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A Study on Proximate Composition Assessment of Fruits of *Cipadessa baccifera*

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A B S T R A C T

The aim of this study was to evaluate and estimate the proximate composition of fruits of *Cipadessa baccifera*. The plant samples for investigation were collected in and around Bengaluru, and identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971. The dried fruit samples were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses. The fruit sample was analyzed for proximate composition *viz.* moisture content, total ash content, total fat content, total protein, crude fibre, total carbohydrates, minerals, and vitamins using standardized assay methods. Results revealed that the fruits *C. baccifera* were found to be high in moisture, rich in crude fibre & total carbohydrate content. Whereas, protein and fat content in the fruit were found to be low. The ash content of the fruit was estimated as2.22%.The major mineral elements such as calcium, potassium and phosphorous were found in higher concentration in the fruits of *C. baccifera*. Magnesium was present in considerable quantity (36.3 mg/100g). Whereas, the amount of sodium, zinc and iron was found to be lesser. The fruits of *C. baccifera* contains only traces of vitamin B_9. In conclusion, the proximate analysis of the wild fruits of *C.baccifera* revealed high moisture, crude fibre and total carbohydrate content. Whereas protein and fat content was comparatively low. The fruits showed significant amount of the major mineral elements such as calcium, potassium and phosphorous, while vitamin B_9 was present in minimal concentration. The present investigation clearly indicates that the wild fruits of *Cipadessa baccifera* which have been reported to be edible are nutritive and could be used as dietary supplements.

Introduction

In the last few decades there is a resurgence of interest in natural drugs and herbal products used in traditional medicinal systems due to the widespread belief that it is healthier, more effective and less toxic than synthetic medicines. According to World Health Organization's estimation [1], in spite of the great advances made in

modern medicine, nearly 80% of the world's population is still dependent on traditional medicines to meet their health care needs. The WHO's Commission on intellectual property and innovation in public health has recognized the promising role of traditional medicines in providing affordable health solutions [2]. Ethno-directed research is therefore helpful in overcoming limitations of modern therapeutics. Furthermore, there is a growing need to identify alternative bio-nutritional sources in order to provide food security. Many of the lesser-known wild variety of fruits though not tasty may be edible and rich in nutrients hence could play a significant role in meeting the dietary requirements of tribal and ethnic communities. Nutritional anthropologist Ann Fleuret has stressed on the role of edible wild plants and fruits in providing nutrients to the local diet of indigenous societies.

Sundriyal and Sundriyal, analyzed the nutritive value of 27 wild edible plants which included 22 wild edible fruits that are an important part of the traditional diets in Sikkim, Himalayas, and indicated that the nutritive value of wild fruits was comparable with the commercial fruits. Cultivation of wild varieties could be adopted in the traditional agro-forestry systems to help benefit poor farmers [3]. The nutritional composition of edible wild fruits such *Spondias mombin, Dialium guineense* and *Mordii whytii* of South-west and Middle belt of Nigeria were analyzed using standard methods of AOAC. The proximate analysis revealed the moisture content to be 82.3, 71.5 and 4.0 g/100 g for *S. mombin, M. whytii* and *D. guineense* respectively. The crude protein content was 2.6-8.3 g, crude lipid 1.6- 2.0 g, crude fibre 0.6-11.8 g, ash 1.0-6.8 g/100 g and total carbohydrate was 7.9, 8.9 and 84.0 g/100 g for S. mombin, *M. whytii* and *D. guineense* respectively. The fruit pulps were found to be rich in sodium (360.0-400 mg/100g), magnesium (300-465 mg/100g) and potassium (260-410 mg/100g). *M. whyti* was rich in calcium (300 mg/100g) and phosphorus (170 mg/100g). The iron and zinc concentration ranged between 2.0-4.1 mg and 0.2-2.2 mg, respectively; while retinol equivalents ranged between 84.9-361.5 g/100 g fresh sample. Since the anti-nutritional factors of the fruit pulps were low they could serve as good source of micronutrients [4].

Hussain *et al.,* investigated the proximate composition and levels of metals in four commonly consumed species, *Sonchus eruca, Melia azadirachta, Withania coagulans* and *Fagonia indica*, and delineated that Among the four species tested, *M. azadirachta* was found to be rich in fibre content, energy values and also showed high concentrations of the metals like Cu, Mn, Cr and Fe [5]. Nazarudeen screened 218 fruit plants used by the tribal communities of Kerala, of which ten wild fruits were subjected to proximate analysis. The results revealed that although these fruits were not tasty their nutritional composition i.e., moisture, protein, fats, reducing, non-reducing, total sugars, fibre, total mineral, vitamin C, iron, sodium, potassium and energy value was higher when compared to the ten common cultivars [6].

Misra and Malaya, have reported that the ripened fruits of *Cipadessa baccifera*, a shrub or small tree found growing in scrubs and open mixed forests are edible and can be eaten raw [7].However, to the best of our literature knowledge reports on phytochemical composition and nutritive value of *C. baccifera* was scare. Hence, in the present study we aimed to evaluate and estimate the proximate composition of fruits of *C. baccifera*.

Materials and Methods

Collection of Plant Material

The plant samples for investigation were collected in and around Bengaluru. The plant under study was identified as *C.baccifera* (Roth) Miq. as per Flora of Hassan (1976) and Flora of Karnataka (1996) by Saldana [8,9].Further, the identification was authenticated by National Ayurveda and Dietetics Research Institute, Bangalore; vide voucher specimen number, RRCBI-8971.

Sample Processing

The samples such as fruits of *C. baccifera* were collected in clean and sterile polythene bags for various analyses. The collected samples were washed thoroughly in running tap water to remove dust and soil particles and were blotted dry. Healthy and infection free plant parts *viz.*, leaves, bark, fruits and roots were separated and shade dried for 20 days. The dried plant parts were pulverized in an electric blender and the powdered material was stored in air tight containers for further analyses.

Proximate Analysis of Fruits

Proximate analysis is the quantitative evaluation of food to determine the percentage of the six fractions it divides food materials into viz., moisture, total ash, fat or ether extractives, protein, crude fibre and carbohydrates.

Moisture content

A clean petri dish was taken, dried in an oven, then cooled in a desiccator and its weight was recorded as (W1). Around 5 g of fruit sample was weighed into a dried dish and its total weight was recorded (W2). The petri dish with sample was placed in the hot air oven at 70°C for about 8 hours with lid open. The dish with dried sample was allowed to cool in a desiccator and its weight was noted (W3). With the lid open, the dish was allowed to stand at room temperature for 1 hour to constant the weight. Difference in the dried weight after 1 hour should not exceed 10 mg [10]. The moisture content in the fruit sample was calculated using following formula:

$$\text{Percentage of moisture content} = \frac{[(W2-W1)-(W3-W1)]}{(w2-w1)} \text{ X } 100$$

Where, W1 = weight of clean and dry empty petri dish

W2 = weight of petri dish + wet sample

W3 = weight of petri dish + dry sample

Total ash content

Ash is the inorganic residue remaining after water and organic matter have been removed by heating and refers to the total mineral content. The ash content of a known weight of fruits was determined by dry ashing method involving the incineration of the sample in Muffle furnace at a high temperature of 550°C until all carbon has been removed and a light gray or white powder remains. Silica crucibles were cleaned and dried in a Muffle furnace and cooled in a desiccator. The weight of empty silica crucible was recorded.

The weight of about 5 g of fruit sample taken in dried silica crucible was recorded. The sample in crucible was pre-ignited to remove carbonaceous matter and then kept in furnace at 550°C for about 4 hours. The ashed sample was removed and cooled in

desiccator. The weight of the dish was noted as W3. Again the ashed sample was allowed to stand for another 1 hour to achieve constant weight. Difference in dried weight should not exceed 10 mg [10]. The ash content in the fruit sample was calculated using following formula:

$$\text{Percentage of Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ X } 100$$

Total fat content

Weight of a dried empty 250 mL beaker was taken. The weight of about 5 g of the fruit sample taken in to the beaker was recorded. Then 50 mL of distilled water and 5 mL of concentrated HCl were added into the beaker and the solution was warmed for about 10 minutes in water bath at 60°C.

The contents were then transferred to a separating funnel by filtering with Whattman No.1 filter paper. About 100 mL of petroleum ether was added to separating funnel, swirled slowly and the pressure was released. A saturated solution of NaCl was then added to remove the emulsion and shaken vigorously. The layers were allowed to separate and the ether layer was collected.

The separation of aqueous solution was continued similarly with 100 mL petroleum ether. Petroleum ether was collected; 100 mL of water was added to it for washing, until it is neutral to acid.

The sample was passed through sodium sulphate and the petroleum ether fraction was collected in a pre-dried 250 mL beaker. The petroleum ether was evaporated to near dryness and kept in an oven for about 1 hour. The beaker was cooled in desiccator and the weight of fat was calculated [10].

The total fat content in the fruit sample was determined by following formula:

$$\text{Percentage of Fat} = \frac{Weight of fat}{weight\ of\ sample} \text{ X } 100$$

Protein content

The total protein content of *C. baccifera* fruits was calculated from the nitrogen content in the fruit, by modified Kjeldahl method. About 1 g of fruit sample was weighed into Kjeldahl flask and 1 g of $CuSO_4$, 10 g of Na_2SO_4 and 25 mL of H_2SO_4 were added to it. The sample was heated for digestion on a heating mantle till it turns into green or blue colour. It's then cooled and the contents were transferred to a round bottomed distillation flask and set up. On the receiver end, a beaker containing 25 mL of 0.1N H_2SO_4 with 2 drops of methyl red indicator was placed. The acid in the beaker was neutralized by adding 40% NaOH. Distillation process was continued till about 50 mL of distillate was collected. The distillate was titrated with 0.1N NaOH till the appearance of a persistent yellow colour. The titre volume in mL was recorded. About 25 mL of 0.1N H_2SO_4 was used as a blank [10]. The protein content in the fruit sample was calculated using the following formula:

$$\text{Percentage of Protein} = \frac{(Blank - sample) X\ Normality of\ NaOH\ X\ 14\ X\ 6.25\ X\ 100}{Weight of sample} X\ 1000$$

Crude fibre

About 2.5 g of fruit sample, taken in a dried round bottom flask was weighed. Then 200 mL of 2.5% w/v H_2SO_4 was added to it and the mixture was boiled for 30 minutes with a

condenser attached. The residue was cooled, filtered through linen cloth and washed with water, then transferred into the same round bottomed flask. To the residue 2.5% w/w NaOH was added and boiled for 30 minutes with condenser attached. Again the residue was allowed to cool, filtered, given a water wash and transferred to a pre-weighed silica crucible. The residue was then dried at 110°C for 12 hours to obtain a constant mass and the weight was noted. The crucible with dried residue was placed in a muffle furnace set at 550°C temperature for 3 hours, till constant mass was achieved and the weight was recorded. The crude fibre present in the fruits was calculated as follows:

$$\text{Percentage of Crude fibre} = \frac{W2-W3}{W1} \text{X } 100$$

Where, W1 = weight of crucible with sample

W2 = weight of crucible with residue

W3 = weight of crucible with ash.

Total carbohydrates

The carbohydrate content was determined by difference, using the standard protocol of AOAC [11]. The nitrogen-free extract (NFE) is mainly composed of digestible carbohydrates. The total carbohydrate is calculated by the addition of crude fibre content to NFE. When the sum of the percentages of moisture, ash, crude protein and fat is subtracted from 100, the difference is designated as nitrogen free extract [10]. The Nitrogen Free Extract (NFE) and Total Carbohydrate are determined as follows:

Percentage of N.F.E = 100–(% moisture + % ash + % crude fibre + % fat + % protein)

Percentage of Total Carbohydrate = % Nitrogen Free Extract (N.F.E) + % Crude fibre

Determination of metals and minerals

Sample preparation

The sample preparation was done according to the guidelines of US-EPA 3050b. About 0.3 g of *C. baccifera* fruits was weighed in a 250 mL Erlenmeyer flask and 5 mL of 1:1 HNO_3 was added to it. The solution was heated on a hot plate to 95ºC, refluxed for 15 minutes without boiling. After cooling, about 2.5 mL of concentrated HNO_3 was added; the sample was then refluxed for 30 minutes at 95ºC without boiling and this step was repeated.

Thereafter, the sample was evaporated to 5 mL without boiling. After cooling, about 2 mL of double distilled water was added to the sample. This was followed by slow addition of 3 mL of 30% H_2O_2. The solution was then heated until the effervescence subsided. About 6 mL of 30% H_2O_2 in 1 mL aliquots was added and then solution was refluxed.

After allowing it to cool, 2.5 mL of concentrated HCl was added and the sample was refluxed for 15 minutes without boiling. After cooling to room temperature, the sample was filtered using Whattman No.1 filter paper and then diluted to 50 mL with double distilled water.

All samples were analyzed in triplicates by ICP-OES; Inductively Coupled Plasma–Optical Emission Spectrometer [11]. The measurements were performed using the Perkin Elmer Optima ICP-OES instrument, ICP version; 4.0 software for simultaneous measurement of all analytes wavelengths of interest.

Analysis of Vitamins

Estimation of water-soluble vitamins

Healthy, infection free fruits of *C.baccifera* were finely ground and homogenized. To 2.5 g of this homogeneous sample, 3 mL of 1N NaOH was added and dissolved by adding 25 mL of diluting solution. The sample was sonicated for 10 minutes and the volume was made up to 50 mL with the diluting solution. The extract was filtered using Whattman filter paper No. 42. The filtrate was collected and used for HPLC analysis. About 20 µL of the filtrate was injected into HPLC. The peak obtained at the respective RT was compared with that of the standard vitamin and the concentration of vitamin was calculated [11].

Estimation of fat-soluble vitamins

The fruit samples were finely ground and made homogeneous. Then 25 mg of standard and 1.0 g of homogeneous sample was taken in a 250 mL round bottom flask; about 50 mg of hydroquinone, 30 mL of ethanol and 4 mL of 50% KOH were added. The mixture was heated on a water bath for 1 hour at 60°C. The solution was then cooled and transferred into a separating funnel with water and extract with 3x50 mL of peroxide free petroleum ether. The ether fraction was collected and washed with water until it was free from acid. The ether fraction was passed through anhydrous Na_2SO_4 solution and evaporated on a water bath, set at 60°C temperature. The residue was dissolved in 10 mL of isopropyl alcohol. The extract was then filtered through Whattman filter paper No. 42. The filtrate collected was subjected to HPLC analysis [11].

The amount of vitamins present in fruits was determined by using following calculation:

$$\text{Vitamin concentration in ppm} = \frac{\text{sample area} \times \text{standard weight} \times \text{dilution} \times 100}{\text{standard} \times \text{dilution} \times \text{weight of sample}}$$

Results and Discussion

Proximate Analysis

Evaluation of the proximate composition, viz., total ash, crude fibre, moisture along with the nutritive value of the fruits of *C.baccifera* was carried out and the results are shown in Table 1. The fruits were found to be high in moisture, rich in crude fibre and total carbohydrate content. Whereas, the protein and fat content in the fruit were found to be low. The ash content which is a measure of the total mineral content of the fruit was estimated to be 1.22%.

Mineral Composition

The major mineral elements such as calcium, potassium and phosphorous were found in higher concentration in the fruits of *C.baccifera* (Table 2). Magnesium was present in considerable quantity (35.3 mg/100g), whereas the amount of sodium, zinc and iron was found to be lesser.

Vitamin Composition

Vitamins present in fruits of *C. baccifera* were determined by HPLC technique (Table 3). Only traces of vitamin B_9 were found to be present while rest of the vitamins was not detected.

Proximate analysis is the quantitative evaluation carried out to determine the percentage of moisture, total ash, crude fibre, fat, protein and carbohydrates in order to assess the nutritional content of fruits. In the present study the proximate composition

of fruits of *C. baccifera* revealed high moisture content of 64.10% when compared to earlier findings in some of the wild fruits of Meliaceae such as, *Dysoxylum arborescens* (25%), *Aglaia diffusa* (57.73%) [12], and *Azadirachta indica* (8.43%) [13]. Moisture content in fruits is a measure of the water present, which plays a vital role in the physiological and biochemical activities of the fruit.

High moisture content in fruits characterizes its freshness and indicates low solid matter in the pulp as reported by Tressler *et al.,* [14]. However, when the moisture content exceeds the safe threshold limit of 15% they cannot be preserved for long due to susceptibility of fruits to the development of molds [15,16].

There is a wide variation in the amount of moisture present in wild edible fruits such as, yellow mombin, *Spondias mombin* (83.66%) [17], *Careya arborea* (85.22%), *Melastoma malabathricum* (56.6%) [18], *Nitraria retusa* (76.25%) [19], *Palaquium ellipticum* (92.43%) [20], *Mordii whytii* (71.5%), *Dialium guineense* (4.0%) [4], and common fruits like apple (84.6%), mango (81%) and papaya (90.8%) [21].

The ash content in fruits of *C. baccifera* was found to be low (2.22%), similar findings have been reported in some of the common fruits such as, apple, mango and papaya [21].

While studies on some wild fruits of Meliaceae have reported high ash content of 5.51% and 2.73% in fruits of *Aglaia diffusa* [12], and *Azadirachta indica* respectively [13].

In the present investigation, the percentage of crude fibre in fruits of *C. baccifera* was 27.8%, which was higher than that reported in some common fruits such as, apple, mango, papaya (3.2, 2.0, and 2.6 % respectively) by Seal *et al.,* [21], and wild edible fruits such as, *Spondias mombin, M. whytii* and *D. guineense* (0.6-11.8%) by Tiburski *et al.,* and Adepoju [4, 17]. The high crude fibre present in fruits of *C. baccifera* signifies it to be a good source of dietary fibre.

Crude fibre is reported to have health promoting properties such as; to act better on the digestive system, lower the serum cholesterol level, constipation, reduce risk of coronary heart diseases, diabetes, colon, breast cancer [22, 23], and help in detoxification of poisonous metals [24].

The results of proximate analysis showed that the concentration of proteins (1.43%) and crude fat (1.14%) were found to be minimal in fruits of *C. baccifera* when compared to higher amounts reported in some uncommon wild edible fruits such as, *Spondias mombin* (protein-1.06%, fat-0.62%) [17], *Arbutus pavarii* (protein-2.23%, fat-1.33%), *Nitraria retusa* (protein-1.5%, fat-0.75%) and *Ficus palmata* (protein-2.17%, fat-1.12%) [19]. Previous researches have reported the protein content in some common fruits like apple, guava, mango to be 1.18, 2.5 and 3.0% respectively and crude fat to be 0.3, 1.16 and 0.4 respectively [21].

The Nitrogen Free Extract (NFE) represents digestible carbohydrates such as sugars; fructans, pectins and polysaccharides like starch [25]. The NFE and total carbohydrates could serve as a potential source of energy and was found to be significantly high at 8.31 and 35.11% respectively in the fruits of *C. baccifera*. Previous studies have also reported significant concentration of total carbohydrates in some uncommon wild edible fruits such as, *Arbutus pavarii* (41.18%), *Nitraria retusa* (24.74%), *Ficus palmata* (28.74%) [19], *S. mombin* (7.9%), *M. whytii* (8.9%) and *D. guineense* (84.0%) [4].

Table.1 Proximate analysis of the fruits of *C. baccifera*

Component	Concentration (%)
Moisture	65.10
Total ash	2.22
Total protein	1.43
Fat	1.14
Crude fibre	27.8
NFE	8.31
Total Carbohydrates	35.11

Values are expressed as mean; n=3

Table.2 Mineral composition of fruits of *C. baccifera*

Mineral	Concentration (mg/100 g)
Calcium	732.0
Copper (Cu)	0.20
Iron (Fe)	2.20
Potassium (K)	409.97
Magnesium (Mg)	36.3
Manganese (Mn)	0.67
Sodium (Na)	1.68
Phosphorous (P)	143.47
Zinc (Zn)	0.88

Values are expressed in mean; n=3

Table.3 Vitamin composition in fruits of *C. baccifera*

Vitamin	Concentration (ppm)
Vitamin A	Not detected
Vitamin B_1	Not detected
Vitamin B_2	Not detected
Vitamin B_3	Not detected
Vitamin B_6	Not detected
Vitamin B_9	5.00
Vitamin C	Not detected
Vitamin D	Not detected

Values are expressed in mean; n=3

Minerals are inorganic elements which are usually divided into two groups viz., macro-minerals and micro-minerals. They are also classified as essential and non-essential based on their requirement for nutrition and vital role in the metabolic activities of the body [26]. Fruits generally contain a variety of essential minerals and vitamins. Analysis of the mineral composition in fruits of *C. baccifera* showed the presence of high percentage of macro-minerals such as calcium, potassium and phosphorous, making it good source of dietary mineral requirements. Similar findings have been reported in wild fruits of *Aglaia diffusa* and *Azadirachta indica* which belong to the

same family Meliaceae as reported by Catibog[12], and Igwenyi *et al.,* [13].

Calcium is essential for the formation, maintenance of bones and teeth, for blood clotting and muscle contraction [27]. The calcium content of *C. baccifera* fruit was found to be high (733 mg/100g) when compared to some wild edible fruits, where the concentration was reported to be 11.038 mg/100g in *Spondias mombin* and 300 mg/100gin *M. whyti* [4]. Hence *C. baccifera* could be recommended as one of the sources of daily intake of calcium for an adult, which ranges between 1000-1500 mg according National Research Council in 1989.

Phosphorous is related to calcium for teeth and bone formation and reported to act as a co-factor in the synthesis of many enzymes in the human body [28]. The availability of calcium in the body is dependent on the calcium to phosphorous ratio which should ideally be 1:1 [29]. In the present study the ratio was found to be 5:1, which indicates that phosphorous content of 143.4 mg/100g present in the fruits of *C. baccifera* is comparatively lesser than its calcium content. Nevertheless, it is significant and higher than that reported in some wild edible fruits of *Spondias edulis* (32.8 mg/100g) and passion fruit-*Passiflora edulis* (68 mg/100g) by Tiburski *et al.,* [17], and lower than in fruits of *M. whyti* (170 mg/100g) [4].

In the present study of proximate analysis, significant amount of potassium (410 mg/100g) was found in fruits of *C. baccifera* when compared to previous findings reported in wild edible fruits of *S. mombin, M. whytii* and *D. guineense* (260-410 mg/100g) [4]. The importance of potassium in controlling human blood pressure, maintaining tissue excitability, electrical conductivity of the brain is well established [30]. The fruits of *C. baccifera* help to meet the RDA (recommended dietary allowance) of 1875-5625 mg, according to National Research Council (1989).

In the present study, fruits of *C. baccifera* showed significant amount of magnesium (36.3 mg/100g) which plays a major role in metabolism of bone and circulatory diseases [23]. Iron is an essential micronutrient required for haemoglobin formation and the normal functions of Central Nervous System [31]. The fruits of *C. baccifera* showed significant amount of iron which could help meet the RDA (5 mg/day).

The fruits of *C. baccifera* showed a low level of sodium, a mineral normally found in low concentration in the fruits. Micronutrients such as Fe, Zn, Cu and Mn were present in trace amounts in the fruits of *C. baccifera*, which are essential and play a major role in the medicinal activity of the fruits, whereas higher concentrations could be injurious [32].

In conclusion, the proximate analysis of the wild fruits of *Cipadessa baccifera* revealed high moisture, crude fibre and total carbohydrate content. Whereas protein and fat content was comparatively low. The fruits showed significant amount of the major mineral elements such as calcium, potassium and phosphorous, while vitamin B_9 was present in minimal concentration. The present investigation clearly indicates that the wild fruits of *C.baccifera* which have been reported to be edible are nutritive and could be used as dietary supplements.The proximate analysis revealed the nutritional significance of the fruits of *C. baccifera* as a good source of macro and micronutrients. To the best of our literature knowledge this is the first report with regard to proximate analysis of *C.baccifera* fruits in India.

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