



Proximate composition, mineral content, phytochemical analysis and *in vitro* antioxidant activities of a wild edible fruit (*Grewia sapida* Roxb. ex DC.) found in Assam of North-East India

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ABSTRACT

Aim: The aim of present study was to investigate the nutritional composition, phytochemical constituents and *in vitro* antioxidant activities of a wild edible fruit in order to document data for further basic research needs or to widen its use among common people. **Methods:** The fresh fruits of *Grewia sapida* Roxb. ex DC. were collected from the Chirang district of Bodoland Territorial Area Districts of Assam, identified and freeze dried. Proximate composition, minerals content and phytochemical constituents were analyzed following standard and reported methods. The antioxidant activities of methanol extract were evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2'-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) assays. **Results:** The results obtained were reported and discussed. The phytochemical screening of different solvent extracts revealed that fruits contained a number of medicinally active secondary metabolites. The study showed antioxidant properties having IC₅₀ value of 257.666 ± 2.516 µg/mL (DPPH) and 134.33 ± 4.041 µg/mL (ABTS). The total phenolic and flavonoid contents in *G. sapida* fruit were found to be 294.353 ± 4.696 mg GAE/g dried extract and 116.95 ± 10.71 mg QE/g dried extract respectively. **Conclusion:** The study suggests that *G. sapida* fruit could be a good source of minerals and natural antioxidants, and studied further for its beneficial effect in human health.

KEY WORDS: *Grewia sapida*; Proximate composition; Mineral contents; Phytochemical analysis; Antioxidant activity, DPPH; ABTS; North-East India.

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INTRODUCTION

Wild edible fruits which have been identified to have rich nutritional value as cultivated one play an important role to the rural poor and tribal communities in the form of food and nutrient supplement[1, 2]. Many of these edible fruits are abundantly available in the forest and wild areas, and huge quantities of wild fruits are usually not collected and wasted because their therapeutic properties and potential as subsidiary food sources are practically unknown to the village and rural communities. The study on the nutritional and therapeutic values of forest foods is extremely important as it will encourage people to consume greater quantity of food and provide them with a better balance of nutrients. In recent years, a growing interest has emerged among researchers throughout the world to assess various wild edible fruits and plants for their nutritional and other features for the well-being of human society[3, 4]. It has been considered that wild and cultivated fruits are rich sources of various vitamins, minerals, fibres and polyphenols which could provide several health benefits and consumption of fruits and green leafy vegetables reduces the risk of several diseases like diabetes, cancer, coronary heart disease, neurodegenerative ailment and aging as well[5-7].

Antioxidants are substances that delay or inhibit the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation process in the human body can produce free radicals. The human bodies encounter free radicals every day in daily life through over-exposure to sun, toxins in food, carcinogens present in our environment, polluted air and water, car fumes, and even stress can create free radicals[8, 9]. The human bodies are always in a constant attack by free radicals or highly reactive atoms or species and as a result the chain reaction starts in a cell which ultimately can cause damage or even death to the cell in the body. Antioxidants can inhibit or terminate these chain reactions by removing free radicals, and inhibit other oxidation processes and ultimately prevent the progress of chronic diseases such as cancer, heart disease, stroke, cataracts and premature aging[10-13]. Antioxidants include both synthetic and natural. Synthetic antioxidants have been found to be harmful while the natural antioxidants are considered to be an alternative to synthetic ones because of their potential health benefits. Vitamins and polyphenols found in various fruits and vegetables are considered to have strong antioxidant property and hence, fruits and vegetables are rich sources of natural antioxidants[14, 15].

North-East (NE) India is the eastern-most region of India which has eight different states comprising of 'Seven Sisters' states viz. Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura, and the Himalayan state of Sikkim. Assam (89°50' E to 96°10' E and 24°30' N to 28°10' N) is one of the cultural and biodiversity rich states of NE India with total area of 78,438 sq. km of which 26,832 sq. km is forest area according to Forest Survey of India, 2011. The region has friendly climatic conditions with greenness of land, variety of plants many of which have their own biological values, and the rural and tribal people of the region mostly depend on biological resources for their food, shelter, medicare and other cultural purposes[16, 17].

Grewia sapida Roxb. ex DC., locally known as *Kusra pitai* in Bodo, belongs to the family Malvaceae. It is a small under shrub with a perennial woody rootstock and grows to about 1–1.5 m in height. It is distributed mainly in tropical and subtropical regions of the world. The plant is native to South Asia and mainly occurs in India, Pakistan, China and Nepal. In India, it is spread over Assam, West Bengal, Bihar, Gujarat, Haryana and Karnataka. The genus *Grewia* comprises approximately 150 species of small trees and shrubs and is the only genus in the family that yields edible fruits[18]. There is no information available in the literatures on nutritional composition, minerals content, phytochemical constituents and antioxidant properties of *G. sapida* fruit. Therefore, the aim of this study is to determine the nutritional composition and phytochemical constituents of the fruits along with antioxidant properties in order to document data for further basic research needs or to widen its use among common people and to preserve the plant.

MATERIALS AND METHODS

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) and quercetin were purchased from Himedia Laboratories Pvt. Ltd., Nashik, Mumbai, India, gallic acid from Central Drug House Pvt. Ltd., Daryaganj, New Delhi, India, Folin-Ciocalteu's reagent and ascorbic acid from Merck, Mumbai, India. All other chemicals and solvents used in this study were of analytical grade and used without further purification.

Collection and identification of plant

The fresh fruits of *Grewia sapida* Roxb. ex DC. were collected in the month of April and May, 2015 from the Chirang district of Bodoland Territorial Area Districts (BTAD) of Assam and the plant was authenticated by the Botanical Survey of India, Shillong, Meghalaya.

Preparation of the extracts

The collected fruits were washed with water to remove dirt and processed for determination of vitamin C content of

the fresh fruits on the same day. For other parameters, the fruits were freeze dried for 72 hours. Thereafter, the dried sample was ground into fine powder with a mixer grinder and stored in air tight container prior to use for analysis. For phytochemical analysis, the powder in 1:10 ratio (w/v) was subjected to solvent extraction separately with methanol, hexane, chloroform, acetone and aqueous by dipping for 72 h and shaking at room temperature. It was then filtered with Whatman No. 1 filter paper, filtrate was collected and evaporated to dryness under reduced pressure using Rotary evaporator (Buchi Rotavapor R-215, Switzerland). The dried extracts were stored in small dry containers at 4 °C until further use.

Determination of moisture content

The moisture content was determined following AOAC method[19]. 5 g of the freeze dried sample was heated in a hot air oven at 105 °C for 2 h, cooled in desiccator, weighted and the moisture content was calculated by the following formula.

$$\text{Moisture (\%)} = \frac{(\text{Weight of sample} - \text{Dry weight})}{\text{Weight of the sample taken}} \times 100$$

Determination of ash content

The ash content was determined by the AOAC method[19]. Silica crucible was first heated in a muffle furnace, cooled in a desiccator and the initial weight was taken. 5 g of the sample was heated in a muffle furnace at 550 °C for 6 h, cooled in desiccator, weight of the ash was taken and ash content calculated.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

Determination of crude fat

The crude fat content was determined following AOAC method[19]. The initial weight of the flask was taken by heating in a hot air oven for overnight at 105 °C followed by cooling in a desiccator. 3–5 g of the sample was extracted with petroleum ether using Soxhlet apparatus for about 6 h. The extracted fat was dried in a rotary evaporator and the weight was measured.

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of the sample}} \times 100$$

Determination of crude protein

Crude protein was determined by Kjeldhal method following the AOAC method[19]. 1 g of the sample was digested with 20 mL concentrated H₂SO₄ and Kjeldhal catalyst (9 parts of K₂SO₄ and one part of CuSO₄) in a

digestion chamber until it becomes clear. The blank test was performed without the sample. After digestion, it was distilled in Kjeldhal distillation chamber (Buchi Kjelflex K-360). The evaporated ammonia was condensed and then titrated against the known concentration (0.1 N) of HCl. The concentration of nitrogen was calculated by the following formula.

$$\text{Nitrogen (\%)} = \frac{(A - B) \times N \text{ of HCl} \times 14}{\text{Weight of the sample}} \times 1000$$

Where, A = Volume (mL) of (0.1 N) HCl used in sample titration.

B = Volume (mL) of (0.1 N) HCl used in blank titration.

14 = Atomic weight of Nitrogen.

The nitrogen content thus obtained was multiplied by a protein conversion factor of 6.25 to get the crude protein content.

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25.$$

Determination of crude fibre

Crude fibre was also determined according to AOAC method[19]. 1 g of the dry sample was boiled with 0.25 N H₂SO₄ for 30 min followed by filtration with muslin cloth, washed with hot water and again boiled with 0.313 N NaOH. It was again filtered, washed with hot water followed by 0.5 N H₂SO₄ and 50% ethanol. The residue was dried in an oven at 130 °C for 2 h. The dry weight of the digested sample was taken, incinerated in a muffle furnace at 600 °C for 30 min, cooled in a desiccator and weight of the ash was measured. The crude fibre content was calculated based on 100 g of the freeze dried sample using the following formula.

$$\text{Crude fibre (\%)} = \frac{(\text{Dry weight of digested sample} - \text{Weight of ash})}{\text{Weight of sample}} \times 100$$

Determination of total carbohydrate

The total carbohydrate content was determined by the difference method[20] according to the following formula.

$$\text{Carbohydrate (\%)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Crude protein (\%)} + \text{Crude fat (\%)}].$$

Dry matter of fruits

The dry matter or total solid was determined by subtracting percentage of moisture from the hundred[20]. Dry matter = 100 – Moisture (%).

Calorific value of fruits

Calorific value or the total energy value of fruits in kcal/100

g was calculated[21] with the help of following equation.

$$\text{Calorific value (kcal/100 g)} = 4 \times \text{Protein (\%)} + 9 \times \text{Fat (\%)} + 4 \times \text{Carbohydrate (\%)}.$$

Determination of vitamin C

The vitamin C content present in fresh fruit was determined by the iodine titration method[22].

Minerals determination

Minerals such as sodium, potassium, calcium, magnesium, iron, zinc, copper, manganese, and cobalt were determined using Atomic Absorption Spectrometer (AAS-ICE 3500, Thermo Scientific, UK) at Sophisticated Analytical Instrumentation Centre (SAIC), Tezpur University. Sample was digested using wet ashing method with concentrated HNO₃. Results obtained were converted to mg/100 g of freeze dried sample.

Preliminary phytochemical screening

The extracts of freeze dried powders of *G. sapida* fruit were subjected to qualitative analysis for the presence of different phytochemical constituents by following the standard methods[23, 24].

Test for alkaloids

Wagner's and Dragendroff's tests

2 mL extract was mixed with 2 mL of 1% aqueous HCl, taken into two separate test tubes and 6 drops of Wagner's and Dragendroff's reagents were added. The formation of a reddish brown precipitate with Wagner's reagent and orange-red precipitate with Dragendroff's reagent indicated the presence of alkaloids.

Test for saponins

1 mL extract was shaken vigorously with 20 mL of distilled water in a graduated cylinder for 15 minutes. Persistence of froth indicated the presence of saponins.

Test for cardiac glycosides

Keller-Killiani's test

2 mL extract was treated with 1 mL of glacial acetic acid containing few drops of FeCl₃ solution. To this, 2 mL of concentrated H₂SO₄ was poured from the side carefully. The formation of reddish brown at the junction of two layers and the formation of bluish green at upper layer indicated the presence of deoxysugar and hence cardiac glycosides.

Tests for steroids

Liebermann Burchard Test

1 mL of the extract was taken in a test tube and then 2 mL of acetic anhydride and few drops of conc. H₂SO₄ were

added to it. Green coloration was the indication for the presence of steroids (terpenoids).

Salkowski's test

2 mL of the extract was taken in a test tube and then 2 mL of chloroform and 2 mL of concentrated sulphuric acid were added to it and shaken well. Chloroform layer appearing red and acid layer showing greenish yellow fluorescence was considered as an indication for the presence of steroids.

Test for anthraquinones

Modified Borntrager's test

About 0.5 g of the extract was taken in a dry test tube and 5 mL chloroform was added and shaken for 5 min. The extract was filtered, and the filtrate was shaken with an equal volume of 100% ammonia solution. A pink, violet or red colour in the ammonical layer (lower layer) indicated the presence of anthraquinones.

Test for coumarins

3 mL of 10% NaOH was added to 2 mL of extract and formation of yellow color indicated the presence of coumarins.

Test for phenols

Ferric chloride test

5 mL of extract was allowed to react with 1 mL of 5% ferric chloride solution. Greenish black coloration indicated the presence of phenols.

Test for tannins

Gelatin test

1% gelatin solution containing 10% sodium chloride solution was added to the extract. Formation of white precipitate indicated the presence of tannins.

Test for flavonoids

Shinoda's test

To the test solution, a small piece of magnesium ribbon was added followed by drop wise addition of concentrated hydrochloric acid. Pink-tomato red color indicated the presence of flavonoids.

Test for carbohydrates

Molisch's test

2 mL of extract was treated with few drops of 15% ethanolic α -naphthol solution in a test tube and 2 mL of concentrated sulphuric acid was added carefully along the sides of the test tube. The formation of a reddish violet ring at the junction of two layers indicated the presence of carbohydrates.

Fehling's Test

2 mL of the extract was added in 1 mL of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few minutes. Red precipitate formation was an indication for the presence of carbohydrates (reducing sugars).

Iodine test for starch

The crude extract was mixed with 2 mL of iodine solution. A dark blue or purple coloration indicated the presence of starch (carbohydrate).

Test for anthocyanins

2 mL of extract was added to 2 mL of 2N HCl and ammonia. The appearance of blue-violet color indicated the presence of anthocyanins.

Test for proteins

Ninhydrin test

Few drops of Ninhydrin reagent were added to the extract. Appearance of blue color indicated presence of amino acid.

Millon's test

Crude extract when mixed with 2 mL of Millon's reagent, white precipitate appeared which turned red upon gentle heating that indicated the presence of protein.

Test for phlobatannins

The crude extract of sample was boiled with 2% HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Test for lignin

2 mL of 2% (w/v) furfuraldehyde was added to the test solution. Formation of red color indicated the presence of lignin.

Determination of antioxidant properties

DPPH free-radical scavenging assay

Antioxidant activity of methanol extract of freeze dried *G. sapida* fruit was performed by stable DPPH free radical [25]. 1 mL methanolic solution of each extracts (2, 5, 10, 50, 100, 200, 400, 500 μ g/mL) was added to 3 mL working solution of DPPH (0.1 mM DPPH in methanol) and incubated in the dark. After 30 min, the absorbance of all extracts were read at 517 nm with an UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA) and compared to that of standard ascorbic acid with similar concentrations. 1 mL of methanol with 3 mL working solution of DPPH served as blank. The % inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample or standard}) \text{ after } 30 \text{ min}}{(\text{Absorbance of blank}) \text{ after } 30 \text{ min}} \times 100$$

The IC₅₀ value in methanol extract of freeze dried sample and standard ascorbic acid were calculated by plotting a graph of % inhibition versus concentration.

ABTS free radical scavenging assay

Antioxidant activity of methanol extract was also investigated by ABTS assay [26] with slight modification. ABTS radical cation (ABTS⁺) was produced by the reaction of 7 mM ABTS solution with potassium persulphate (2.45 mM). The ABTS⁺ solution was diluted with methanol to an absorbance of 0.706 ± 0.01 at 734 nm. The mixture was stored in the dark at room temperature for 12 h before use. After addition of 25 - 250 μ L of sample extract or ascorbic acid used as standard to 2 mL of diluted ABTS⁺ solution, absorbance was measured at 734 nm with an UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA) after exactly 6 min. The decrease in absorption was used for calculating scavenging effect values. The % inhibition was calculated as:

$$\% \text{ Inhibition} = 100 \times (\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank}$$

Determination of total phenolic content

Total phenolic content in dried extract of methanol obtained from freeze dried sample was determined spectrophotometrically using Folin-Ciocalteu's reagent [25]. 1 mL methanolic solution of extract (1 mg/mL) or standard solutions of gallic acid (10, 20, 40, 60, 80, 100 μ g/mL) was added to 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in distilled water and incubated for 5 min. After 5 min of incubation, 2 mL of 7.5% Na₂CO₃ solution was added to the mixture, incubated in dark for 30 min at 24 °C, and the absorbance was measured at 765 nm with an UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA). Blank was concomitantly prepared containing 1 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent and 2 mL of 7.5% Na₂CO₃ solution. The results were calculated using the calibration curve of gallic acid and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram extract (mg GAE/g dried extract).

Determination of flavonoid content

Total flavonoid content was also determined spectrophotometrically at 510 nm with slight modification [27]. 1 mL methanolic solution of extract (1 mg/mL) or standard solutions of quercetin (10, 20, 40, 60, 80, 100 μ g/mL) was added to 0.5 mL of 5% NaNO₂ solution and 0.5 mL of 10% AlCl₃ solution. After 5 min, 2 mL of 4% NaOH solution was added and incubated for 15 min at room temperature and the absorbance was measured against the blank at 510 nm with an UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA). Blank reagent was prepared by adding the entire reagent without adding sample or standard. A calibration curve was being constructed using standard quercetin and the total flavonoid content was expressed as milligrams of quercetin

equivalents per gram extract (mg QE/g dried extract).

Statistical analysis

All the experiments were carried out in triplicates and data were expressed as mean \pm standard deviation.

RESULTS

The proximate composition, calorific value and vitamin C content of the fruits studied are presented in Table 1. The results were based on 100 g of freeze dried sample and vitamin C on 100 g of fresh weight. The proximate analysis of *G. sapida* fruit revealed moisture content of 16.25 ± 0.02 g per 100 g of freeze dried sample and 81.06 ± 0.75 g per 100 g of fresh fruit. The fruit was found to contain 0.29 ± 0.027 g of ash with 0.13 ± 0.013 g as acid-insoluble ash and 0.14 ± 0.02 g as acid soluble ash. Crude fat, crude protein, crude fibre and total carbohydrate contents found were 2.5 ± 0.26 g, 0.78 ± 0.017 g, 1.71 ± 0.026 g and 80.18 ± 0.02 g per 100 g of freeze dried sample respectively. The dry matter obtained was 83.75 ± 0.03 g. The calorific or nutritive value of *G. sapida* fruit was found to be 346.34 ± 0.04 kcal/100 g. The vitamin C estimated in fresh fruit was 8.6 ± 0.30 mg/100 g.

Table 1. Proximate analysis of *G. sapida* fruit per 100 g of freeze dried sample

Parameters	Composition
Moisture (g)	16.25 ± 0.02 (81.06 \pm 0.75 ^a)
Ash (g)	0.29 ± 0.027
Acid insoluble ash (g)	0.13 ± 0.013
Acid soluble ash (g)	0.14 ± 0.02
Crude fat (g)	2.5 ± 0.26
Crude protein (g)	0.78 ± 0.017
Crude fiber (g)	1.71 ± 0.026
Total carbohydrate (g)	80.18 ± 0.02
Dry matter (g)	83.75 ± 0.03
Calorific value (kcal)	346.34 ± 0.04
Vitamin C (mg/100 g fresh fruit)	8.6 ± 0.30

Values were expressed as mean of 3 replicates \pm standard deviation.

^aMoisture content of fresh fruit.

Table 2 shows the minerals content in mg per 100 g of freeze dried fruit. Sodium content of the fruit was found to be 3.873 ± 0.019 mg/100 g. The fruit was found rich in minerals like potassium (1243.788 ± 8.707 mg/100 g), calcium (472.555 ± 0.945 mg/100 g) and magnesium (122.004 ± 0.244 mg/100 g). The amount of iron and zinc detected in *G. sapida* fruit were 7.574 ± 0.015 mg/100 g and 1.318 ± 0.004 mg/100 g respectively. The amount of copper, manganese and cobalt detected in the fruit were 0.905 ± 0.047 mg, 3.208 ± 0.003 mg and 0.299 ± 0.017 mg per 100 g, respectively.

Table 2. Mineral analysis of *G. sapida* fruit

Minerals	Content (mg/100 g FD)
Sodium	3.873 ± 0.019
Potassium	1243.788 ± 8.707
Calcium	472.555 ± 0.945
Magnesium	122.004 ± 0.244
Iron	7.574 ± 0.015
Zinc	1.318 ± 0.004
Copper	0.905 ± 0.047
Manganese	3.208 ± 0.003
Cobalt	0.299 ± 0.017

Values were expressed as mean of 3 replicates ± standard deviation. FD, freeze dried.

The qualitative phytochemical analysis results of different solvent extracts are shown in **Table 3**. The freeze dried *G. sapida* fruit extracts of five different solvents viz. methanol, chloroform, hexane, acetone and aqueous were investigated for the presence of various bioactive compounds like alkaloids, saponins, cardiac glycosides, steroids, anthraquinones, coumarins, phenols, tannins, flavonoids, carbohydrates, starch, anthocyanins, proteins, phlobatannins and lignin. The fruit showed the presence of alkaloids, saponins, steroids, phenols and carbohydrates in all the five extracts. Cardiac glycoside was not detected in aqueous extract, while it was found to be present in methanol, chloroform, hexane and acetone extracts. Methanol, acetone and aqueous extracts of fruit showed the presence of anthraquinones and coumarins, while these were not detected in chloroform and hexane extracts. Tannins gave positive results in methanol and acetone extracts. Chloroform extract showed negative result for

flavonoids, while these were found to be present in all other four extracts. Chloroform, hexane and aqueous extracts gave positive results for starch. Methanol, hexane, acetone and aqueous extracts were found to contain anthocyanins. Methanol and aqueous extracts showed the presence of proteins (Millon's test) and phlobatannins were detected in acetone and aqueous extracts of the fruit. Lignin was found to be present in four different solvent extracts except methanol.

In this study, the DPPH and ABTS methods were employed to evaluate the *in vitro* antioxidant activities of methanolic extract of *G. sapida* fruit. The DPPH and ABTS free radical scavenging activities of *G. sapida* freeze dried fruit extract in methanol and standard ascorbic acid are shown in **Table 4**. The study showed that the DPPH free radical scavenging activity increased with the increasing concentrations of sample extract showing highest % of inhibition (85.433 ± 0.251%) in 500 µg/mL, while that of standard ascorbic acid was found to be 95.066 ± 0.450% in 500 µg/mL. The IC₅₀ value found in the present study with DPPH assay was 257.666 ± 2.516 µg/mL. The ABTS radical scavenging activity also increased with the increasing concentrations of sample extract showing 66.95 ± 0.29% inhibition at 250 µg/mL. The results shown in **Table 4** revealed that the ABTS free radical scavenging assay showed higher antioxidant capacities with IC₅₀ value 134.33 ± 4.041 µg/mL than DPPH assay (IC₅₀ = 257.666 ± 2.516 µg/mL). The total phenolic and flavonoid contents in methanol extract of the fruit are shown in **Table 5**. The total phenolic content was 294.353 ± 4.696 mg GAE/g dried extract and flavonoid content detected was 116.95 ± 10.71 mg QE/g dried extract.

Table 3. Phytochemical screening of freeze dried *G. sapida* fruit with different solvent extracts

Phytochemical constituents	Test	Methanol	Chloroform	Hexane	Acetone	Aqueous
Alkaloids	Wagner's reagent	+	+	+	+	+
	Dragendroff's reagent	+	+	+	+	+
Saponins	Froth test	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	+	+	-
Steroids (Terpenoids)	Liebermann-Burchard test	+	+	+	+	+
	Salkowski's test	+	+	+	+	-
Anthraquinones	Modified Borntrager's test	+	-	-	+	+
Coumarins		+	-	-	+	+
Phenols	FeCl ₃ test	+	+	+	+	+
Tannins	Gelatin test	+	-	-	+	-
Flavonoids	Shinoda's test	+	-	+	+	+
	Molisch's test	+	+	+	+	+
Carbohydrates	Fehling's test	+	+	+	+	-
	Iodine test	-	+	+	-	+
Anthocyanins		+	-	+	+	+
Proteins	Ninhydrin test	-	-	+	-	-
	Millon's test	+	-	-	-	+
Phlobatannins		-	-	-	+	+
Lignin		-	+	+	+	+

(+), present; (-), absent.

Table 4. DPPH and ABTS free radical scavenging activities of freeze dried *G. sapida* fruit extract

Standard/ Extract	DPPH assay			ABTS assay		
	Conc. ($\mu\text{g/mL}$)	Inhibition (%)	IC ₅₀ value ($\mu\text{g/mL}$)	Conc. ($\mu\text{g/mL}$)	Inhibition (%)	IC ₅₀ value ($\mu\text{g/mL}$)
Ascorbic acid	2	15.8 \pm 0.556	16.666 \pm 2.516	25	36.093 \pm 0.875	73.666 \pm 3.214
	5	27.1 \pm 0.754		50	38.52 \pm 1.176	
	10	36.433 \pm 0.702		75	55.55 \pm 1.023	
	50	93.233 \pm 0.404		100	66.856 \pm 0.661	
	100	93.6 \pm 0.50		150	73.506 \pm 0.810	
	200	94.166 \pm 0.550		250	79.426 \pm 1.168	
	400	94.333 \pm 0.650				
	500	95.066 \pm 0.450				
Methanol	2	6.733 \pm 0.270	257.666 \pm 2.516	25	29.69 \pm 0.58	134.33 \pm 4.041
	5	13.966 \pm 0.750		50	32.5 \pm 0.656	
	10	16.566 \pm 0.375		75	42.866 \pm 0.512	
	50	21.246 \pm 0.427		100	46.72 \pm 1.391	
	100	26.166 \pm 0.251		150	55.89 \pm 1.045	
	200	35.833 \pm 0.404		250	66.95 \pm 0.29	
	400	75.466 \pm 0.450				
	500	85.433 \pm 0.251				

Conc., Concentration; Results are expressed as mean of 3 replicates \pm standard deviation.

Table 5. Total phenolic and flavonoid contents in methanol extract of freeze dried *G. sapida* fruit

Parameters	Result
Total phenolic content (mg GAE/g dried extract)	294.353 \pm 4.696
Flavonoid content (mg QE/g dried extract)	116.95 \pm 10.71

Results are expressed as mean of 3 replicates \pm standard deviation.

DISCUSSION

The proximate analysis includes moisture, ash, crude fat, crude protein, crude fibre and total carbohydrate (Table 1). The moisture content is dependent on humidity, temperature and harvest time of the species. The ash content in the fruits is generally low which contains salt of metals and trace minerals. Lower value of acid-insoluble ash indicates the presence of a small amount of non-physiological components like silica and silicates. Higher value of acid-soluble ash suggests larger amount of acid soluble compounds like oxalates, carbonates, phosphates and oxides[25]. The crude fat obtained in this study is almost similar to the values of jujube and tamarind fruits reported by Salih *et al.*[28]. The crude protein content is also comparable to jujube fruit (0.8 g) reported by Pareek[29], while Salih *et al.*[28] reported higher levels of crude protein in their work. The crude fibre contained in *G. sapida* fruit was close to the values reported for the fruits of milk apple, malay apple and water apple by Lim *et al.*[30]. The total carbohydrate content reported in this study is close to the values reported by Gnansounou *et al.*[31]. The important nutrients of life are carbohydrates, fats and proteins. The

fruits rich in carbohydrate content provide more energy. The key role of carbohydrate in the body is to supply energy and is responsible for doing various activities in our daily life[32, 33]. The calorific value of *G. sapida* fruit (Table 1) is higher in contrast to that of *Carallia brachiata* fruit (310.25 kcal/100 g) reported by Patil *et al.*[34]. Similarly, calorific value of jackfruit and chempedak reported by Tang *et al.*[35] were 301 kcal/100 g and 490 kcal/100 g, respectively. Seal *et al.*[36] also reported the calorific value of five wild edible fruits of Meghalaya, India and found to be ranged from 342.15 \pm 0.13 to 419.09 \pm 0.06 kcal/100 g. Vitamin C also known as ascorbic acid is the main vitamin supplied by fruits and vegetables in the human diet. Fruits and vegetables normally can provide about 90% of a person's dietary vitamin C requirement. The average vitamin C requirement for an adult human being is about 50 mg per day[7, 37]. Vitamin C is needed during various growth stages of human life and being a powerful reducing agent, it plays an important role in absorbing and neutralizing free radicals and thus protects the body from harmful effects[7, 38].

Minerals play a key role in maintaining proper function and

good health in the human body. Insufficient consumption of minerals in the diet is often linked to an increased susceptibility to infectious diseases due to the weakening of the immune system[39]. Fruits, vegetables, and drinking water are important sources of essential elements[40]. Sodium, potassium, calcium, and magnesium are macro-minerals, whereas iron, zinc, copper, manganese, cobalt and nickel are micro-minerals which are also known as trace elements. The trace elements are required in very trace quantities as they are important for the physiological and biological functions of the human body[41, 42]. These elements can sometimes act as antioxidants. The functions of antioxidants are associated with decreased DNA damage, diminished lipid peroxidation, maintained immune function and inhibited malignant transformation of cells[39, 43]. Deficiency and excess of these metals leads to metabolic disorders[44]. The sodium content of the fruit (Table 2) may depend on sodium of soils. Sodium plays an important role in fluid distribution, blood pressure, and cellular work[34]. The *G. sapida* fruit was found to contain rich macro-minerals like potassium, calcium and magnesium (Table 2). Potassium plays an important role in controlling skeletal muscle contraction and nerve impulse transmission[45, 46]. It was reported by Nair *et al.*[47] that high amount of potassium is helpful for the people taking diuretics to control hypertension and it also increases the utilization of iron. The recommended dietary allowance of sodium and potassium intake level per day for a healthy adult is not more than 2400 mg and 4700 mg respectively[39], while the recommended daily allowance value of magnesium is 400 mg per day for men of 19-30 years old and 310 mg per day for women of 19-39 years old[48]. Calcium has been reported to be effective in the building of skeletal structures, muscle functioning, while magnesium being a macro-element is important in the ionic balance and enzyme co-factors[49]. Magnesium cooperates with calcium in the muscular contraction and blood coagulation[31]. The amount of iron in *G. sapida* fruit detected is higher in comparison to *Satkara* fruit (0.15 ± 0.02 mg/100 g) and *Taikor* fruit (0.08 ± 0.01 mg/100 g) reported by Islam *et al.*[48]. Similarly, the iron content of five cultivars of Chinese jujube reported by Li *et al.*[50] ranged from 4.68 to 7.90 mg/100 g. Gnansounou *et al.*[31] also reported 14.75 ± 0.25 mg of iron per 100 g in *Dialium guineense* fruit which is superior to that of *G. sapida* fruit of this study. The recommended daily intake of iron for adult male is 8 mg and for adult female is 18 mg[39]. Trowbridge *et al.*[51] stated that more than one billion people are affected by anaemia which is caused by the deficiency of iron. Iron deficiency also causes immune system dysfunction[48, 52]. Iron is an essential trace element and a vital component of proteins involved in oxygen transport and metabolism. About 15% of the body's iron stored for future supplies is mobilized when dietary iron consumption is not sufficient[53]. It is necessary for normal functioning of the central nervous system, haemoglobin formation and in the oxidation of carbohydrates, fats and proteins[46, 54]. The zinc content was found higher than the fruits

of *Citrus macroptera* (0.21 ± 0.01 mg/100 g) and *Garcinia pedunculata* (0.15 ± 0.01 mg/100g) reported by Islam *et al.*[48]. The recommended dietary allowance for zinc is 8 mg per day for adult women and 11 mg per day for adult men which appear to be sufficient to prevent deficiency in most individuals[39]. Excessive intake of zinc has been reported to be toxic[55]. Zinc is a very important element for human growth which also increases resistance to infection[48]. It is a cofactor for the antioxidant enzyme super oxide dismutase and is required for the functioning of over 300 different enzymes, and for a number of enzymatic reactions involved in carbohydrate and protein metabolism[53]. Copper is essential for the production of enzyme in the body and plays an important role in biological electron transport[56, 57]. Copper deficiency causes reduced energy production, abnormal glucose and cholesterol metabolism, and increased oxidative damage[58]. Copper complexes can act as anticancer, antiulcer, anticonvulsant, and antidiabetic agents[59]. Manganese is an essential component of metalloenzymes and plays essential role in a number of physiologic processes as a constituent or activator of some enzymes which are essential for the metabolism of carbohydrate, cholesterol and amino acid[58]. Cobalt is an important nutritional trace element having therapeutic value in pharmacological doses. Cobalt has also been established to improve the effects of insulin and its action[58, 60].

Phytochemical analysis of the fruit showed the presence of a number of medicinally active secondary metabolites (Table 3). Plant cells produce two types of metabolites. Primary metabolites *viz.* carbohydrates, lipids and proteins are involved directly in growth and metabolism. Most natural products are compounds derived from primary metabolites such as carbohydrates, fatty acids and amino acids and are generally known as secondary metabolites. Secondary metabolites *viz.* alkaloids, saponins, phenolics, flavonoids, tannins, essential oils, terpenes, steroids, lignin *etc.* are considered as the products of primary metabolites. These secondary metabolites are not involved in metabolic activities and are the main sources of pharmaceuticals, food additives, fragrances and pesticides[61, 62]. Fruits and vegetables constitute an important source of bioactive compounds which differ widely in terms of structure, biological properties, and mechanisms of actions. Various phytochemical constituents of plants are known to be responsible for antioxidant, antimicrobial, anti-larvicidal, and anti-inflammatory activities[49]. Alkaloids are the plant substances known for their pharmacological effects rather than for their toxicity[63]. Isolated pure alkaloids and the synthetic derivatives have been used as basic medicinal agents as they possess analgesic, anti-spasmodic and anti-bacterial properties[64, 65]. Alkaloids cause gastrointestinal trouble and neurological disorders when their content in plants is high. Alkaloids content more than 20 mg/100 g are not safe for human consumption[63]. Steroids have analgesic, antibacterial and anti-inflammatory properties[65, 66]. Saponins show anti-inflammatory and hemolytic activities. They have bitterness and cholesterol

binding properties[67-69]. Steroids and saponins are responsible for the activities of central nervous system[70]. Glycosides have anti-diarrhoeal activity[71] and can reduce blood pressure[67]. Fruits are good sources of phenolics, flavonoids, and anthocyanins which are responsible for antioxidant, anti-carcinogenic and health-promoting properties[72, 73]. Phenolic compounds are one of the main and most abundant groups of plant metabolites[65]. Natural antioxidants are generally derived from plants in the form of phenolic compounds such as phenolic acids, flavonoids, tocopherols *etc.*[74]. Flavonoids, a group of phenolic compounds, are free radical scavengers which prevent oxidative cell damage through their water soluble property and also possess strong anti-cancer activity[24]. Tannins are used for the treatment of diarrhoea and dysentery, and have received immense attention in many fields due to their physiological activities like antioxidant, antimicrobial and anti-inflammatory[24, 64]. Coumarins act as potential antioxidants with the ability of scavenging free radicals and chelating metal ions[24, 75].

In this study, DPPH and ABTS assays were used to evaluate the antioxidant activities in methanolic extract of *G. sapida* fruit and both the methods showed antioxidant activities (Table 4). The IC₅₀ value of DPPH assay was found to be higher than that of ABTS assay and ABTS assay showed more antioxidant capacities. Similarly, Khomdram *et al.*[7] reported the DPPH assay IC₅₀ value of some wild endemic fruits of the Manipur region of India that ranged from 181.21 ± 2.0 µg/mL to 2717.46 ± 363.6 µg/mL. The ABTS assay is known to be a rapid method for the investigation of antioxidant property and employed to screen samples in order to know high content of natural antioxidants in foods[26]. Antioxidants react with DPPH, a stable free radical having a characteristic absorption at 517 nm and convert 1, 1-diphenyl-2-picrylhydrazyl (DPPH, deep violet color) to 1, 1-diphenyl-2-picrylhydrazine (yellow color or bleached product) due to their hydrogen donating ability at a very rapid rate resulting in a decrease in absorbance at 517 nm. More rapidly the absorbance decreases, the higher is the antioxidant activity of the extract, and lower is the IC₅₀ value greater is the antioxidant activity[7, 25]. IC₅₀ value is a measure of inhibitory concentration where the active crude extract will exhibit 50% of antioxidant activity and exhibit 50% of inhibition[76]. The total phenolic content reported in this study was more than flavonoid content (Table 5). The concentration of total phenolic and flavonoid in plant extracts depends on the polarity of solvents used in the extract preparation[76, 77]. Fruits are good sources of phenolics, flavonoids, and anthocyanins. Phenolic compounds known as phenolic acids and flavonoids are major ingredients of fruits which play an important role in the nutritional and commercial properties of the fruits[78]. Several literatures have revealed that the phenolic compounds in the plants have antioxidant activities which may be due to their redox properties, hydrogen donating abilities, and singlet oxygen quenchers[25, 49, 79].

CONCLUSION

The study revealed that *G. sapida* fruit is rich in potassium, calcium and magnesium. The investigation also showed high calorific value, rich phenolic and flavonoid contents. Methanolic extract of the fruit showed *in vitro* antioxidant activities with DPPH and ABTS assays. The ABTS method showed higher antioxidant capacity with lower IC₅₀ value than DPPH method. This study and the presence of various phytochemical constituents in *G. sapida* fruit suggest that it can be a good source of minerals, natural antioxidants and value added products to pharmaceutical industries. Hence, further biological studies of this wild fruit are needed to explore the beneficial effect in human health.

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