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Nutraceutical studies of *Polygonum plebeium* R. Br. (small knotweed) a promising vegetable for future

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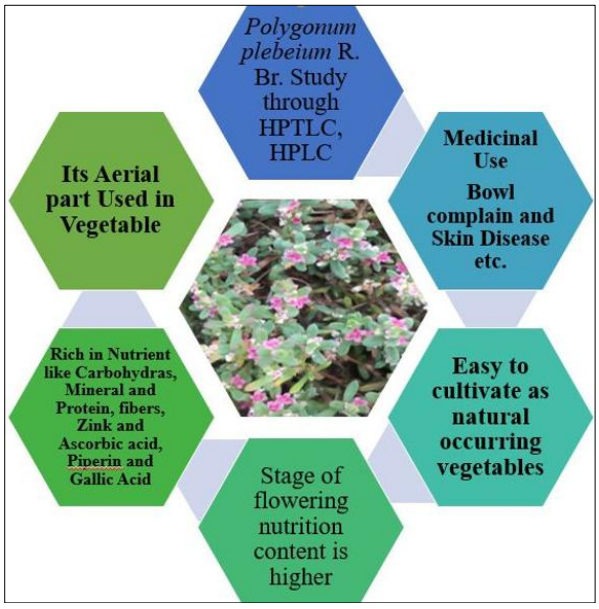
Abstract
Polygonum plebeium R. Br. is often found near waterlogged areas in many parts of the world. In some parts of India, it is widely consumed as local vegetable and also used medicinally. However, in other parts it is not included in diet due to many reasons. Thus an attempt is made to look for the nutritional and proximate analysis of the species collected from wild near the regions of district Haridwar, Uttarakhand, India. Plant material were collected from wild sources in the field and a study was conducted to analyse the nutritional potential of arial parts by evaluating carbohydrates, fat, proteins, energy, total ash, and water content by standard methods. However, UHPLC profiling as well as HPTLC was performed using an in-house protocol developed at Patanjali Research Institute, Haridwar, Uttarakhand. The aerial parts of the plant were found to contain all essential nutrients as carbohydrate (69.63%w/w), protein (1.30%w/w), total ash (11.68%w/w), moisture (13.10%w/w) and energy (140.26 Kcal /100g). The HPLC analysis confirms the presence of gallic acid (0.049 µg/mg) and piperine (0.234 µg/mg) in the aerial parts of the plant.The study was performed with the objective of nutritional composition analysis, qualitative analysis of secondary metabolite so that it can be recommended for its utilization for edible purposes. Graphical abstract of the study has been given in Figure.

Graphical Abstract



Keywords: Nutraceutical value, piperine, *Polygonum plebeium* R. Br. proximate analysis, vegetable

Introduction
The world population is steadily growing, resulting in excessive burden to produce food crop leading to utilization of massive amounts of chemical and pesticide fertilizers to increase yield, thereby affecting common man health. Thus there is an urgent need to reduce the burden on the planet's traditional food supplies, therefore exploring naturally occurring edible sources is urgently needed.

Authors in this series have consistently highlighted the use of wild plants as a food source in the form of vegetables that are high in vitamins and minerals and the investigation of these dietary details are examined in these kinds of plants. According to the work plate, *Polygonum plebeium* R. Br. is utilized as a vegetable in some countries and regions of the world where it grows wild [1]. The plant attains the height of 10-40 cm papillose-scrabidulous in nature. The leaves are triangular with approximate size of 2.5-3 mm. and grows in the less waterlogged parts of swamps, ponds, ditches, puddles, rice fields etc., thus is commonly known to be a frequent wetland plant widespread in many parts of the world, especially the palaeotropics [2-4]. In many places specially in north eastern parts of India it is locally used as vegetable. In Jharkhand the leaves of this plant are used in the form of Sag (green leafy vegetable with dry semiliquid preparation), eaten raw or dried and stored for uses throughout the year [5].

It also provides rural household with income opportunities through their sale in market. The leafy vegetables are rich source of phytochemicals helping to protect cell from oxidative damage induced by free radicals and thereby reduce the oxidative stress [6]. The herbal recipe is prepared from the leafy part of this plant during the festival of Bohag or Rongali Bihu in Assam [7].

P. plebeium belongs to family Polygonaceae and is widely referred to as "small knotweed" and bisort in English however names as "Chemti Sag", "Dubia Sag", "Anjaban" and Lalbuti are its indigenous names. The tribals of Uttar Pradesh, Bihar, Jharkhand and Orissa utilize this plant as famine food and also use for treatment of various ailments including menstrual issues [8].

It is also employed as a traditional medicine in numerous cultures worldwide. In India, it has traditionally been used orally, in the rural areas of Shahjahanpur, Uttar Pradesh, to treat digestive problems and pneumonia. Menstrual irregularities are treated with a mixture of leaf powder and mishri (candied sugar). The plant is rich in wide range of phytochemicals, including tannins, alkaloids, flavonoids, phenols, unsaturated sterols, triterpenoids, essential oils, saponins, etc. It also exhibits various pharmacological properties, such as antinociceptive, antioxidant, cytoprotective, and anticancer properties. *P. plebeium* extract is used to treat diarrhoea, eczema, ring worm, inflammation, dysentery, and other conditions due to its extensive pharmacological effects. It is galactagogic, and its roots are used in bowel complaints and powdered plant in pneumonia in the name of Sarpakshi (Ayurveda), Muniyaara (Bihar), Raniphul in Santhali. Cream prepared from the crushed leaves is used for treatment of eczema and ring worms [9-15].

Botanical description

Plant is purplish green homophyllous prostrate, white hairy, much-branched from the base, not wiry, 10-40 cm, papillose-scabridulous. Leaves: ocrea single-veined, 2.5-3 mm, base cylindric, distal part laciniate; sessile or petiole short to 1 mm; blade dark green, narrowly elliptic or oblanceolate, 5-16 × 1-4 mm, margins entire, apex obtuse to acute, papillose-scabridulous; stem leaves longer than adjacent branch leaves to ca 2 times, distal leaves covering the flowers. Inflorescence axillary; solitary or cymes occurred with clusters of 2-3-flowers. Pedicels exerted from ocreae, 3-5 mm. Flowers closed; perianth short, tube ¼ to the perianth length; tepals overlapping, green with white or pink margins, petaloid, without keel, elliptic; midveins prominent; stamens 5-8; styles 3. Achenes enclosed in perianth, black-brown, broadly ovate, 2-3-edged, sub-equal, apex edges concave, shiny, smooth presented in fig. 1. Flowering May-October [16].

Fig 1: Habit of *Polygonum plebeium* R. Br.

Nutritional and Medicinal attributes

The plant is rich in minerals like calcium, magnesium, potassium, cobalt, manganese, chromium, iron, sodium along with biochemical compounds as proteins, carbohydrates, fat, lipids, vitamin C etc. However, the presence of few anti nutritional compounds like oxalate, nitrate and tannins have also been reported from it [17, 18]. The presence of various phytochemicals as triterpenoids, epifriedlanol, oleanolic acid, betulinic acid, flavonoids guaijaverine, rutin, quercetin, phytosterols, beta sitosterol have also been reported [10].

Plant is used for hepatitis, jaundice, diarrhoea, bowel complaints. The plant and bark of *Butea superba* Roxb. ex Willd. and adventitious roots of *Ficus benghalensis* L. is used to cure dysentery. The oil and ash is used for eczema, powdered herb for pneumonia. The decoction of plant is used as cooling agent. Balm prepared from crushed leaves is used for ringworms and eczema. The plant is also used as blood purifier [19].

Each and every plant contains some phytochemcials that help to enhance or boost the power of immunity in humans, therefore a good number of plants acclaimed for their medicinal utility are nowadays tried to analyse for their nutritional values so that they can be included in a common man diet.

Materials and Methods

The aerial parts of *P. plebeium* R. Br. were collected from Haridwar areas during the month of July, and following studies were conducted in fresh vegetable samples:

The proximate analysis of aerial parts of the species were evaluated using standard protocols. The protein estimation was performed by Kjeldahl method wherein total protein was determined by multiplying the evaluated nitrogen by 6.25 (IS 7219). The total ash content and moisture content was determined using method as prescribed in IS:561 taking 2-4 gm of sample. The results were expressed as ash (% w/w) = Difference in weight of ash/weight of sample × 100. Two gram of each sample was taken in a flat bottomed dish and kept overnight in an air oven at 100-110ºC and weighed. The loss in weight was regarded as a measure of moisture (water content). The carbohydrate content was calculated

accordingly as given in IS:1656. Percentage of available carbohydrate was calculated using the formula,% of carbohydrate = 100 - [% of ash+% of fat+% of protein +% of fiber]. The total energy was calculated with the procedure described as per IS:14433. The total energy (percent by mass) is calculated using formula 9 × A + 4 (B + C), where A= percent by mass of fat, B= percent by mass of total protein, C= Percent by mass of carbohydrate.

The analysis of total polyphenols and total flavonoids in the leaves of *P. plebeium* R. Br. were determined by UV spectrometry taking reference standards of gallic acid and quercetin respectively. 20 gm of each reference standard was dissolved in 20 ml water (for gallic acid) and methanol (for quercetin). One ml of each reference standard was further diluted to 10 ml (in respective diluents) to prepare concentration of 100 ppm and also further diluted to prepared different concentration to plot linearity. The fresh leaf sample was crushed in pestle mortar. The powdered material was used for proximate analysis. About 500 mg of sample was dissolved in 10 ml milli Q water and solution was sonicated for 20 minutes and centrifuged, clear supernatant was used for the analysis.

About 1ml of sample was mixed with 1 ml of Folin- Ciocalteu reagent (1:10 with distil water). The samples were incubated at room temperature for 5 minutes. Then 1 ml of 10% sodium carbonate (Na2CO3) was added to the mixture. The mixture was kept in the dark at room temperature for 60 minutes. Then the absorbance was taken at 760 nm using gallic acid as standard.

About 1 ml of sample was mixed with 0.4 ml of 10% aluminium chloride (AlCl3). Then 0.4 ml of 3M sodium acetate (C2H3NaO2) and 3 ml ethanol was added to the mixture. The mixture was kept at room temperature in dark for 30 minutes. The absorbance was taken at 450 nm using quercetin as standard.

The quantitative estimation of important phytoconstituents present in leaves of *P. plebeium* R. Br. were determined by using High Performance Liquid Chromatography. The reference standards used were gallic acid (TCI/ product code: G0011/potency: 100%) and piperine (sigma aldrich/ product code: P49007/ potency: 97%). These compounds were dissolved in methanol to prepare 1000 µg/ml standard solution individually. 0.05 ml of 1000 ppm from each standard stock solution was diluted to 1 ml in methanol to prepare 50 ppm of standard mix working solution. About 2.0 gm of leaves sample was diluted with 20 ml of methanol and sonicated for 30 minutes and then centrifuged at 10000 rpm and filtered using 0.45µm nylon filter. This filtered solution was used for the analysis. The chromatographic analysis was performed by Prominence-XR UHPLC system (Shimadzu, Japan) equipped with quaternary pump (Nexera XR LC-20AD XR), DAD detector (SPD-M20 A), auto-sampler (Nexera XR SIL-20 AC XR), degassing unit (DGU-20A 5R) and column oven (CTO-10 AS VP). Separation was achieved using a Shodex C18-4E (5 µm, 4.6*250 mm) column subjected to binary gradient elution. Two solvents were used for the analysis, water containing 0.1% v/v ortho-phosphoric acid in water (solvent A) and acetonitrile (solvent B). Column temperature was kept 35°C and flow was set 1.0 mL/min during the analysis. 10 µl of standard and test solution were injected. Wavelengths were set at 270 nm. given in table 1.

Table 1: Chromatographic conditions of the study

Time (min)	A%	B%
0	100	0
20	85	15
40	70	30
50	50	50
60	20	80
65	10	90
66	100	0
70	100	0

Results

The results of the proximate analysis are given in table 2, figs. 2, 3.

In a study the aqueous methanolic extract of *P. plebeium* R. Br. did not showed the presence of fats and fixed oils along with glycosides however the alkaloids, saponins, tannins, phenols, proteins, carbohydrates gums and mucilage were detected[19]. The standard plots for gallic acid and quercetin are shown in table 3 and figure 4.

The overlay chromatogram of standard gallic acid and piperine and aerial parts of *P. plebeium* R. Br. confirms the presence of gallic acid (0.049) and piperine (0.234) respectively in aerial parts when compared with the standards used. In a study the gallic acid from methanolic extract of whole plant of *P. blebeium* R. Br. is reported to be antioxidant, antimicrobial, anti-inflammatory and anticancer activity [19]. The compound piperine is shown to have antibacterial property [20].

Table 2: Nutritional value of *Polygonum plebeium* R. Br.

S. No.	Parameters (%w/w)	Result
1.	Moisture	13.10
2.	Total Ash	11.68
3.	Total Fat	4.29
4.	Protein	1.30
5.	Carbohydrate	69.63
6.	Energy (Kcal/100g)	140.26

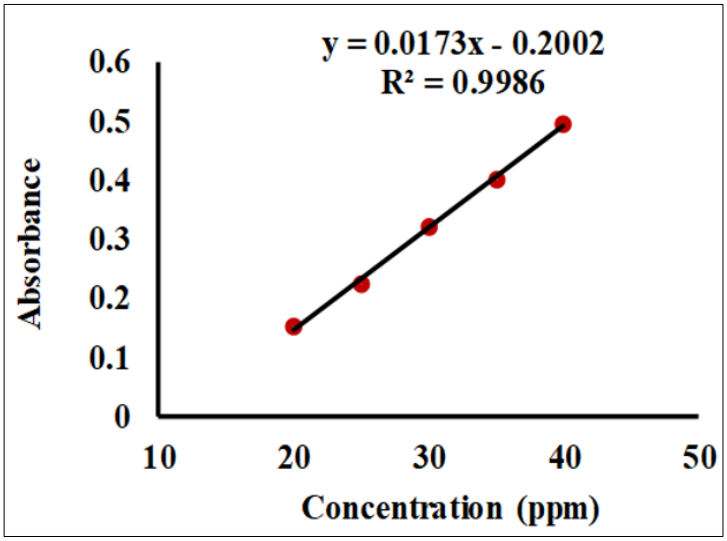


Fig 2: Linearity plot of Gallic acid for determination of polyphenol content

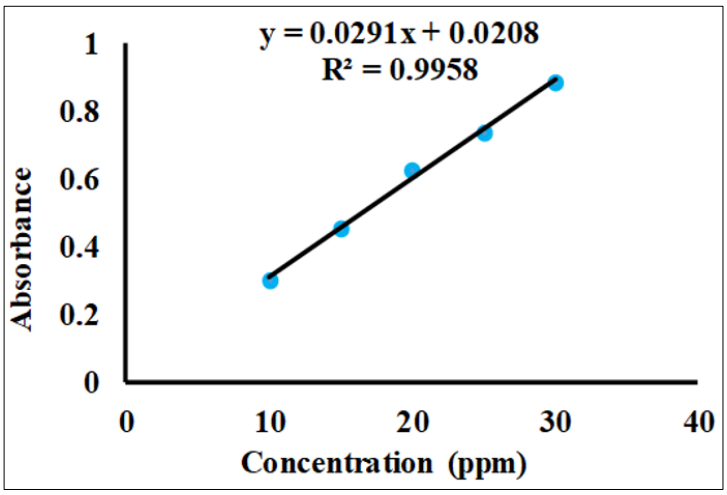


Fig 3: Linearity plot of Quercetin for determination of flavonoid contents

Table 3: Percentage value of gallic acid and piperine in arial parts of *Polygonum plebeium* R. Br.

S. No.	Name of compound	*Polygonum plebeium* R. Br. aerial part
1	Gallic acid	0.049
2	Piperine	0.234

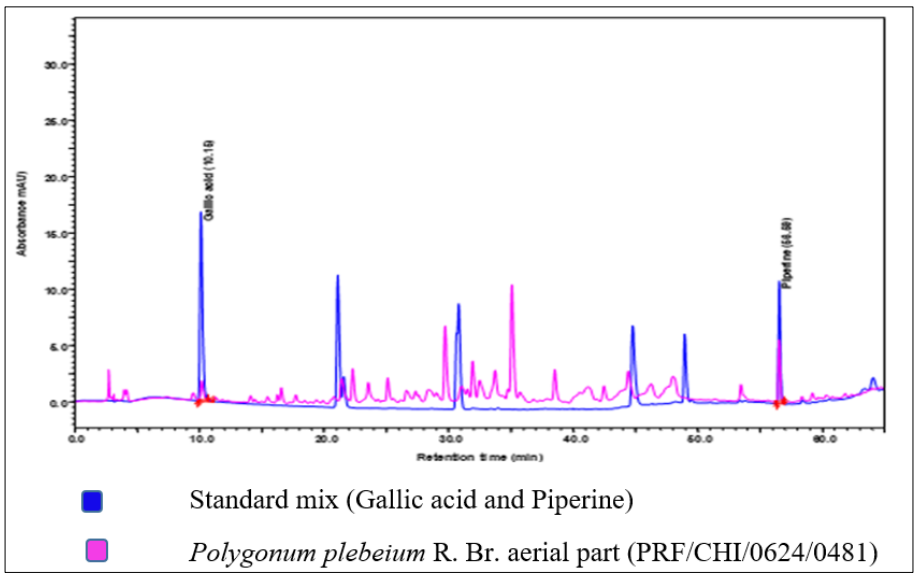


Fig 4: Overlay chromatogram of standard mix (Gallic acid and Piperine) and *Polygonum plebeium* R. Br. aerial part (PRF/CHI/0624/0481) at 270 nm

Discussion

The plant *P. plebeium* widely grows in moist places and commonly available in nature in the tropical areas, and the local people can consume it as vegetable. Accordingly, instead of adding more fertilizer and pesticides in the crop field, one should collect these types of vegetable and sell in the vegetable shops for promoting to use wild vegetables growing with no application of pesticides and chemical fertilizers. As in the states of Northeast India, number of locally available vegetables are collected from wild and sold in local vegetable shops. Some of the important local vegetable are *Amaranthus spinosus* L., *A. viridis* L., *Basella alba* L., *Diplazium esculentum* (Retz.) Sw., *Phlogacanthus thyrsiformis* (Roxb. ex Hardw.) Mabb., *Gronostegia hirta* (Blume) Miq., *Houttuynia cordata* Thunb., *Nelumbo nucifera* Gaertn, *Solanum nigrum* L., *S. torvum* Sw., *S. violaceum* Dunal, and several others.

It has been observed that the standing crop biomass of *P. plebeium* increased as the plant grew older and the energy content rose until the fruiting stage, at which point it fell. Up to the stage of flowering, the concentrations of calcium, sodium, phosphorus, potassium, and nitrogen increased; after that, they decreased.[21] The potassium content in this species is reported to higher in comparison to many other vegetables used regularly like *Amaranthus viridis* L., *Centella asiatica* (L.) Urb. *Colocasia esculenta* (L.) Schott, *Crotalaria juncea* L., *Meyna spinosa* Roxb. ex Link, and *Portulaca oleracea* L., etc [22].

In many proximate studies the antioxidant potential of *P. plebeium* has been reported and used for the cure of several diseases [23-26]. The ongoing research in the field of nutraceuticals and exploration of the wild plants for nutrition shows that this plant is considered to be an important one. A recipe of the aerial parts of this plant using other common ingredients used in households has been made, however the presence of tannin is also reported but considerably has not affected its taste after preparation [4, 10]. Seeking out the need of the health benefits in suggesting this plant for inclusion in daily diet, the authors carried out the present study and found appreciable results for this inclusion.

Conclusion

As a result of the present study, it is concluded that the plant *P. plebeium* R. Br. contains rich amount of nutrients like carbohydrate, crude proteins, fibres ascorbic acid, carotenoids and phenols and the minerals like calcium, cobalt, copper, chromium, iron, magnesium, manganese, sodium and zinc to supplement the required dietary components. The anti-nutritional contents like oxalate, nitrate and tannin are in low quantity than the leafy vegetable, which are regularly eaten. Besides, its use in regular diet helps in preventing the body from various ailments like bowel complaints, skin diseases, etc. This is also to conclude that the plant use as vegetable is fruitful till the stage of flowering and gradually the biomass as well as nutritional contents get decreased. Since nutritional constants in this plants are higher up to the stage of flowering and lowers in later stages, it is better to use the plant before flowering starts. The main concern to highlight in the present paper is to evaluate its nutritional aspects so that it can be widely utilized as vegetable food with ease. Thus additional research is need of the hour so that its precise nutrient analysis can be performed required for its consumption as a popular vegetable.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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