Bioactive constituents and nutritional composition of Bridelia stipularis L. Blume fruits

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**ABSTRACT**

*Bridelia stipularis* (L.) Blume is a fruit-yielding climbing shrub native to Southern Asia and various parts of the plant have been used in traditional systems of medicines to treat a range of diseases. Proximate, mineral, phytochemical analysis of *Bridelia stipularis* fruit pericarp and seeds were carried out in the present study to assess nutritional and phytochemical status. The pericarp and seeds were rich in carbohydrate (38.78 and 33.46 g/100 g dry mass), protein (8.94 and 44.40 g/100 g dry mass), fiber (3.86 and 2.83 g/100 g dry mass) and minerals, in addition to these, seeds also contain oil (9.10 g/100 g dry mass). Pericarp and seeds possess higher concentrations of phenolics (9.84–125.59 mg GAE/g dry mass), flavonoids (7.17–44.67 mg QE/g dry mass), tannins (11.79–17.71 mg TAE g dry mass) and lesser concentrations of antinutritive factors, such as phytate (0.06–0.26 g/100 g dry mass) and oxalate (0.23–0.46 g/100 g dry mass). The physicochemical characteristics and fatty acid profile revealed that *B. stipularis* seed oil could be used for edible purposes. The seed oil is abundant with linolenic acid (36.7 g/100 g of oil), oleic acid (23.39 g/100 g oil) and hence, it could be used in soap and detergents.

**Introduction**

*Bridelia stipularis* (L.) Blume (Synonym: *Bridelia scandens* (Roxb.) Willd., Common name: Climbing bridelia, Family: Phyllanthaceae) is a woody, climbing shrub which is distributed in South Asian countries including Bangladesh, China, India, Indonesia, Malaysia, Myanmar, Nepal, Sri Lanka and Vietnam. It is a fruit-yielding plant; fruits are small (8 mm long, 5–8 mm in diameter), ripened fruits are black in color, edible and taste like currants. Various parts of this plant are used in the treatment of amoebic dysentery, constipation, diarrhea, and skin disease. The decoction prepared out of leaves and bark is used for curing cough, fever, jaundice. \cite{1} Bioactive terpenoids were isolated from the bark of this species which depicted antimicrobial activities. \cite{2}

Wild and underutilized fruits are rich source of nutrients especially dietary fibers, vitamins, and minerals; they are also abundant with bioactive compounds. \cite{3,4} Wild fruits are a source of dietary supplements and studies on their nutritional and phytochemical status and exploitation are very much useful for overcoming food security problems of tropics and subtropics. \cite{5} As there is a paucity of data on the nutritional composition of fruits of *Bridelia stipularis*, the present study was aimed to examine

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the chemical composition and nutritional value of fruits. Additionally, we analyzed the amount of seed oil present in the seed of Bridelia stipularis, and assessed the physicochemical composition of seed oil.

**Materials and methods**

**Collection of fruit samples**

Ripe fruits of Bridelia stipularis (L.) Blume (Synonym: Bridelia scandens (Roxb.) Willd., Common name: Climbing bridelia, Family: Phyllanthaceae) were randomly collected in three different lots from plants grown in the botanical garden, Karnatak University, Dharwad, India (Figures 1A and 1B). The fruits (1C and 1D) were washed thoroughly with sterile distilled water the pericarp and seeds (Figure 1E) were separated from the fruits and samples were dried at 40°C in a hot air oven till the constant weight was reached. All dried pericarp and seeds were powdered using a blender and used for nutritional compositions and phytochemical analysis.

![Figure 1. Bridelia stipularis: A. Morphology of plant; B. Flowering twig; C. Twig bearing unripe fruits; D. Ripened fruits; E. Pericarp.](image-url)
**Chemicals and reagents**

Analytical-grade chemicals were used throughout the experiment. Folin-Coicalteu reagent were procured from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Gallic acids, quercetin, tannic acid and Folin-Danis reagent were purchased from Sigma Aldrich Chemical Co. (USA).

**Proximate analysis and nutrient analysis**

Dried and powdered pericarp and seed samples were analyzed for protein, fat and moisture content using official methods of analysis of Association of Analytical Chemists. [6] The carbohydrate content in the samples was estimated by the anthrone method. The fiber content in the samples was determined by the method described by AOAC. [6] Ash content in the samples was determined by igniting the oven-dried samples in the muffle furnace at 750°C. The ash content is expressed as a percentage of the mass of the oven-dried samples. [6]

**Analysis of minerals**

Various minerals such as calcium, copper, iron, magnesium, manganese, nitrogen, potassium, phosphorous, Sulfur, and zinc contents of different samples were estimated by Atomic absorption spectrophotometer and flame photometry. [6]

**Estimation of phytochemicals**

The quantitative estimation of phenolics was determined spectrophotometrically using Folin and Ciocalteu method. [7] 100 µL of acetone, ethanol and water extract was mixed with 2.5 mL of distilled water, then 0.1 mL of Folin Ciocalteu reagent and allowed to stand for 6 min and then 0.5 mL of 20% sodium carbonate reagent was added. After 30 min of incubation at room temperature, absorbance was measured at 760 nm using a spectrophotometer. The phenolic content in the sample was expressed as gallic acid equivalents (mg GAE g⁻¹ of dry mass). Similarly, seed ethanol extract was prepared and phenolics were estimated.

The flavonoid content was determined spectrophotometrically by the method of Prior et al. [8] Acetone, ethanol and water extract (0.25 mL) was mixed with 1.25 mL of distilled water, followed by the addition of 75 µL of 5% sodium nitrate solution. After 6 min of incubation, 0.15 mL of 10% aluminum chloride solution was added and then absorbance was measured using spectrophotometer at 510 nm. The amount of flavonoid was expressed as quercetin equivalent (µg QE g⁻¹ of dry mass). The seed ethanol extract was used for the estimation of flavonoids.

The amount of oxalate was determined using the method of Oke. [9] One gram of material was mixed with 6 M hydrochloric acid were heated at 90°C for 4 hours. The obtained supernatant was titrated with concentrated ammonia solution in presence of methyl orange until the color changed to faint yellow. The warm solution was precipitated with 5% calcium chloride solution. The precipitate was dissolved with 25% sulfuric acid and titrated against 0.05 M potassium permanganate till the appearance of persistent pale pink color.

The amount of tannin was estimated by adding 0.5 mL of acetone, ethanol and water extract were mixed with Folin-Danis reagent and sodium carbonate solution. Absorbance was measured spectrophotometrically at 750 nm by comparing it with the tannic acid standard. [10] For estimation of tannins from seed cake 10 g of seed cake was boiled with 100 mL of distilled water for 1 h. The supernatant was subsequently used for the estimation of tannins by using the above mentioned method.

The quantification of phytic acid was carried out by the spectrophotometric method. [11] Aliquots of 1 mL of calcium chloride solution (10 mg L⁻¹), 0.2 mL of glyoxalbis (2-hydroxyaniline) solution (1 mg L⁻¹), 1.0 mL of borate buffer solution (pH 12.5) containing cetyl trimethylammonium bromide (CTAB, 1 mmol L⁻¹), 10 mL of sample or phytic acid and 1.8 mL of ethanol/methanol mixture
were taken in falcon tube and after 20 min absorbance measured by using a spectrophotometer at 500 nm.

**Physico-chemical characterization of seed oil**

The seed oil was extracted by a heat-reflux method using 100 mL of petroleum ether (b. p. 40–60°C). The percent of oil presented was determined gravimetrically after removal of the solvent. The specific gravity and refractive index were determined by using the pycnometer method and the Abbe refractometer using the analytical method. Iodine, ester, peroxide and saponification values were also determined by standard AOAC methods.

**Determination of fatty acid profiles of seed oil**

The fatty acid composition of seed oil was determined by gas chromatography using Fatty acid methyl esters (FAMEs). FAMEs were prepared using catalyst boron trichloride according to the AOAC method. The gas chromatograph was equipped with BP x 70 (50 m x 0.32 mm x 0.25 µm films). The detector temperature was programmed for 260°C with flow rate of 0.3 ml/min. The injector temperature was set at 240°C, Nitrogen was used as the carrier gas. Identification of the peaks was performed by comparing retention times with those of genuine standards analyzed under the same conditions.

**Statistical analysis**

All experiments were conducted in triplicate using three different lots of fruits. Data are presented with standard error.

**Results**

**Proximate composition**

In the present study, we evaluated the chemical composition and nutritional status of *Bridelia stipularis* fruits and the proximate composition of fruit pericarp and seeds are presented in Table 1. The carbohydrate content was 38.78 g/100 g of dry mass and 33.46 g/100 g of dry mass in pericarp and seeds. Fruit protein content in the pericarp and seeds was found to be 8.94 g/100 g of dry mass and 44.40 g/100 g of dry mass. Whereas fiber and ash content was 3.86 g/100 g of dry mass, 1.92 g/100 g of dry mass in fruit pericarp and 2.83 g/100 g of dry mass, 1.20 g/100 g of dry mass in seeds respectively. The crude fat content was 0.78 g/100 g of dry mass in fruit pericarp, however, seeds were possessing 9.10 g/100 g of dry mass crude fat.

| Fruit part | Moisture (g/100 g fresh mass) | Crude fat (g/100 g dry mass) | Protein (g/100 g dry mass) | Carbohydrate (g/100 g dry mass) | Total ash (g/100 g dry mass) | Crude fiber (g/100 g dry mass) |
|------------|-------------------------------|-------------------------------|----------------------------|---------------------------------|-----------------------------|-----------------------------|
| Pericarp   | 45.70 ± 0.73                  | 0.08 ± 0.03                   | 0.94 ± 0.01                | 38.78 ± 0.32                    | 0.92 ± 0.01                 | 0.38 ± 0.26                  |
| Seed       | 0.84 ± 0.67                   | 0.10 ± 0.68                   | 4.40 ± 3.96                | 33.46 ± 2.06                    | 0.20 ± 0.24                 | 0.28 ± 0.33                  |

*Data are mean ± standard error (n = 3)
Table 2. Mineral analysis of Bridelia stipularis fruit.

| Fruit part | Minerals |
|------------|----------------|
|            | N  | P  | K  | S  | Mg | Ca | Zn | Fe | Mn | Cu |
| Pericarp   | 7130.66 | 8918.00 | 1433.33 | 501.33 | 14566.67 | 27989.67 | 35.38 | 116.67 | 10.00 | 5.83 |
| Seeds      | 28200.00 | 6050.00 | 3448.66 | 1238.00 | 16791.00 | 34116.67 | 80.63 | 182.16 | 45.88 | 8.21 |

All values in the table represent the content in mg/kg of dry mass.

**Mineral content**

The mineral content of Bridelia stipularis fruit pericarp and seeds is shown in Table 2. The amount of nitrogen (N), phosphorus (P), potassium (K), sulfur (S) and calcium (Ca) were 7130.66, 8918.00, 1433.33, 501.33 and 27989.67 mg/kg dry mass respectively in pericarp, whereas, zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) levels were 35.38, 116.67, 10.00, 5.83 mg/kg dry mass respectively. The concentration of N, P, K, S, Ca, Zn, Fe, Mn and Cu were 28200.00, 6050.00, 3448.66, 1238.00, 16791, 34116.67, 80.63, 182.16, 45.88 and 8.21 mg/kg dry mass in seeds of Bridelia stipularis.

**Phytochemical constituents**

Phytochemicals such as total phenolics and flavonoids are extracted Bridelia stipularis fruit pericarp and seeds are presented in Table 3. The total phenolic content was 125.59 GAE/g dry mass and 9.84 mg GAE/g dry mass in fruit pericarp acetone and seed ethanol extract respectively. The total flavonoid content in the fruit pericarp acetone extract and seed ethanol extract was 44.67 mg QE/g dry mass and 7.17 mg QE/g dry mass respectively.

The total tannins content of Bridelia stipularis was 11.79 mg TAE/g dry mass and 17.71 mg TAE/g dry mass in fruit pericarp acetone extract and seed water extracts respectively. The oxalate amount was 0.17 g/100 g dry mass and 0.06 g/100 g dry mass respectively in pericarp and seeds. The phytate values were 0.46 g/100 g dry mass and 0.41 g/100 g dry mass in pericarp and seeds of Bridelia stipularis (Table 3).

**Physico-chemical properties of seed oil**

The amount of oil in the seeds of Bridelia stipularis was 9.10 g/100 g and the oil was liquid at room temperature and yellow. The specific gravity, refractive index of oils were 0.91 and 1.47 respectively. The acid value, saponification value, free fatty acid value, iodine value, peroxide value and ester values were 6.03 mg of KOH/g, 343.11 mg of KOH/g, 3.02% (as oleic acid), 115.02 mg/100 g, 4.13 mEq O2/kg and 336.97 mg/kg respectively (Table 4).

**Fatty acid profile of seed oil**

The gas chromatogram of the fatty acid profile of Bridelia stipularis is presented in Figure 2. Major fatty acids present in the seed oil Bridelia stipularis were linolenic acid (36.73 g/100 oil), oleic acid (23.39 g/100 oil), linoleic acid (15.00 g/100 oil), stearic acid (12.06 g/100 oil), and pamitic acid (11.55 g/...
Other fatty acids such as myristic acid, palmitolic acid, arachidic acid, and behenic acid were present in minor concentrations (Table 5). The amount of total saturated and unsaturated fatty acids were 24.84 g/100 oil and 75.15 g/100 oil respectively.

**Discussion**

**Proximate analysis**

Wild fruits are rich source of nutrients including carbohydrates, proteins, and lipids. They are also rich in dietary fiber, vitamins and minerals and a large number of phytochemicals with medicinal value. Therefore, it is essential to evaluate the nutritional and phytochemical constituents of wild fruits so that they could be used as an alternative source of nutrients. The results of proximate analysis of fruits of *Bridelia stipularis* shows that the pericarp is rich in carbohydrates (38.78 g/100 g dry mass), protein (8.94 g/100 g dry mass) and crude fiber (3.86 g/100 g dry mass), whereas, the seed is abundant with protein (44.40 g/100 g dry mass), carbohydrates (33.46 g/100 g dry mass), fat (9.10 g/100 g dry mass) and 100 oil. Other fatty acids such as myristic acid, palmitolic acid, arachidic acid, and behenic acid were present in minor concentrations (Table 5). The amount of total saturated and unsaturated fatty acids were 24.84 g/100 oil and 75.15 g/100 oil respectively.

**Table 4. Physico-chemical properties *Bridelia stipularis* seed oil.**

| Properties                      | Value*       |
|---------------------------------|--------------|
| Oil yield (g/100 g)             | 9.10 ± 0.68  |
| Specific gravity                | 0.91 ± 0.01  |
| Refractive index                | 1.47 ± 0.00  |
| Acid value (mg of KOH/g)        | 6.03 ± 0.96  |
| Saponification value (mg of KOH/g) | 343.11 ± 2.89 |
| Free fatty acid (%) as oleic acid | 3.02 ± 0.26  |
| Iodine value (mg/100 g)         | 115.02 ± 1.46|
| Peroxide value (mEq O2/kg)      | 4.13 ± 0.003 |
| Ester value (mg/kg)             | 336.97 ± 2.47|
| Physical state at RT            | Liquid       |
| Color                           | Yellow       |

*Data are mean ± standard error (n = 3)

Figure 2. Gas chromatogram showing fatty acid profile of *Bridelia stipularis* seed oil.
mass), fiber (2.83 g/100 g dry mass) and ash (1.20 g/100 g dry mass), (Table 1). The proximate composition of fruit pericarp was similar to that reported for other wild fruits belongs to phyllanthaceae such as Uapaca kirkiana (wild loquat) in which protein, crude fat, carbohydrates, fiber and ash levels were 0.1–0.5 g/100 g dry mass, 0.3–0.4 g/100 g dry mass, 28.7 g/100 g dry mass, 1.8–2.3 g/100 g dry mass and 0.6–1.1 g/100 g dry mass. The protein and carbohydrate content of the seeds was higher than that of Cordia dichotoma and Adansonia digitata seeds. The crude fat content of Bridelia stipularis seeds was similar to that of Adansonia digitata (12.2 g/100 g dry mass, but lower than that of Cordia dichotoma seeds.

### Mineral composition

The mineral composition data reveals that both pericarp and seeds are rich in varied mineral elements such as nitrogen (N), phosphorous (P), potassium (K), sulfur (S) and calcium (Ca) (Table 2). The mineral content of Bridelia stipularis fruit pericarp and seeds is shown in Table 2. The fruit pericarp and seeds are excellent source of calcium, magnesium, nitrogen, phosphorous and potassium. Mineral values of Bridelia stipularis were higher than that of Bridelia machrantha and Upaca kirkiana. Calcium is essential for bone development and regulation of heart beat and blood clotting. Magnesium, sodium and potassium inorganic elements which involve in varied biological processes in human and livestock. Calcium and magnesium of Bridelia stipularis fruits are over above the levels of Food and Drug Administration (FDA, USA) recommended dietary allowance and tolerable upper levels, and thereby Bridelia stipularis fruits could serve as a good source of calcium and magnesium for human consumption. The results of both proximate and mineral composition suggest that the fruits of Bridelia stipularis could be used as an alternative source of nutrients and seeds could be used as roughage.

### Phytochemical constituents

The phytochemical analysis of Bridelia stipularis fruits is presented the Table 3 and results show that pericarp is rich in phenolics (125.59 mg GAE/g dry mass), flavonoids (44.67 mg QE/g dry mass) and tannins (11.79 mg TAE/g dry mass). Similarly, Akinpelu and Shelembe reported the occurrence of flavonoids in the fruits of Bridelia ferruginea and Bridelia micrantha respectively. Polyphenols especially flavonoids are responsible for varied biological activities and the antimicrobial, anthelmintic, antioxidant activities are reported from Bridelia micrantha. Since, the content of phenolics,
flavonoids and tannins are rich in *Bridelia stipularis* fruits, these fruits may also responsible for antioxidant and antimicrobial activities, and however, research in this area is very much essential. The oxalate and phytate components of the fruits are considered as anti-nutritional phytochemicals which reduces the availability of essential nutrients of fruits and vegetables.\(^{[20]}\) For example oxalate content of the fruits creates the availability of free calcium ions for utilization.\(^{[21]}\) The oxalate amount was 0.17 g/100 dry mass and 0.06 g/100 dry mass respectively in pericarp and seeds. The phytate values were 0.46 g/100 dry mass and 0.41 g/100 dry mass in pericarp and seeds of *B. stipularis* (Table 3). The oxalate and phytate values were below the established toxic levels.\(^{[22]}\)

**Physicochemical characteristics of seed oil**

The seeds of *Bridelia stipularis* are rich in oil content (9.10 g/100 g; Table 4). The refractive index oil was 1.47, which is comparable to *Garcinia xanthocymus* (1.47)\(^{[23]}\) and higher than *Garcinia livingstonei* (0.90)\(^{[24]}\) and *Lavandula bipinnata* seed oil (0.97)\(^{[25]}\). Peroxide and free fatty acid values are the two parameters that determine the stability of the seed oil during storage. The peroxide and free fatty acid values of *Bridelia stipularis* seed oil were 4.13 mEq O2/kg and 3.02% of respectively (Table 4) and these values are comparable to the seed values of *Garcina livingstonei* seed oil.\(^{[23]}\) The peroxide and free fatty acid values suggest that of *Bridelia stipularis* seed oil could be stored for a longer duration without deterioration. The iodine value of the seed oil depicts the amount of unsaturation of the oil and the iodine value of *Bridelia stipularis* seed oil was 115 mg/100 g and this is due to higher levels of unsaturated fatty acids such as lineolenic and oleic acids (Table 5).

**Fatty acid profile of seed oil**

Fatty acid profile of *Bridelia stipularis* seed oil reveals that linolenic acid (36 g/100 g seed oil), oleic acid (23 g/100 g seed oil), linoleic acid (15 g/100 g seed oil), stearic acid (12 g/100 g seed oil) and palmitic acid are the major fatty acids (Table 5 and Figure 2). Linolenic acid belongs to the group of omega-3 fatty acids, which are essential fatty acids for human and animal nutrition. The other sources of linolenic acid are chia (64 g/100 g seed oil), flax (55 g/100 g seed oil) and hemp (11 g/100 g seed oil). Varied studies have shown that linolenic acid may be useful in the management of rheumatoid arthritis, premenstrual breast pain, and diabetic neuropathy\(^{[26]}\) and hence the *Bridelia stipularis* seed oil could be used as an alternative source of linolenic acid. The Oleic acid content of *Bridelia stipularis* seed oil is on par with grape-seed oil (15–20 g/100 g seed oil)\(^{[27]}\). Oleic acid as its sodium salts is a major component of soap as an emulsifying agent and thus *B. stipularis* seed oil could be used as an emulsifying agent. The levels of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) of *Bridelia stipularis* seed oil was 75:25 g/100 g seed oil and it is very close that of *Garcinia livingstonei* seed oil.\(^{[24]}\)

**Conclusion**

The present study on the nutritional composition of *Bridelia stipularis* fruits revealed that fruits are a rich source of carbohydrates, protein and seed oil. The pericarp and seeds also contain all the essential mineral elements and a reduced amount of antinutrients. Hence, the fruits of *Bridelia stipularis* are an alternative source of the nutrients. The physicochemical properties of the seed oil suggested the good stability and can be stored for a longer duration. The higher proportion of linolenic acid and oleic acid in seed oil and phenolics in pericarp signify the various health benefits.

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