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Extraction, Phytochemical Screening and Potential Applications of Natural Pigments from Beetroot (*Beta vulgaris L.)*

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Abstract: *Beta vulgaris* (beetroot) is a nutritionally rich root vegetable known for its diverse bioactive compounds and therapeutic potential. The present study aims to perform preliminary phytochemical screening of the ethanolic root extract of *Beta vulgaris* using standard qualitative methods.

The extract was subjected to various phytochemical tests, which confirmed the presence of major bioactive constituents such as carbohydrates, phenolic compounds, flavonoids, saponins, and betalains, while alkaloids and terpenoids were absent or detected in trace amounts. Betalains, the characteristic red-violet pigments of beetroot, are well recognized for their strong antioxidant properties.

The presence of phenolic compounds and flavonoids further indicates significant antioxidant potential of the extract. These findings highlight the potential application of beetroot as a natural source of bioactive compounds in food, pharmaceutical, and nutraceutical industries. The study supports its traditional use and emphasizes its role in health promotion and disease prevention.

Keywords: *Beta vulgaris, Phytochemical Screening, Betalains, Phenolic Compounds, Flavonoids, Antioxidant Activity, Natural Pigments.*

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I. INTRODUCTION

Beta vulgaris, commonly known as beetroot, is a root vegetable widely consumed for its nutritional and medicinal value. It belongs to the family Amaranthaceae and is cultivated in many parts of the world. Beetroot is rich in carbohydrates, vitamins (such as vitamin C and folate), minerals (including iron, potassium, and magnesium), and dietary fiber (USDA, 2019).

Beyond its nutritional importance, beetroot contains several bioactive phytochemicals, including betalains, phenolic compounds, flavonoids, tannins, saponins, and glycosides. Betalains are nitrogen-containing water-soluble pigments responsible for the red-violet color of beetroot and are known for their strong antioxidant properties (Loponen et al., 2000; Georgiev et al., 2010). Phenolic compounds and flavonoids play a crucial role in scavenging free radicals and reducing oxidative stress, thereby contributing to disease prevention (Kujala et al., 2000; Khan, 2016).

Previous studies have reported that beetroot exhibits antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities due to its phytochemical constituents (Clifford et al., 2015; Tesoriere et al., 2004). These biological activities make beetroot a promising natural source of therapeutic agents.

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Phytochemical screening is a preliminary analytical technique used to identify the presence of various secondary metabolites in plant extracts. It provides a scientific basis for validating the traditional medicinal uses of plants (Harborne, 1998; Trease & Evans, 2009; Sofowora, 2008).

Therefore, the present study aims to perform qualitative phytochemical screening of beetroot root extract to identify the major bioactive compounds present in it and to evaluate its potential as a natural source of antioxidants.

II. MATERIALS AND METHODS

➢ *Required Materials*

- *Plant Material*

Fresh beetroot roots (*Beta vulgaris*) were procured from the local market and authenticated based on morphological characteristics (Sofowora, 2008).

Fig 1 Fresh Roots of *Beta vulgaris* Used for Extraction.

- *Chemicals and Reagents*

All chemicals used were of analytical grade. The following reagents were employed for extraction and phytochemical screening (Harborne, 1998; Trease & Evans, 2009):

✓ Ethanol
✓ Distilled water
✓ Hydrochloric acid (HCl)
✓ Sulfuric acid (H_2SO_4)
✓ Glacial acetic acid
✓ Sodium hydroxide (NaOH)
✓ Ferric chloride ($FeCl_3$)
✓ Chloroform

- *Specific Reagents for Phytochemical Tests*

✓ Wagner's reagent (alkaloids detection)
✓ Hager's reagent
✓ Fehling's solution (reducing sugars)
✓ Benedict's reagent
✓ Molisch's reagent
✓ Keller–Killiani reagent (glycosides detection)

- *Glassware*

✓ Beakers (50 mL, 100 mL, 250 mL)
✓ Conical flasks
✓ Test tubes
✓ Measuring cylinder
✓ Pipettes
✓ Funnel
✓ Glass rod
✓ Watch glass

- *Equipment*

✓ Mechanical grinder
✓ Weighing balance
✓ Water bath
✓ Filter paper (Whatman No.1)
✓ Test tube holder
✓ Hot plate

III. METHODOLOGY

➢ *Collection of Plant Material*

Fresh roots of *Beta vulgaris* were collected from the local market. The samples were washed thoroughly with running tap water followed by distilled water to remove soil and other impurities.

➢ *Drying and Powder Preparation*

The cleaned beetroot roots were cut into small pieces and shade-dried at room temperature (25–30°C) for 5–7 days. The dried material was ground into fine powder using a mechanical grinder and stored in an airtight container for further analysis.

➢ *Preparation of Extract*

Approximately 20 g of powdered beetroot sample was soaked in 100 mL of ethanol in a conical flask. The mixture was kept at room temperature for 48 hours with occasional shaking (maceration method) as described by Harborne (1998).

After extraction, the mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated using a water bath at 40°C to obtain a semi-solid extract. The extract was stored at 4°C until further use.

➢ *Preliminary Phytochemical Screening*

The ethanolic extract was subjected to qualitative phytochemical tests to detect various bioactive compounds following standard phytochemical procedures (Harborne, 1998; Trease & Evans, 2009; Sofowora, 2008).

- *Test for Betalains*

Dilute HCl was added to the extract.

✓ *Observation:*

The red-violet color remained stable. On heating, the color intensity slightly decreased.

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This test confirms the presence of betalain pigments (Georgiev et al., 2010).

- *Test for Saponins (Foam Test)*

The extract was shaken vigorously with distilled water.

✓ *Observation:*

Formation of stable persistent foam lasting for 10–15 minutes indicated the presence of saponins (Sofowora, 2008).

- *Test for Tannins*

One milliliter of extract was mixed with 1 mL of 5% $FeCl_3$ solution.

✓ *Observation:*

Dark blue or greenish-black coloration indicated the presence of tannins (Harborne, 1998).

- *Test for Quinones*

One milliliter of extract was treated with 1 mL of concentrated H_2SO_4.

✓ *Observation:*

Red coloration indicated the presence of quinones (Trease & Evans, 2009).

- *Test for Flavonoids (Alkaline Reagent Test)*

One milliliter of extract was mixed with 1 mL of 2N NaOH.

✓ *Observation:*

Yellow coloration indicated the presence of flavonoids (Harborne, 1998).

- *Test for Glycosides*

One milliliter of extract was mixed with 3 mL of chloroform followed by addition of 10% ammonium solution.

✓ *Observation:*

Pink coloration indicated the presence of glycosides (Trease & Evans, 2009).

- *Test for Phenols*

One milliliter of extract was mixed with 2 mL distilled water and a few drops of 10% $FeCl_3$ solution.

✓ *Observation:*

Blue-green coloration confirmed the presence of phenolic compounds (Harborne, 1998).

- *Test for Coumarins*

One milliliter of extract was treated with 1 mL of 10% NaOH.

✓ *Observation:*

Yellow coloration indicated the presence of coumarins (Sofowora, 2008).

- *Test for Anthocyanins and Betacyanins*

One milliliter of extract was mixed with 1 mL NaOH and heated for 5 minutes in a boiling water bath.

✓ *Observation:*

- Bluish-green color indicated anthocyanins.
- Yellow coloration indicated betacyanins (Georgiev et al., 2010).

- *Test for Carbohydrates*

✓ *Molisch's Test*

Two milliliters of extract were mixed with two drops of Molisch's reagent. Concentrated H_2SO_4 was added along the side of the test tube.

- *Observation:*

Formation of a violet ring at the junction confirmed carbohydrates (Harborne, 1998).

✓ *Fehling's Test*

Equal volumes (1 mL each) of Fehling's solution A and B were mixed with 1 mL extract and heated in a boiling water bath for 10 minutes.

- *Observation:*

Brick-red precipitate indicated reducing sugars (Trease & Evans, 2009).

✓ *Benedict's Test*

Equal volumes of Benedict's reagent and extract were mixed and heated for 5–10 minutes.

- *Observation:*

Color change from green to yellow to red indicated reducing sugars.

- *Test for Alkaloids*

✓ *Hager's Test*

A few drops of Hager's reagent were added to 1 mL of filtrate.

- *Observation:*

Yellow precipitate indicated alkaloids (Sofowora, 2008).

✓ *Wagner's Test*

Two milliliters of Wagner's reagent were added to 1 mL of filtrate.

- *Observation:*

Reddish-brown precipitate confirmed the presence of alkaloids (Trease & Evans, 2009).

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Table 1. Qualitative Phytochemical Screening of *Beta vulgaris* Extract

Bioactive Compounds	Result	Biological Significance
Betalains	Positive	Natural pigment, antioxidant
Flavonoids	Positive	Free radical scavenging
Phenolic compounds	Positive	Strong antioxidant activity
Saponins	Positive	Antimicrobial activity
Glycosides	Positive	Cardioprotective effects
Quinones	Positive	Antioxidant activity
Coumarins	Positive	Anti-inflammatory activity
Carbohydrates	Positive	Energy source
Alkaloids	Negative	-

The qualitative screening indicates that beetroot is rich in secondary metabolites responsible for antioxidant and potential therapeutic activities. The absence of alkaloids suggests that its biological effects are primarily associated with phenolic and betalain constituents rather than nitrogen-containing alkaloidal compounds.

Fig 2 Qualitative Phytochemical Screening of Ethanolic Extract of *Beta vulgaris* Showing Characteristic Color Changes Indicating the Presence of Betalains, Tannins, Flavonoids, Phenolic Compounds, Quinones, and Saponins.

IV. RESULTS AND DISCUSSION

The preliminary phytochemical screening of the ethanolic extract of *Beta vulgaris* revealed the presence of several important bioactive constituents, including flavonoids, phenolic compounds, tannins, carbohydrates, saponins, quinones, glycosides, coumarins, and betalains, while alkaloids were absent or present only in trace amounts.

The presence of phenolic compounds and flavonoids indicates strong antioxidant potential, as these compounds are well known for their free radical scavenging activity. Betalains, the characteristic red-violet pigments of beetroot, also contribute significantly to antioxidant activity and are reported to exhibit anti-inflammatory and anticancer properties.

The detection of saponins and glycosides suggests possible pharmacological activities such as antimicrobial and cardioprotective effects. The absence of alkaloids indicates that the biological activity of beetroot is mainly associated with non-alkaloidal phytoconstituents, particularly phenolics and betalains.

The results obtained in this study are consistent with previously reported literature, confirming that *Beta vulgaris* is a rich source of natural antioxidants and bioactive compounds. These findings support the potential use of beetroot extract in nutraceutical and therapeutic applications.

➢ *Applications of Beetroot Pigments*

Beetroot pigments, particularly betalains, have gained significant attention due to their natural origin and wide range of applications.

- *Food Industry:*

Betalains are used as natural food colorants as they are non-toxic and safe alternatives to synthetic dyes.

- *Pharmaceutical Applications:*

The antioxidant, anti-inflammatory, and anticancer properties of beetroot extracts make them useful in the development of therapeutic formulations.

- *Nutraceutical Uses:*

Beetroot is widely used in dietary supplements due to its health-promoting properties and ability to reduce oxidative stress.

- *Cosmetic Industry:*

Natural pigments from beetroot are used in cosmetic formulations as coloring agents and skin-benefiting compounds.

- *Textile Industry:*

Beetroot pigments can be used as eco-friendly natural dyes for fabrics, reducing environmental pollution caused by synthetic dyes.

V. CONCLUSION

The present study confirms that *Beta vulgaris* is a rich source of bioactive phytochemicals, including flavonoids, phenolic compounds, saponins, glycosides, and betalains. The presence of these compounds indicates significant

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antioxidant potential and supports its traditional medicinal use.

The study highlights the importance of beetroot as a natural and sustainable source of bioactive compounds with potential applications in food, pharmaceutical, nutraceutical, cosmetic, and textile industries.

Further quantitative analysis and detailed pharmacological studies are recommended to explore its full therapeutic potential. The study also highlights the potential of beetroot pigments as eco-friendly alternatives to synthetic dyes."

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