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QUALITATIVE ANALYSIS OF ROOT OF *BETA VULGARIS L.*

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ABSTRACT:

A significant member of the Chenopodiaceous family is the therapeutic herb Beta vulgaris Linn. It is often referred to as garden beet or beet root. In Indian traditional medical practices, the root is primarily used to treat issues with the reproductive system, high blood pressure, cancer, and the urinary tract. The standardization of the herb's roots was deemed to be important due to the herb's widespread use in Indian traditional medical systems. The morphological and microscopic characteristics, physical-chemical parameters, such as plant extractive with different solvents, ash levels, foreign organic matter, loss on drying, and Ph of aqueous solution, were used as quality control measures. The present study deals with the phytochemical investigation on root of *Beta Vulgaris L* for presence of saponins, tannins, terpenoids, flavonoids, polyphenols, steroids etc. Extraction is performed using the Soxhelt extraction apparatus, as well as macro and microscopical parameters are studied. Various root sections were taken to investigate and photograph the anatomical properties. Physiochemical investigation revealed loss on drying (11.74±0.32 %), total ash (10.84±0.43), water soluble ash (1.70± 0.24), acid insoluble ash (0.21± 0.31), alcohol soluble extractive (11.21± 0.18), water soluble extractive (21.31± 0.35). The HPTLC technique was used for qualitative determination of components from methanolic extracts of root solvent system Toluene; Ethyl Acetate; Formic acid Volume 5:4:1 that revealed the Rf values for terpenoids, flavonoids, gallic acid, quercitine. The HPTLC technique of alcoholic extract showed the presence of six and seven spots at 254 nm and 366 nm. The study done will provide relevant data used for proper identification and authentication of used herbal plant.

KEYWORDS: *Beta Vulgaris L*, Ayurveda, Pharmacognostic, Physicochemical Evaluation, HPTLC.

I. INTRODUCTION:

Beetroot, often known as red beet, is the taproot of the beet plant (Beta vulgaris L.). In many countries around the world, including India, beetroot is a widely consumed vegetable. High concentrations of bioactive compounds in beetroot, such as betalains and inorganic nitrate, may have a variability of positive health effects.[1] Because it is a perishable vegetable, it can be dehydrated, and its mineral content can rise due to the loss of water mass. And it is heavy in both fiber and sugars. The colour of food can play an important part in lavor perception. Food colouring is classified as follows four classifications [2]

A. Natural Colour

B. Nature-Identical Inorganic Colour

C. Mayank is A Synthetic Colour

Betalains is a kind of pigments that are commonly used as natural food colorants used in several culinary industries. Beetroot is high in nutrients such as vitamins (B complex and C), minerals, fibre, proteins, and a diversity of bioactive phenolic substances, primarily betalains, and other antioxidant-rich components such as coumarins, carotenoids, sesquiterpenoids, triterpenes, and flavonoids. Beetroot has been employed as an ingredient or preservative in food processing due to its firmness, nontoxic, noncarcinogenic, and nonpoisonous properties. Beetroot and its biochemical components have been shown to have antioxidant, anti- inflammatory, antiapoptotic, antibacterial, antiviral, and other properties. [3]

Figure 1: Beet Plant

I. MATERIALS AND METHODS:

A. Collection of Plant Material:

The material was purchased from market of Ale, Junnar. The material was Identified and Authenticated from Botanist Dr Ranangdale Savita Sanjaykumar, M.Sc. Ph.D., FIAAT, FAA BSc,Department of Botany, Balasaheb Jadhav College of Art,Commerce & Science , Ale Junnar, Pune University Maharashtra with Herbarium collection number 619-*Beta Vulgaris*.Also authenticated by Dr.R.K Chaudhary,Scientist, Agharkar Research Institute, Autonomous Body under DST, GOI, Pune. Herbarium specimen has been preserved in laboratory voucher specimen specimen no.23-94 For Beta Vulgaris. [4]

B. Method:

- **Morphological and Microscopical evaluation**

  The root of *B.Vulgaris L* was examined for various organoleptic properties. These studies include parameters such as taste, odour, shape, margin, venation, size, surface and apex. The microscopically study of *B.Vulgaris L* was done with the help of Swift Ives camera lucida microscope. The air-dried plant material was then, pulverized into a coarse powder and used for research work. [5]

- **Determination of Physicochemical constants**:

  Physicochemical constants of B.Vulgaris L root were determined water soluble ash, total ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value as per the method described in Pharmacopoeias. [6]

- **Ultraviolet screening of leaf powder of *Beta Vulgaris L***

  The fluorescence study of powder with different reagents under ordinary day light and UV light (long & short) shows distinct characteristics fluorescence. [6]

III. PREPARATION OF EXTRACTS:

Beta Vulgaris L root were cleaned under running water and dried in the shade for seven days. Dried root were mechanically crushed to a coarse powder, sieved, and stored at room temperature in an airtight container. The extraction method was chosen based on the presence of active ingredients in the medicine. By using the soxhlet extraction method, dried powder (500 g) was extracted with Acetone, ethanol, methanol and distilled water. The extracts were dried by distilling the solvent at low temperatures with a rotary evaporator. The extracts were kept in a refrigerator at 4^0 C.

A. Phytochemical Screening:

The Phytochemical screening of the extracts were assessed to detect the presence of different phytoconstituents such as alkaloids, flavanoids, saponins, triterpenoids, steroids, carbohydrate, tannin, coumarins, phenols, carboxylic acid, amino acid and proteins by performing chemical tests.[9,10]

B. HPTLC analysis of extract [11-16]

HPTLC analysis of methanolic extract of root of *Beta Vulgaris L* was done by lane analysis. HPTLC analysis was done to access presence of components.

C. TLC instrumentation and conditions

- **Sample Preparation**: Sample Dissolved in methanol & incubated over night for 24 hrs to 48 hrs followed by concentrating the sample by Rotary evaporator method.
- **Sample loading:** About 5 µl of extract of Beta Vulgaris L is diluted with methanol and standard solution of Quercetin and Gallic acid were loaded as 6.0 mm 60F 254 TLC plate with use of

Hamilton Syringe.

- **Scanning:** TLC developed was dried to evaporate solvent and then placed in photo documentation chamber and images were captured at 254nm and 366nm.
- **Band Size** : 5mm
- **Analysis Type :** Lane Analysis
- **Seperation Technique**: Ascending
- **Test :** Methanolic extract of Beta Vulgaris L
- **Standards:** Quercetin, Gallic acid
- **Mobile Phase**: Toluene; Ethyl Acetate; Formic Acid (5:4:1)

IV. RESULTS AND DISCUSSION MICROSCOPIC CHARACTERS:

The transverse section of Beta Vulgaris L in Figure 1(a, b, c, d, e, f) displays deep red-colored cell vacuoles of the cortical parenchyma and the pith. The conductive vessel and rhizodermis are colourless. Starch is absent from the roots of sugar beetroot and red beetroot. A secondary structure of roots is present, comprising a concentric circle of conductive tissues that are created from cells with cellulose walls and are penetrated by large rays of parenchyma. The cross section of the beetroot revealed a well-represented xylem vessel, with the root centre (to pith) showing evidence of proto-xylem and outward association with meta-xylem. Under the phloemis cambium, supplied by the pericycle, pith, or pith rays. This cambium, which produces more secondary xylem than secondary phloem, is responsible for the majority of the root thickness. 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.

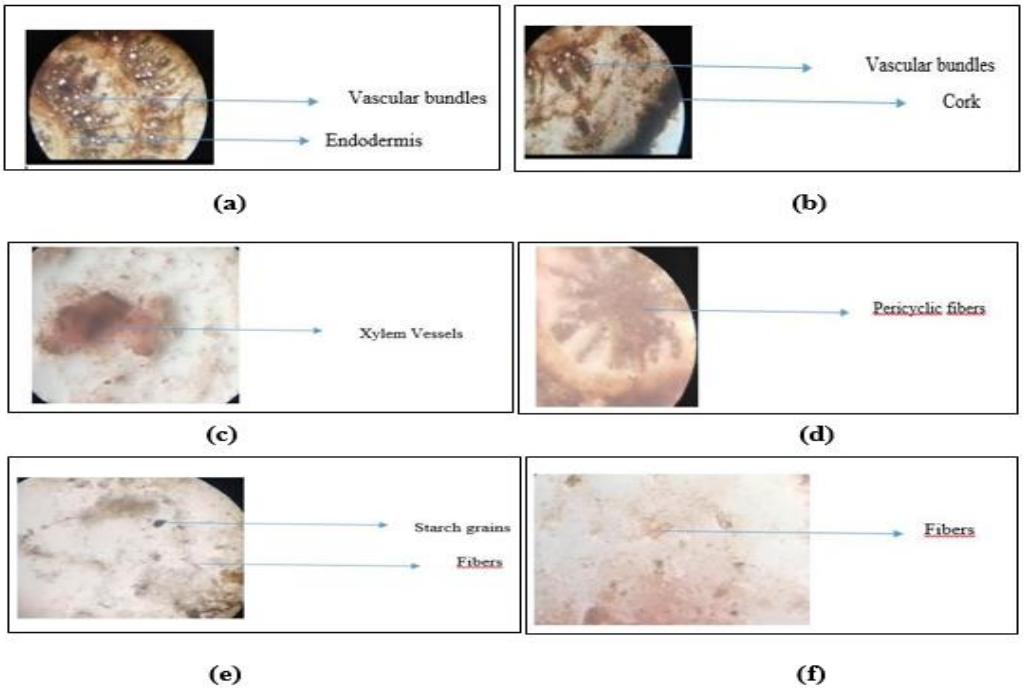

Figure 2: Microscopic Evaluation of *Beta Vulgaris L* Root

Table 1: Description of *Beta Vulgaris root*

Plant	An erec, sprawling perennial plant up to 60 cm (2 ft) high
Root	Dark green, leathery, shiny rosette root with wavy & rough triangular lower root and narrow and oval upper root. grow 20–40 cm (7.9–15.7 in) in length
Fruit	enclosed by the leathery and incurved perianth, and is immersed in the swollen, hardened perianth base
Root	swollen and fleshy long main red root
Flowers	green and tiny with the sepals thickening and hardening reach 1–2 m (3.3–6.6 ft) in height
Seed	The horizontal seed is lenticular, 2–3 mm, with a red-brown, shiny seed coat. The seed contains an annular embryo and copious perisperm

a. Ultraviolet screening of *Beta Vulgaris L* root powder

The fluorescence study of powder with different reagents under ordinary day light and UV light shows distinct characteristic fluorescence

Table 2: UV fluorescence studies of root powder of *Beta Vulgaris L*

Powder + reagent	Ordinary light	UV short wave (254nm)	UV long wave (365nm)
Only Powder	Dark red	Green	Dark Green
Powder + 5 % NaOH	Green	Dark green	Yellowish Brown
Powder +Chloroform	Yellow	Red	Green
Powder +1% KOH	Reddish yellow	Blue	Dark blue
Powder +conc. HNO3	Pale yellow	Green	Black
Powder +H2SO4	Brown	Dark brown	Brown
Powder +conc.HCl	Purple	Blue	Blue
Powder + Acetone	Light green	Red	Light green
Powder + FeCl3	Black	Blue	Blue
Powder + H2SO4	Light brown	Brown	Brown

b. Physical Analysis of Root powder of *Beta Vulgaris*

Various physical analyses are done for various parameters like foreign organic matter, loss on drying, ash content, extractive values.

Table 3: Physical Analysis of Root powder of Beta Vulgaris

Sr no	Parameters	Results
1	Foreign Organic matter	0.74 % (w/w)
2	LOD 110^0C	11.74 % (w/w)
3	Total Ash Content	10.84 % (w/w)
4	Water soluble ash	1.70 % (w/w)
5	Acid insoluble Ash	0.21 % (w/w)
6	Ethanol extractive value	11.21% (w/w)
7	Aqueous extractive value	21.31% (w/w)
8	pH	7.1

c. Extraction of root of *Beta Vulgaris L*

The % extraction yield of in aqueous, Ethanol, Acetone and Methanol are 23.61% w/w, 21.49 % w/w, 9.7% w/w and 25.37% w/w respectively. Figure 2 indicates that % w/w yield of root extract is higher in methanolic extract.

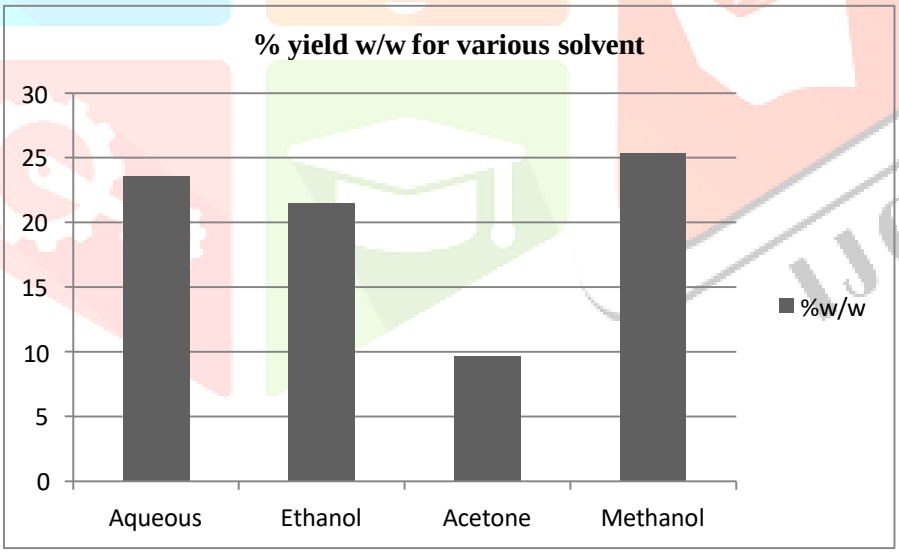

Figure 3: Extraction yield percentage in different solvents

d. Phytochemical Screening of *Beta Vulgaris L.*

Preliminary phytochemical screening of various extract of *Beta Vulgaris* linn root revealed phytoconstituents i.e. Alkaloids, Glycosides, Saponins, Phytosterols ,Tannins, Flavonoids, Carbohydrates, Proteins & amino acids, Fixed oils & fats, Gums and Mucilage, Volatile oil. Methanolic extracts shows the presence of most numbers of components Methanolic root extract has shown that it has extracted most of the compounds. Table clearly indicates clearly that methanol can be used as a principle extracting solvent and also evaporation of methanol is easy phytochemical constituents detected in crude extracts of root of *Beta Vulgari L*

Table 4: Phytochemical screening of *Beta Vulgaris* root extrac

Sr. No.	Tests	Aqueous	Ethanol	Methanol	Acetone
1	**Tests for Alkaloids**				
	Mayer's test	-	+	+	-
	Wagner's test	-	+	+	-
	Hager's tests	-	+	+	-
	Dragendorff's test	-	+	+	-
2	**Tests for Carbohydrates**				
	Molish test	+	+	+	-
	Fehling test	+	+	+	-
	Barfoed's test	+	+	+	-
	Benedict's test		+	+	-
3.	**Tests for Glycosides**				
	Borntrager's test	+	+	+	+
	Legal's test	+	+	+	+
4.	**Test for Saponins**				
	Test solution+20ml distilled H20	+	+		+
5.	**Tests for Proteins & amino acids**				
	Millon's test		-		
	Biuret test		-		
	Ninhydrin test		-		
6.	**Test for Phytosterol**				
	Libermann-Burchard's test	+		+	
7.	**Tests for Fixed oils & fats**				
	Spot test				
	Saponification test				
8.	**Tests for Tannins**				
	Ferric chloride test	+		+	+
	Gelatin test	+		+	+
	Aqueous bromine test	+		+	+
9.	**Tests for Flavonoids**				
	Lead acetate	+	+	+	+
	Alkaline reagent test	+	+		
10.	**Test for Gums &Mucilages**				
	Ext. + dis. H_2O +abs. alc. + stirring				
11.	**Test for Volatile oil**				
	50 gm. of powder subjected to hydro-distillation	-	-	-	-

Various tests were performed to find phytochemical constituents present in extracted material of different solvents. Various tests have been performed to find out the phytochemical constituents. Methanolic extracts shows the presence of most numbers of components.

Methanolic root extract has shown that it has extracted most of the compounds. Above table clearly indicates clearly that methanol can be used as a principle extracting solvent and also evaporation of methanol is easy.

e. HPTLC analysis of Methanolic extract

Phytochemical profiling and quantification of Quercetin and Gallic acid in the methanolic extract of root of *Beta Vulgaris L* was studied and results were obtained in the form chromatograms depicted in figures 3and 4. Chromatograms from standards and test samples were obtained by CAMAG TLC scanner III at short (254 nm) (fig. 3) and long (366 nm) (fig.4) wavelength.

Table 4: HPTLC details of methanolic extract of root *of Beta Vulgaris L*

Band number	Rf Value		Assigned substances
	254 nm	366 nm	
1	0.526,0.265,0.126,0.210	0.692,0.542,0.471, 0.238	Methanolic extract of *Beta Vulgaris L* root
2	0.202	0.238,0.455, 0.452,	Gallic acid
3	0.526,0.412,	0.551,0.452	Quercetin
4	0.455	0.455	Flavanoids

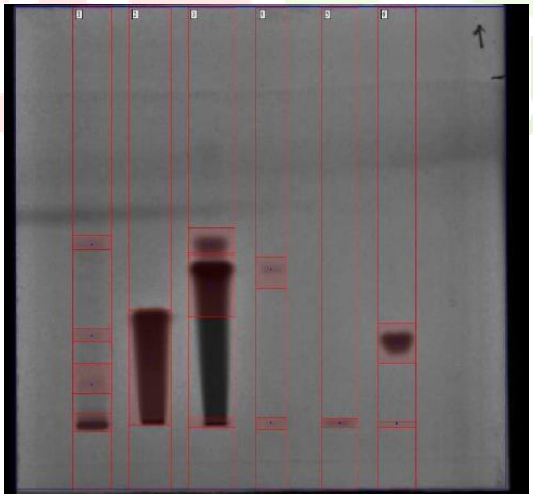

Figure 4-image of TLC plate at 254nm

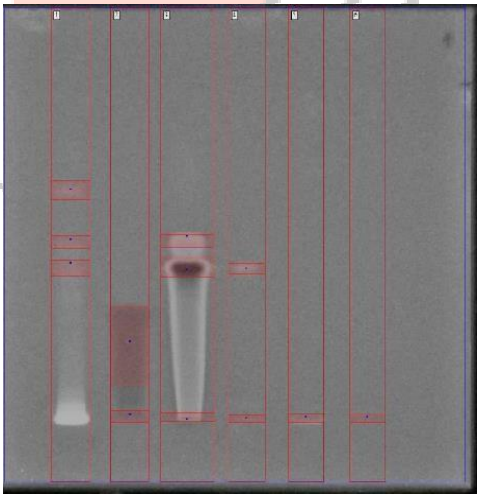

Figure 5-image of TLC plate at 366nm

Figure 4 and 5 indicates the presence of Gallic Acid and Quercetin in the methanolic extract of root of *Beta Vulgaris L.*

V. CONCLUSION:

The current study advances science by making pioneering preliminary findings regarding physicochemical properties, the presence of useful constituents through HPTLC, microscopic diagnostic characteristics through powder microscopy, and sufficient scientific material to initiate future studies As HPTLC phytochemical profiling reveals the presence of several bioactive compounds like Quercetin and gallic acid in the methanolic extract of Beta Vulgaris reveals that can be further explored up to their identification and future application in pharmacological treatment.

VI. FUTURE SCOPE:

The current study may contribute to research pioneering preliminary study with respect to pharmacognostical physiochemical, phytochemical and advanced parameters like HPTLC so that benefits of Beetroot reaches out their therapeutic values.

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