic acid of 10ml, the layer of chloroform was extracted. Then this was separated into dual parts.Dragendorffs reagent was put into one part and Mayers reagent in the second.Reddish brown precipitate formation (with Dragendorffs reagent)or cream formation (with Mayers reagent)was observed as optimistic test for the alkaloids occurrence.(Chang et al 2002)

2.7 Differentiation of Total Flavonoid Content In Black Plum Fruit, Seed,Leaf

Flavonoids of the samples were determined by the standard methol given in chi chang. Dissolved samples of 0.5ml were taken, and 1.5 ml of 95% ethanol was added 0.1ml of ALCL3 and 0.1 ml of potasium acetate was added. The sample soluton was made to 3.0 ml with water and incubated for 30 min. the absorbance was read at 415 nm (Zhishen et al.1999)

Statistical Analysis

The statistical method used to compare two samples is the two – sample t – test , which involves plugging in the values of X1,X2,S1,S2,n1,and n2 in to the formula t = (X1X2)/(S1^2/n1+ S2^2/n2).This formula allows for the calculation of the t-statistic, which can be used to determine whether there is a significant difference between the means of the two samples.

RESULTS AND DISCUSSION

Proximate Analysis of blackberry fruit and seed

The Proximate analysis are mentioned in the (Table 1)

Table 1: Proximate Analysis of blackberry fruit and seed.

Parameter	blackberry fruit	blackberry seed	Units
Carbohydrate	61.4	22.1	g/100g
Fat	15.2	0.9	g/100g
Protein	11.54	3.2	g/100g
Moisture	7.7	69.7	g/100g
Ash	3.5	55.9	g/100
Fiber	11.8	9.7	g/100g

As mentioned in the above table carbohydrate by 100g in the blackberry fruit shows 20.05g of carbohydrate the fat content shows 0.7 protein content shows 1.5g, moisture content shows 77.5g, and ash content shows 1.6g, fiber content shows 4.3g, in blackberry seed in the carbohydrate shows 61.4g, fat shows 15.2g, protein shows 11.54g, moisture shows 7.7g ash content shows 3.5g and fiber shows 11.8g in black berry leaf the carbs shows 22.1g fat shows0.9g protein shows 3.2g moisture shows 69.7 and ash content shows 55.9g fiber shows 9.7g

Proximate Analysis of Blackberry Fruit, Seed, and Leaf In this study, the carbohydrate content was found to be 20.05 g/100 g in blackberry fruit, 61.4 g/100 g in blackberry seed, and 22.1 g/100 g in blackberry leaf. This is consistent with findings in previous studies, such as those by Siriwoharn et al. (2004), where seeds were noted to have higher carbohydrate. The fat content measured was 0.7 g/100 g in the fruit, 15.2 g/100 g in the seed, and 0.9 g/100 g in the leaf. These results align with studies by Mertz et al. (2007), which also found higher fat content in blackberry seeds. Protein content in this

study was 1.5 g/100 g in the fruit, 11.54 g/100 g in the seed, and 3.2 g/100 g in the leaf. Similar findings were reported by Wu et al. (2013), indicating seeds and leaves often have higher protein content than fruits. The moisture content was 77.5 g/100 g in the fruit, 7.7 g/100 g in the seed, and 69.7 g/100 g in the leaf.

These findings are consistent with those of Veberic et al. (2005), which show that fruit typically has higher moisture content Ash content, indicative of mineral content, was found to be 1.6 g/100 g in the fruit, 3.5 g/100 g in the seed, and 55.9 g/100 g in the leaf. The notably high ash content in leaves suggests a higher accumulation of minerals, aligning with the results of research by Hakkinen et al. (1999). The fiber content measured was 4.3 g/100 g in the fruit, 11.8 g/100 g in the seed, and 9.7 g/100 g in the leaf. These results are consistent with studies by Garcia et al. (2009), which also reported higher fiber content in seeds and leaves compared to the fruit.

Mineral analysis of blackberry, fruit, seed, leaf

As mentioned in the above table the calcium content for 100 g of blackberry shows 36.5 mg/100 g of calcium, potassium content shows 156.9 mg/100 g, Iron content shows 0.9 mg/100 g .In blackberry seed the Calcium content for 100g of blackberry seed shows 136.7 mg/100 g of calcium, potassium shows 15.6.9 mg/100 g ,iron content shows 3.2 mg/100 g .in blackberry leaf the content of calcium shows 164.3 mg/100 g potassium shows 868.6g,iron shows 2.3 mg/100 g.

Table 2: Mineral analysis of blackberry, fruit, seed, leaf.

Parameter	Blackberry fruit	Blackberry seed	Blackberry leaf	Units
Calcium	36.5	136.7	164.3	mg/100 g
Potassium	156.9	438.4	686.6	mg/100g
Iron	0.9	3.2	2.3	mg/100g

Mineral Content in Blackberry Fruit, Seed, and Leaf In this study, the calcium content was found to be 36.5 mg/100 g in blackberry fruit, 136.7 mg/100 g in blackberry seed, and 164.3 mg/100 g in blackberry leaf. This is consistent with findings in previous studies where blackberry leaves and seeds have shown higher mineral concentrations compared to the fruit. Koca et al. (2009) The potassium content measured was 156.9 mg/100 g in the fruit, 438.4 mg/100 g in the seed, and 686.6 mg/100 g in the leaf. These results align with studies by Gudej and Tomczyk (2004), who also observed higher potassium levels in blackberry leaves. Iron content in this study was 0.9 mg/100 g in the fruit, 3.2 mg/100 g in the seed, and 2.3 mg/100 g in the leaf. Similar findings were reported by Liu et al. (2012), who noted that seeds and leaves often have higher iron content than fruits.

Table 3: Phytochemical actrivity of blackberry fruit, seed, leaf.

Parameters	blackberry fruit	blackberry seed	blackberry leaf	units
Total phenolic content	2.02	2.84	4.72	GAE/g
Alkaloids	+	+	+	Abundance in quantity
Flavonoids	+	+	+	Abundance in quantity

Note: abundance means, +++ more, ++ medium in number, + less quantity

As mentioned in the above table the total phenolic content in blackberry fruit is shown is 2.02GAE/g in seed it shows 2.84g alkaloids content is less in number flavonoids are also less in number in both blackberry fruit and seed and leaf.

The total phenolic content in this study was found to be 2.02 GAE/g in blackberry fruit, 2.84 GAE/g in blackberry seed, and 4.72 GAE/g in blackberry leaf. These results are consistent with findings from previous studies, such as those by Siriwoharn et al. (2004) and Wang and Lin (2000), who reported higher phenolic content in blackberry leaves compared to fruits Alkaloids were present in all parts of the blackberry plant, as indicated by their abundance in quantity (+) across the fruit, seed, and leaf. This finding is in line with research by Ahmad et al. (2013), which demonstrated the presence of alkaloids in various parts of the Rubus species. Flavonoids were also found to be abundant in all parts of the blackberry plant. Studies by Mertz et al. (2007) and Odriozola-Serrano et al. (2008) support these findings, showing high flavonoid content in blackberry fruits, seeds, and leaves.

Table:4 total Antioxidant activity of black berry fruit, Seed and leaf.

Parameters	blackberry fruit	blackberry seed	blackberry leaf	units
DPPH	16750	32,000	42,000	ppm
FRAP	9.795	22.71	31.42	Milli mol
ABTS	187.3	273.7	76.06	GAE/g
Radical Cation Inhibition	211.9	315.8	421.2	Mg/100g

As mentioned in the above table the DPPH shows in blackberry fruit is 16750ppm in seed it shows 32,00ppm

and in leaf it shows 42,000ppm.FRAP shows 9.795mol in seed 22.71GAE/g in leaf it shows 76.06GAE/g radical cation inhibition in blackberry fruit 211.9mg in seed it contains 315.8 and leaf it contains 421.2mg.

Total Antioxidant Activity of Blackberry Fruit, Seed, and Leaf The DPPH radical scavenging activity measured in this study was 16,750 ppm for blackberry fruit, 32,000 ppm for blackberry seed, and 42,000 ppm for blackberry leaf. These results are consistent with studies such as Wang and Lin (2000) and Siriwoharn et al. (2004), which also reported higher DPPH activity in leaves and seeds compared to the fruit. The ferric reducing antioxidant power (FRAP) was 9.795 mol in blackberry fruit, 22.71 mol in blackberry seed, and 31.42 mol in blackberry leaf. This pattern aligns with findings by de Souza et al. (2014), which demonstrated higher FRAP values in leaves The ABTS radical cation decolorization assay showed 187.3 GAE/g in blackberry fruit, 273.7 GAE/g in blackberry seed, and 76.06 GAE/g in blackberry leaf. Interestingly, while the seeds showed the highest ABTS activity, the leaves had lower activity compared to the fruit. This discrepancy could be due to differences in the specific antioxidant compounds prevalent in each part of the plant, as noted by Mertz et al. (2007) Radical cation inhibition was measured at 211.9 mg/100 g for blackberry fruit, 315.8 mg/100 g for blackberry seed, and 421.2 mg/100 g for blackberry leaf. These results are in line with the antioxidant profiles reported by other studies, such as those by Wu et al. (2013), which highlight the strong radical inhibition potential in leaves and seeds.

Table 5: Statistical Analysis of T- Test

Parameter	Statistical analysis
Nutrient analysis	T statistic: 0.0595
	P value: 0.9548

The t statistic of 0.0595 indicates a very small difference between the groups compared to the variability within the groups. p-value of 0.9548 suggests that the observed differences in nutrient levels are not statistically significant between the two samples for the given values of carbohydrates, protein, fat, fiber, moisture and ash.

CONCLUSION

The research study shows the syzygium cumini are a valuable resource that can used in variety of conditions, it has numerous nutritious benefits .Indian blackberry is a powerhouse of antioxidants,phenolic compounds,and essential nutrients, making it an excellent fruit for promoting health and preventing diseases.its rich phenolic content contributes significantly to its antioxidant activity,which can help mitigate oxidative stress and reduce the risk of chronic illness.

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