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Evaluation of nutritional, phytochemical and anti-diabetic properties of two underutilized leafy vegetables *Basella alba* L. and *Basella rubra* L.

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Abstract
Green leafy vegetables are known to be a great source of several vital nutrients. Propagation and consumption of such valuable vegetables help to improve healthcare and should be included in our dietary menu. In the present study, the nutritional and anti-nutritional activity of *Basella alba* L and *Basella rubra* L were analysed. Selected plants showed comparatively higher nutritional and vitamin content than commonly used leafy vegetables. Anti-diabetic activity study in both plants indicated significant IC_{50} values. The preliminary phytochemical screening of methanolic leaf extracts confirms the presence of carbohydrate, protein, glycosides, alkaloids tannin. Flavonoids, saponins, and phenols were quantified. Both plants possess a good amount of phenols and an average amount of saponin and flavonoids.

Keywords: nutritional, underutilized, phytochemical, *Basella alba* L., *Basella rubra* L., antidiabetic

Introduction
Man has tremendous knowledge about edible plants, the overlapping nutritional and medicinal benefits of green leafy vegetables, which provide better support for human wellbeing. They are the cheapest of all the vegetables within reach of poor men but richest in their nutritional value. Considering the extra nutritional value, they deserve greater recognition. The lack of knowledge, especially on the nutritive value of these green leafy vegetables, is the main drawback in their lower consumption. Earlier studies have analyzed the essential micronutrient content in leafy vegetables and in a few wild species of plants in India (Gupta *et al.*, 2011) [12]. Medicinal plants are defined as plants with one or more of their parts containing properties that can be used for therapeutic purposes or used as precursors for the synthesis of various drugs (Kasali *et al.*, 2014) [16]. In the light of developments made in the scientific field, the medicinal properties of plants have received great interest because of their low toxicity, pharmacological activities, and economic viability. Green leafy vegetables represent an important proportion of foods with medicinal value (Anugraha *et al*.,2020, Pradesh and Swapna, 2017) [3, 23]. Tribal communities may have access to global vegetables such as cabbage and cauliflower, but these are costly to purchase. There is little interest in consuming global vegetables, as the crops are not part of traditional diets. Several traditional leafy vegetables require processing as they contain anti- nutritional factors such as tannins, saponin, phytate, oxalate, nitrate, and glucosinolates (Makkar and Becker 1997) [20]. Traditional processing methods like soaking, adding tamarind, and prolonged cooking are used in tribal communities to detoxify vegetables with anti-nutrients and render them safe for human consumption (Aregheore 2012) [4].
Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg 1999) [13]. These phytoconstituents have an important role in human health when their dietary intake is significant. Plants with anti-diabetic activity hold an important role in the current situation as Diabetes mellitus is one of the common metabolic disorders acquiring around 2.8% of the world's population. There are many plant species available all over the world which has been used for these multi-beneficial activities. Here, the present study emphasizes two such under-utilized plants, *Basella alba* L. & *Basella rubra* L., which are commonly used by tribal communities.

Materials and Methods
Plant materials
The plants for the present study, namely *B. alba* L. & *B. rubra* L. was collected from various places in Trivandrum, Kerala, India

Basella alba L.
Basella alba (Fig 1) is a perennial twining herb. Dark green colored stem, which is fleshy, stout at the base with slender upper branches.

Fig 1: Basella albaL

Basella rubra L.

The plant is a perennial herb (Fig 2). The stem is very long, purple colored, slender, succulent, glabrous, and much branched.

Fig 2: Basella rubraL.

Nutritional and anti-nutritional analysis

Fresh leaves from *B.alba* & *B.rubra* were collected and subjected to various tests according to standard procedures in order to estimate nutritional (carbohydrate, protein, chlorophylls, carotenoids, lipids, aminoacids, reducing sugar, vitamins) and anti-nutritional (phytate, oxalate, saponins, phenols) parameters.

Anti-diabetic activity

Alpha-amylase inhibition assay

Alpha-amylase inhibition activity was carried out by the starch-iodine method (Wickramaratne 2016) [26]. 10µl of α-amylase solution (0.025mg/ml) was mixed with 390µl of phosphate buffer (0.02M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37°C for 10 minutes, 100µl of starch solution (1%) was added, and the mixture was re-incubated for 1hr. 0.1ml of 1% iodine solution was added, and after adding 5ml distilled water, the absorbance was taken at 565nm. Sample, substrate, and α amylase blank determination were carried out under the same reaction conditions. Inhibition of enzyme activity was

Calculated as (%) = (A-C) ×100/ (B-C). Acarbose was used as standard.

Where A= absorbance of the sample, B= absorbance of blank (without α amylase), and C= absorbance of the control (without starch).

Phytochemical analysis

Sample preparation

Leaves of *B.alba* & *B.rubra* were dried under shade separately. The dried materials were powdered in a mixer grinder, and the powder was stored in containers.

Extraction of plant materials

The powdered leaves were subjected to solvent extraction using methanol. About 5gm of powdered plant materials were subjected to soxhlet extraction for 6-7hrs with 150ml solvents. The extracts obtained were kept in an oven at 50°C for evaporation to remove the excess solvent. The extracts were stored in a cool, dry place for the preliminary analysis of phytochemicals.

Preliminary phytochemical analysis

The presence of phytochemical components including carbohydrate, protein, glycosides, alkaloids and tannin was confirmed by preliminary phytochemical screening using standard procedure.

Phytochemical profiling

Flavonoids, saponins, and phenols were quantified using standard protocols

Results and Discussion

Nutritional analysis

In the present work, the following factors were analyzed to evaluate the nutritive quality of *B.alba* and *B.rubra*. Carbohydrate (mg/g), protein (mg/g), Total chlorophyll (mg/g), Carotenoids (mg/g), lipid (%), Amino acids (mg/g), Reducing sugar (mg/g), Vitamin C, Vitamin B2, Vitamin B3, and Vitamin B1 and results were analysed using statistical tools and shown in table 1.

Table 1: Nutritional Analysis of *B. alba* and *B. rubra.*

Nutritional Parameters	*Basella rubra L.*	*Basella alba L.*
Carbohydrate (mg/g)	72.433±1.64	75.5±2.87
Reducing sugar (mg/g)	0.002±0.00	0.006±0.00
Chlorophyll a (mg/g)	1.71±0.11	2.86±0.49
Chlorophyll b (mg/g)	1.71±0.11	3.73±0.73
Total chlorophyll (mg/g)	1.71±0.16	3.30±0.64
Carotenoids (mg/g)	0.41±0.01	0.54±0.031
Proteins (mg/g)	0.32±0.06	1.26±0.90
Amino acids (mg/g)	0.94±0.14	0.32±0.05
Thiamine (mg/g)	0.11±0.00	0.15±0.00
Riboflavin (mg/g)	10.97±0.77	9.10±1.40
Ascorbic acid (mg/g)	6.32±0.10	3.23±0.14
Niacin (%)	0.00±0.00	0.01±0.00
Lipids (%)	0.34±0.01	0.32±0.00

Carbohydrate content was higher in *B. alba* (75.5±2.87mg/g) followed by *B. rubra* (72.433±1.64mg/g). When compared with the carbohydrate value of *Amaranthus dubius* it was found that 83mg/g was present in *A. dubius*, while it is less in *B. alba* (Alegbejo 2013). Carbohydrate is low in the sample due to the high level of protein content. On analysing nutritional content, it was found that protein content is high in *B. alba* (1.26±0.90mg/g) than the *Amaranthu*s sps. *B. rubra* showed an average amount of protein content (0.32±0.06mg/g). Plant foods that provide more than 12% of their calorific value from protein can be considered good sources of protein (Ali 2009) [2]. The present study showed that *B. alba* and *B. rubra* are all good sources of protein.

Greens are important sources of protective food that are highly beneficial for the maintenance of good health and the prevention of diseases. In this study, the chlorophyll content was higher in the *B. alba* (3.30±0.64mg/g) compared to *B. rubra* (1.71±0.16mg/g). According to Ferruzzi and Blakeslee (2007) [10], chlorophyll counteracts toxins and inhibits the activities of cancer-causing elements. Chlorophyll increases iron levels in human blood, which is especially useful for pregnant or nursing women. Chlorophyll also helps to purify the liver. Chlorophyll regulates blood sugar levels in the human body, which is good for general health and wellness. Chlorophyll is known to be reported for its antioxidant potential and has promising medicinal and health benefits (İnanç, 2011) [15].

The amino acid content was significantly high in *B. rubra* (0.94±0.14mg/g) and *B. alba* (0.32±0.05mg/g). Amino acids can be used as a treatment for all sorts of medical conditions. Both plants were containing good amount of carotenoids, *(B.alba*-0.54±0.031mg/g & *B. rubra* 0.41±0.01mg/g). Green leafy vegetables are rich sources of carotenoids. It has been reported that available β-carotene from greens in India is 95%, and out of this, 90% is contributed by green leafy vegetables (Singh *et al*., 2001). An increased intake of β-carotene rich food in the daily diet may be one of the strategies for improving vitamin A status among children instead of synthetic vitamin A.

The lipid content was found to be higher in *B. rubra* (0.34±0.01mg/g) compared to *B. alba* (0.32±0.00mg/g). Ejoh *et al*., (1996) [9] reported leafy vegetables are poor sources of lipids. The highest amount of thiamine (0.15±0.00mg/g) and Niacine (0.01±0.00mg/g) was observed in *B. alba. B. rubra* possesses a maximum amount of riboflavin (10.97±0.77mg/g) and Ascorbic acid (6.32±0.10mg/gm). Ascorbic acid is a water-soluble antioxidant that promotes the absorption of soluble iron by chelating or by maintaining the iron in its reduced form. Vitamin C cannot be produced or stored by humans, and 34 must be obtained in the diet. They are monosaccharide antioxidant found in both plants and animals, is implicated in the prevention of some cancers, heart disease, and the common cold.

Anti-nutritional analysis

The anti-nutritional factors can be defined as those substances generated in natural food substances by the normal metabolism of species and by different mechanisms such as inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed. The anti-nutritional factors in *B. alba* L. and *B. rubra* L. were estimated using fresh leaves, and results were shown in table 2

Table 2: Anti-nutritional Factors in *B. alba* L. And *B. rubra* L.

Anti-nutritional parameters	*B. alba*	*B. rubra*
Phenol (mg/g)	0.0013±0.00	0.0029±0.00
Oxalate (%)	3.35±0.01	2.3±0.10
Saponin (%)	47±0.12	60.5±0.03
Phytate (mg/g)	6.66±0.05	13.33±14

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. *B. rubra* contains 0.0013±0.00mg/g phenol followed by *B. alba* (0.0029±0.00). Plant polyphenols are economically important because they make major contributions to the taste and flavour (tea and beer) and color (red wine) of our food and drink. In nature, phenolic compounds protect plants from herbivores and act as chemical signals in flowering and pollination and in the process of plant symbiosis and parasitism (Saranraj *et al*., 2016) [24]. It has been recognized for some time that several classes of flavonoids play a significant role in many physiological processes and show antioxidant and fungicidal activity (Larson 1988) [18], and are natural antihistamines. Oxalates are present in many plants and in significant amounts. Oxalates bind to calcium and prevent its absorption in the human body (Dolan *et al*., 2010) [8]. In the present study, *B. alba* and *B. rubra* possess 3.35%, 2.3% oxalate content, respectively. A comparatively higher amount of saponin was present in *B. rubra* (47%) than *B. alba* (60.5%). Despite the fact that saponins are widely distributed in the plant kingdom, only a small number of such plants are actually toxic to mammals. The negative effect of saponins can be reverted by the inclusion of dietary cholesterol, which interferes with the absorption of saponins by forming an insoluble complex with saponins. At the same time, dietary saponin may exert a positive effect by reducing cholesterol levels (Liener 2003). Phytate is another anti-nutrient that was estimated. Phytate represents the major storage form of phosphorus and inositol in plants. Since monogastric animals lack sufficient amounts of appropriate endogenous phytate degrading enzymes, phytate is considered anti-nutritional, having negative effects on the bioavailability of essential dietary minerals (Cosgrove 1980) [6]. Phytate content is comparatively higher in *B. rubra* (6.66±0.05mg/g) followed by *B. alba* (13.33±14mg/g).

Anti- diabetic activity

In the present study, anti-diabetic activity in fresh leaves of *B.alba* and *B. rubra* was evaluated by alpha amylase inhibition assay, and IC_{50} values were calculated (Table 3). Supplementation of antioxidant vitamin C has also been shown to lower glycosylated hemoglobin in diabetic patients (Davie *et al*., 1992) [7]. B. *rubra* shows higher activity than *B.alba* (798µg/ml and 79.458 µg/ml, respectively). *Amaranthus spinosus*, one commonly used leafy vegetable, possesses IC_{50} =55.9 (Kumar *et al*., 2011) [17], which is almost equal to *B.rubra.* So, it can be used as a substitute for true spinach.

Table 3: Percentage of Alpha Amylase Inhibition

Concentration (µg/ml))	Percentage of inhibition (%)	Percentage of inhibition (%)
20	10.90	17.772
40	25.1184	23.696
60	35.781	30.568
80	41.469	38.151
100	70.142	76.066

B. *alba* IC50 = 798µg/ml
B. *rubra* IC50 = 79.458 µg/ml

Moisture content

The moisture content of both *B.alba* and *B.rubra* were estimated according to standard procedure and it showed 93% and 86% of moisture content, respectively.

Phytochemical analysis

Another area of the work was preliminary phytochemical analysis and quantitative estimation of some phytochemical components. Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but they have important properties to prevent or to fight some common diseases. Many of these benefits suggested a possible role for phytochemicals in the prevention and treatment of disease.

Yield of extracts

$$\text{The yield of methanolic leaf extract} = \frac{Weight\ of\ extract}{Weight\ of\ sample} \times 100$$

The yield of *B. rubra* L. = 25.26%
The yield of *B. alba* L. = 42.7%

Preliminary analysis

Preliminary analyses of phytochemical constituents in methanolic leaf extracts are carried out using a standard protocol. The results are summarized in Table 4.

Table 4: Phytochemical Components of *B. alba L* and *B. rubra L*

Phytochemical components	*B.alba L*	*B.rubra L.*
Carbohydrates	+	+
Proteins	+	+
Glycosides	+	+
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Steroids	+	+
saponins	+	+

The preliminary phytochemical analysis of methanolic leaf extract of both plants showed the presence of carbohydrates, proteins, glycosides, alkaloids, tannins, flavonoids, steroids & saponins.

Quantification of phytochemical components

Methanolic leaf extracts of both plants are used for the quantification of phytochemical components. The following results were obtained table 5.

Table 5: Quantification of Phytochemical Components

Phytochemical components	B.*alba L.*	B.*rubra L.*
Flavonoids	32.558 mg/g	46.464 mg/g
Saponins	53%	61%
Phenols	78.91 mg/g	81.017 mg/g

Flavonoids are found in varying amounts in foods and medicinal plants. They exert potent antioxidant activity against the superoxide radical (Hertog *et al.*,1993) [14]. They are synthesized by plants in response to microbial infection and effective antimicrobial substances against a wide array of microorganisms (Pradeesh *et al.*, 2012) [22]. From the results (Table 5), it was found that *B. alba* and *B. rubra* possess a good amount of flavonoids. Comparatively, *B. rubra* containsa high amount of flavonoid (46.464 mg/g) followed by *B. alba* (32.558 mg/g). Saponins are amphipathic glycosides consisting of a polycyclic aglycone that is either a choline steroid attached via C3 and an ether bond to a sugar side chain. This class of phytochemical has hypotensive and cardiac depressant properties. Saponins bind to cholesterol to form insoluble complexes: as a result, humans do not suffer severe poisoning from saponins. Endogenous cholesterol excreted via the bile combined with dietary saponins prevents cholesterol reabsorption and results in a reduction of serum cholesterol (Cheeke, 1971) [5]. In the present, the saponin content of *B. alba* and *B. rubra* were estimated, and from the result, it is clear that *B. rubra* contains more amounts (61%) than *B.alba* (53%).

The phenolic content of leaf extract was also evaluated, and found that *B.rubra* contains 81.017 mg/g and *B.alba* possesses 78.91 mg/g. Many of these phenolics have been shown to contain high levels of antioxidant activities. In nature, phenolic compounds protect plants from herbivores and act as chemical signals in flowering, pollination, and in the process of plant symbiosis and parasitism. It has been recognized that several classes of flavonoids play a significant role in many physiological processes and show enormous pharmacological effects (Gardam, 2000) [11].

Conclusion

Edible green leafy vegetables appear to be underutilized throughout the world and, in some areas, may even be diminished in use (Prasad *et al*., 2008). In the present study, *B. alba* and *B. rubra*, two underutilized plants, were used as the study material. The nutritional and anti-nutritional study was carried out in fresh leaves by evaluating several nutritional parameters such as carbohydrate, reducing sugar, total chlorophyll, carotenoids, lipids, proteins, amino acids, and vitamins like thiamine riboflavin, niacin, and ascorbic acid. From the results, it was found that *B.alba* possesses more amount of carbohydrates, reducing sugar, total chlorophyll, carotenoids, proteins, thiamine, and niacin than *B.rubra*. But *B.rubra* has a comparatively higher amount of amino acids, lipid, riboflavin, and ascorbic acid. In comparison with other commonly used leafy vegetables, these plants exhibit more nutritional contents. They contain a low amount of phenols, oxalate, and phytate, and a moderate amount of saponins. Methanolic leaf extract of *B.alba* and *B. rubra* was used for preliminary phytochemical analysis using a standard protocol, which revealed the presence of carbohydrate, protein, glycosides, alkaloids tannin. Flavonoids, saponins, and phenols were quantified. Both plants possess a good amount of phenols and an average amount of saponin and flavonoids. Anti-diabetic activity of B. *alba* and B. *rubra* was checked, and IC50values were determined. *B. rubra* showed a significant IC50 value for alpha-amylase inhibition assay than *B. alba*.

The present study reveals the potentiality of these inexpensive, easily accessible, but lesser-known leafy vegetables as a source of unconventional food with nutritive value and many other health benefits. Consumption of such valuable vegetables should be encouraged through awareness and should be propagated. The inclusion of these plants into the diet will add more nutritional benefits to our body in a low cost.

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