Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X

RESEARCH ARTICLE

Received on: 08-10-2013
Accepted on: 28-11-2013
Published on: 21-12-2013

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Conflict of Interest: None Declared !

Pharmacognostic specifications of roots of *Beta vulgaris* cultivated in India

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Abstract
Beta vulgaris Linn. is an important medicinal plant of family Chenopodiaceae. It is commonly known as Beet root or garden beet. The root is used in Indian traditional systems of medicine specifically for the treatment of fertility, hypertension, cancer and urinary tract disorders. As the herb is used widely in the Indian traditional systems of medicine, it was thought worthwhile to undertake the standardization of its roots. The quality control parameters like morphological and microscopic characters, physico-chemical parameters *viz.* extractive of plant with different solvents, ash values, foreign organic matter, loss on drying and pH of aqueous solution were determined. Total phenolic and total flavonoid content were also determined. The results obtained from preliminary pharmacognostic standardization of root of *Beta vulgaris* are very helpful in determination of quality and purity of the crude drug and its marketed formulation.

Keywords: *Beta vulgaris,* Chenopodiaceae, quality standards, WHO guideline.

Cite this article as:
Adil Ahmad, S.H. Ansari, Javed Ahamad, Kamran J. Naquvi. Pharmacognostic specifications of roots of Beta vulgaris cultivated in India. Asian Journal of Biomedical and Pharmaceutical Sciences; 03 (26); 2013; 5-10.

1. INTRODUCTION

Beta vulgaris Linn. (Chenopodiaceae) root is a succulent, tuberous with mostly basal, ovate to oblong-ovate leaves and greenish flowers. It is cultivated as a vegetable almost throughout India. The two verities which are commonly grown in India are Crimson Globe and Detroit Dark Red, both having globular-oval roots (Anonymous, 1988). Mainly the beet root is used in Indian traditional systems of medicine specifically as haemagglutination, antifertility, antifungal, anticlastogenic, anti-cancer activity, antiprotozoal, antiviral and diuretic activities and effects on respiration. Its nutritive value of the plant has been reported by several workers. The amino acid composition of leaf protein was reported as threonine, valine, cystine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, aspartic acid, serine, glutamic acid, proline, glycine, alanine and tyrosine (Subba Rao *et al.,* 1972; Rao *et al.,* 1956). The cooked beet root, on steam distillation yielded 17 volatile constituents of which the major ones were, pyridine (5.6 %) and 4-picolene (54.4 %) (Sengupta and Pal., 1971). It is a rich source of a group of red and yellow pigments known as betalains comprising the red violet betacyanins and yellow betaxanthins. Betanin is the major constituent (75-95%) of the red pigment and vulgaxanthine I, the principal pigment of the yellow betaxanthin group. It also contains isobetanin, isobetanidine, prebetanin, isoprebetanin, and vulgaxanthin II (Anonymous, 1988). Anthocyanins and betalains have never been reported in the same plant, seeming to be mutually exclusive in the plant kingdom (Stafford *et al.,* 1994). The present study deals with development of quality standards of *B. vulgaris* root as per WHO guidelines.

2. MATERIALS AND METHOD

2.1 Plant material and chemicals

The root of *Beta vulgaris* procured from a local vegetable market of Delhi and identified by Dr. H. B. Singh, Scientist, National Institute of Science Communication and Information Resources and a voucher specimen number NISCAIR/RHMD/Consult/-2012-13/2159/165 was deposited in RHMD of NISCIR, New Delhi. All analytical grade solvents and reagents were obtained from S.D. Fine Chemicals, Mumbai, India.

2.2 Morphological character

Detailed study of the morphological characters can be helpful in differentiating them. The macroscopy of a drug includes its visual appearance to the naked eye. It depends to a large extent on the part of the plant from which the drug is obtained. For each particular morphological group, a particular systemic examination can be carried out. Size, Colour, odour and taste are important parts of morphology of a particular drug.

2.3 Microscopical character

Microscopical examination of epidermal trichomes and calcium oxalate crystals is extremely valuable, especially in powdered drugs; as the cells are most likely broken expect lignified cells. The cell contents such as starch granules, calcium oxalate crystals, aleurone grains were determined.

2.4 Preliminary phytochemical screening of *Beta vulgaris* root extracts

The qualitative chemical tests were performed for different extracts according to the methods described by Farnsworth et al., 1996 with some modifications.

2.4.1 Determination of presence/absence of alkaloids

The alkaloids were extracted by refluxing the sample with sufficient amount of water for about 2 hr. The extract was concentrated on a rotor vapor, basified with NH_4OH and was extracted with $CHCl_3$ (three times). Then the content was concentrated and 2 drops were spotted separately on a thin layer chromatography (TLC) plate. After development the plate was dried, Dragendorff's reagent was sprayed onto them. Alkaloids give an orange color with Dragendorffs reagent.

2.4.2 Determination of presence/absence of steroid glycosides

The extracts were dissolved in equal volumes of acetic anhydride and $CHCl_3$. The mixture was transferred to a dry test tube and conc. H_2SO_4 acid was added at the bottom of the tube. Formation of a reddish brown or violet brown ring at the interface of the 2 liquids indicates presence of steroids.

2.4.3 Determination of presence/absence of polyphenolic compounds

Two to three drops of 1% $FeCl_3$ solution was added to 2 ml portions (1%) of each extract. Phenolic compounds produce a deep violet color with ferric ions.

2.4.4 Determination of presence/absence of saponins

The extract is taken in test tube with small amount of water and shaken vigorously for one minute and observed for formation of rich lather, which is stable for more than ten minutes.

2.4.5. Determination of presence/absence of flavonoids

The extracts were dissolved in methanol (50 %, 1-2 ml) by heating. Then metal magnesium and 5-6 drops of concentrated hydrochloride acid (HCl) were added. The solution turns red when flavonoids are present. Other chemical tests for phytoconstituents were

performed as per method described by Mukherjee, 2002.

2.5 Determination of physico-chemical parameters of *Beta vulgaris* roots.

Physicochemical parameters were determined for root of *Beta vulgaris* according to methods described in WHO guidelines.

2.5.1 Determination of total ash content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600 °C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccator. The content of total ash (in mg/g) of air-dried material was calculated as follows:

$$\% \text{ Total ash} = \frac{\text{weight ash}}{\text{weight of sample}} \times 100$$

2.5.2. Determination of acid insoluble ash content

HCl (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

$$\% \text{ Acid insoluble ash} = \frac{\text{weight ash}}{\text{weight of sample}} \times 100$$

2.5.3 Determination of water soluble ash content

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water soluble ash content was calculated using the following equation.

$$\% \text{ Water soluble ash} = \frac{\text{total ash content-water insoluble residue in total ash}}{\text{weight of sample}} \times 100$$

2.6 Foreign matter analysis

Foreign matter presence may be due to faulty collection of crude drug or due to deliberate mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated (Mukharjee, 2002).

2.7 Determination of moisture content

The powdered material (10 g) was placed in a moisture dish and dried to a constant weight in an oven at 100-105^{0}C. The loss of weight (in mg/g) of air dried was calculated as follows:

$$\% \text{ Moisture content} = \frac{\text{weight loss}}{\text{weight of sample}} \times 100$$

2.8 Determination of pH

The pH of 1 and 10 % were determined by making appropriate concentration of powdered drug in aqueous solution, filtered and checked pH of the filtrate with a standardized glass electrode (Anonymous, 1988).

2.8 2.9 Determination of methanol soluble extractive matter

Accurately weighed powdered material (4 g) and was placed in a glass stoppered conical flask. Methanol (100 ml) was added to the flask and it was weighed to obtain the total weight, including the flask. Then, the flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h, and then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of methanol. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25 ml of the filtrate was transferred to a tarred flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h and cooled in a desiccator and weighed. The content of extractable matter (% w/w) air-dried material was calculated as follows:

2.10 Determination of water, petroleum ether and chloroform extractable matter

$$\% \text{ Methanol soluble extractive matter} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 4 \times 100$$

The same procedure as described for the methanol extractable matter was followed for the determination of water, petroleum ether and chloroform extractable matter using distilled water instead of methanol.

2.11 Fluorescent analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (255 and 366 nm) and after treatment with different reagents.

2.12 Determination of Total Phenolic Content

TPC of the methanolic extracts was measured using Folin-Ciocalteu method as described by Azlim Almey, 2010. Gallic acid was used as standard. 0.5 mg/ml stock

standard solution of gallic acid was prepared by dissolving 250 mg of dry gallic acid in 1 ml of extracting solvent and then diluted to 500 ml of distilled water. The stock solution was stored at 4 ºC. Working standards of between 50 and 500 mg/l were prepared by diluting the stock solution with distilled water. The extract was prepared at concentration of 1 mg/ml. 100 µl of extract was transferred into a test tube and 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) was added and mixed. The mixture was allowed to stand at room temperature for 5 min. Then, 0.75 ml of 6 % (w/v) sodium carbonate was added to the mixture and mixed gently. After standing at room temperature for 90 min, the absorbance was read at 725 nm using Perkin Elmer Lambda 25 UV/Vis spectrophotometer. The standard calibration curve of gallic acid (50 - 500 mg/ml) was plotted.

2.13 Determination of Total flavonoid content

The total flavonoid content was determined using the (Ramamoorthy and Bono, 2007). 5 ml of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (10 mg/ml). Absorption readings at 415 nm using PerkinElmer UV-VIS lambda 25 spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 ml extract solution with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with catechin (5 - 100 µg/ml) as the standard. Total flavonoid content is expressed as mg of rutin equivalents /g of extract.

3. RESULT AND DISCUSSION

The results of standardization parameters are:

3.1 Morphological Characters

Proper examination of the untreated sample of root of the chosen plant was carried out under diffused sunlight and artificial source similar to day light.

Organoleptic properties

Shape	: globular
External colour	: reddish purple
Size	: 8cm
Surface	: tuberculated
Texture	: no hair
Odour	: slight
Taste	: strongly astringent

3.2 Microscopical Characters

The Figure 1 shows the microscopic characters of beet roots. The cell vacuoles of cortical parenchyma and the pith, are coloured in a deep red. Rhizodermis and conductive vessel are not coloured. Sugar beet, red beet roots do not contain starch. There is a secondary structure of roots, which contain concentric circle consisting of conductive tissues, penetrated by parenchyma as wider rays, formed from cells with cellulose walls. Beet root showed in the cross section well represented xylem vessel, revealing to the root center (to pith) a protoxylem, outwards associated with a metaxylem. Under the phloem is cambium, provided from the pith rays, pericycle or pith. Most of the root thickness due to this cambium, which produces more secondary xylem than secondary phloem. Beet vascular bundles are collateral type, the phloem is located in the back. Between two primary tissues persists meristematic tissues namely cambium, from which secondary xylem and phloem are forming.

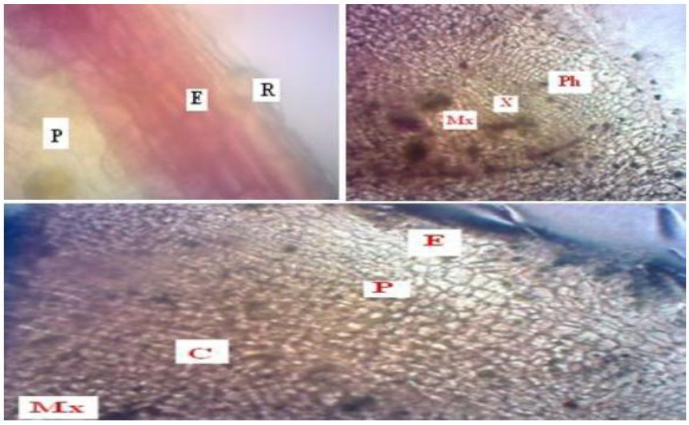

Figure 1. Microscopic characters of *B. vulgaris*
(R-rhizodermis; E-epidermis: P-parenchyma: C-cambium; Ph-phloem; X-xylem; Mx-metaxylem)

3.3 Phytochemical Screening

The results of phytochemical screening were given in Table 1.

Extract constituents	Pet. ether	Chloroform	Methanol	Hydro-alcohol	Aqueous
Alkaloids	-	+	+	+	-
Carbohydrates	-	-	+	+	+
Glycosides	-	+	+	+	+
Tannin	-	-	+	+	+
Phenolics	-	-	+	+	+
Flavonoids	-	+	+	+	+
Proteins & amino acids	-	-	-	-	+
Saponins	-	-	-	-	-
Acidic compounds	-	-	-	-	-
Mucilage	+	-	-	-	-
Resins	-	-	-	-	-
Lipids/fats	+	-	-	-	-

Table 1. Results of phytochemical screening
(absent - , present +)

3.4 Physico-chemical parameters

Parameters	Results (n= 3, Mean ± SD)
Total ash (% w/w)	11.93 ± 1.43
Acid insoluble ash (% w/w)	1.33 ± 1.26
Water soluble ash (% w/w)	7.99 ± 1.41
Foreign organic matter (% w/w)	1.35 ± 0.34
Loss on drying (% w/w)	13.52 ± 1.74
pH of 1 % aqueous solution	6.34 ± 0.08
pH of 10 % aqueous solution	5.75 ± 0.13

Table 2. Summary of results of physico-chemical parameters

Results of physico-chemical parameters are summarized in Table 2.

3.5 Extractive values

The results of different extractive values were summarized in Table 3.

Extractive value (%)	Pet. ether	Chloroform	Methanolic	Hydro-alcoholic	Aqueous
Hot extraction	2.53±1.23	6.180±0.17	14.67±2.13	18.82±1.86	23.87±2.96
Successive Extraction	1.62±0.32	2.43±0.13	6.05±0.17	9.51±0.75	6.77±0.17
Cold extraction	1.04±0.16	1.625±0.15	9.06±0.74	12.81±0.73	18.39±1.97

Table 3. Results of extractive values (n= 3, Mean ± SD)

3.6 Fluorescence Analysis

The behavior of beet root powder with different chemicals was summarized in Table 4.

S. No.	Treatment	Day light	UV (254nm)	UV (366 nm)
1.	Powder as such	Dark red	green	Dark green
2.	5% NaOH	Green	Light green	Black
3.	H_2SO_4	Light brown	Dark brown	Brown
4.	Conc. HCL	Reddish purple	Light blue	blue
5.	Acetone	Light green	Dark red	Light green
6.	Chloroform	Greenish yellow	Red	Dark green
7.	Conc. HNO_3	Pale yellow	green	Bluish black
8.	$FeCl_3$	Black	Light blue	Dark blue
9.	KOH (1%)	Reddish yellow	Light blue	Dark blue
10.	Acetic acid	Light red	Black	Black

Table 4. Results of fluorescence analysis

3.7 Total Phenolic Content

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Azlim Almey, 2010, using gallic acid as a standard phenolic compound. A linear calibration curve of gallic acid with R^2 value of 0.9917 was obtained (not shown). Figure 2 shows mean TPC of the *B. vulgaris* extracts measured using the GAE equation of Y = 0.001x +0.0631 (R^2 = 0.9917), whereby Y = absorbance at 765nm and X = concentration of total phenolic compounds in mg per ml of the extract. The methanolic extracts, *B. vulgaris* showed the TPC (11.23 ±0.13 mg/g).

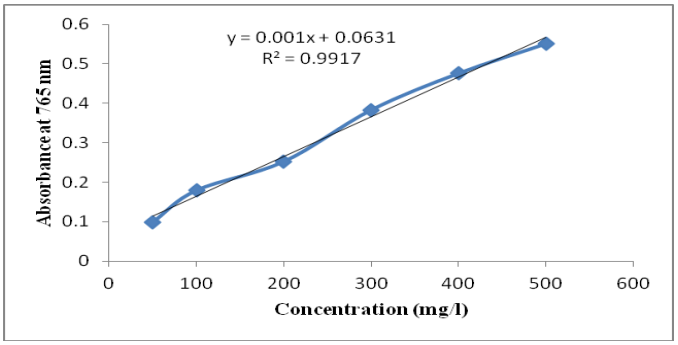

Figure 2. Calibration curve of standard Gallic acid

Standardization of crude drug is an integral part of establishing its correct identity. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. Phytochemical screening revealed the presence of alkaloids, polyphenolic compounds, flavonoids, amino acids and steroid glycosides in methanolic and aqueous extracts mainly. The physicochemical analysis of plant drugs is an important for detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity and quality of drugs. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition (Singh and Sharma, 2010). The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and nonphysiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing (Mukherjee, 2002). Acid insoluble ash indicates contamination with silica, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles (Mukherjee, 2002). The roots of *B. vulgaris* also contain a measurable quantity of total phenolic and total flavonoid contents and hence can be used as a potent antioxidant plant. All these parameters, which are being reported, could be useful in identification of distinctiveness features of the crude drug and used for establishing quality of

Beet roots. In conclusion, the results obtained from phytochemical screening studies and physico-chemical parameters can be used to standardize roots of *B. vulgaris*.

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