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Qualitative Analysis of Secondary Metabolites in Beetroot Extract (*Beta vulgaris L.*) through Phytochemical Screening

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ABSTRAK

Beetroot (*Beta vulgaris L.*) is a plant rich in bioactive compounds and has significant potential for development in the pharmaceutical field as a natural antioxidant source and raw material for health related products. Secondary metabolites such as flavonoids, tannins, saponins, and alkaloids play an important role in providing biological activities; therefore, their identification is necessary to determine their pharmacological potential. However, scientific information regarding the profile of secondary metabolites in beetroot extract through phytochemical screening remains limited and requires further investigation. This study aimed to analyze the presence of secondary metabolites in beetroot (*Beta vulgaris L.*) extract through qualitative phytochemical screening. The research employed a laboratory experimental method, with extraction carried out using the maceration technique for three days with 96% ethanol as the solvent. A total of 200 grams of beetroot simplicia were extracted to obtain a concentrated extract, which was subsequently subjected to phytochemical analysis. The screening included the identification of flavonoids, tannins, saponins, alkaloids, steroids-terpenoids, and betacyanin, conducted in triplicate with one blank sample. The results indicated that the beetroot extract positively contained flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and betacyanins. These findings demonstrate the presence of various secondary metabolites in beetroot extract, which may contribute to its biological activities and support further studies to evaluate its potential applications in the pharmaceutical field.

Keywords: *Beta vulgaris*, secondary metabolites, phytochemical screening, bioactive compounds

INTRODUCTION

Beetroot (Beta vulgaris L.) is a horticultural plant that has been increasingly utilized in the health sector due to its rich content of bioactive compounds with potential pharmacological effects. The characteristic purplish-red color of beetroot is derived from betalain pigments, which are known to possess strong antioxidant activity (Utami & Farida, 2022). In addition, beetroot contains various nutrients such as vitamins, minerals, fiber, as well as secondary metabolites that play an important role in maintaining overall health (D. Rahayu et al.,

2024). With the growing public interest in the use of natural products as alternative medicine and raw materials for health-related products, research on the active compound content of beetroot has become increasingly important to be further explored.

The utilization of plants as sources of bioactive compounds has been widely explored in the pharmaceutical field, primarily because plants contain secondary metabolites that can exert various biological activities. Secondary metabolites are organic compounds produced by plants that do not play a direct role in growth processes (Hersila et al., 2023), but function in adaptation and defense against environmental stress. Compounds such as flavonoids, alkaloids, tannins, and saponins are known to exhibit diverse pharmacological activities, including antioxidant, antibacterial, anti-inflammatory, antidiabetic, and anticancer effects (Asrifaturofingah et al., 2024). Therefore, the identification of secondary metabolites through phytochemical screening represents an essential initial step in the development of natural products as sources of medicinal agents and other health-related products.

Flavonoids are a group of phenolic compounds known for their strong antioxidant activity due to their ability to scavenge free radicals in the body (Rahayu et al., 2022). Alkaloids exhibit potential as antimicrobial and analgesic agents, while tannins function as antibacterial and astringent compounds that can support wound healing processes (Rangga et al., 2025). Saponins are recognized for their activities as immunostimulants, antifungal agents, and cholesterol-lowering compounds (Ilyas et al., 2025). The presence of these compounds in a plant can serve as an indicator of its pharmacological potential. Therefore, qualitative analysis of secondary metabolites is necessary to determine the presence of active compounds in plant extracts, including beetroot.

In the pharmaceutical field, beetroot has been widely utilized as a natural ingredient in various health and cosmetic products. The antioxidant content of beetroot has potential applications in cosmetic formulations such as masks, serums, and anti-aging creams, as it helps protect the skin from damage caused by free radicals (Farika et al., 2024). In addition, beetroot extract is used as a natural colorant in pharmaceutical and food preparations due to its higher safety profile compared to synthetic dyes (Lembong & Utama, 2021). Several studies have also reported that beetroot exhibits antihypertensive and hepatoprotective activities, as well as the ability to improve blood circulation, indicating its potential for development as a raw material for herbal medicines and dietary supplements (Alizar, 2020).

Previous studies on beetroot have been widely conducted, particularly focusing on its antioxidant activity and betalain pigment content. However, research specifically addressing the identification of secondary metabolites through phytochemical screening of beetroot extract remains limited and requires further development. Several studies have reported that beetroot extract contains flavonoids, tannins, and saponins; however, the results may vary depending on the extraction method, type of solvent, and identification

conditions applied (Dermawan et al., 2023). Therefore, further studies are needed to provide comprehensive scientific information regarding the secondary metabolite content of beetroot extract through qualitative phytochemical screening.

This study is expected to contribute to the advancement of knowledge in the pharmaceutical field, particularly in the utilization of natural products as sources of bioactive compounds. The findings may serve as supporting data for the development of traditional medicines, herbal cosmetics, and health products derived from beetroot. Furthermore, this study can provide a scientific reference for future researchers interested in exploring the potential applications of beetroot in pharmaceutical and health-related fields.

Based on the aforementioned description, this study aims to analyze the secondary metabolite content in beetroot (*Beta vulgaris L.*) extract through qualitative phytochemical identification, including the detection of flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and betacyanin.

METHODS

This study was a laboratory-based experimental research aimed at identifying the presence of secondary metabolites in beetroot (Beta vulgaris L.) extract through qualitative phytochemical screening. Fresh beetroot samples (4 kg) were obtained from a local market. The samples underwent wet sorting, washing, cutting, and drying to obtain dried simplicia. A total of 200 grams of simplicia was used for the extraction process.

Extraction was carried out using the maceration method with 96% ethanol as the solvent for three days at room temperature with periodic stirring. After maceration, the filtrate was filtered and concentrated using a rotary evaporator to obtain a thick beetroot extract. The resulting extract was then used as the sample for phytochemical screening.

Phytochemical screening was conducted qualitatively to identify flavonoids, tannins, saponins, alkaloids, steroids-terpenoids, and betacyanin. Each test was performed in triplicate to ensure data validity, and one blank sample consisting of extract and ethanol without reagents was used as a control.

The screening procedures were as follows: flavonoid identification was performed by adding 5 drops of concentrated HCl to 2 mL of extract. Tannin identification was conducted by adding 5 drops of $FeCl_3$ solution to 2 mL of sample. Saponin identification involved dissolving 0.5 grams of extract in 10 mL of distilled water followed by vertical shaking for 10 seconds. Alkaloid identification was carried out by dissolving 0.5 grams of extract in 1 mL of 2N HCl and 9 mL of distilled water, followed by heating and filtration; the filtrate was then treated with 5 drops of Dragendorff's reagent. Betacyanin identification was performed by adding 2.5 mL of 2N HCl and NaOH 2M to 0.5 grams of extract. Meanwhile,

steroid and terpenoid identification was conducted by adding a mixture of 1 mL chloroform, 1 mL acetic acid, and 1 mL H_2SO_4 to 0.5 grams of extract.

The results of the phytochemical screening were observed based on color changes, precipitate formation, or foam formation as indicators of the presence of secondary metabolites. The data obtained were analyzed descriptively and qualitatively and presented in Table 1.

Table 1. Positive Indicators of Phytochemical Identification

Identification	**Reagent**	**Positive Indicator**
Flavonoid	Concentrated HCl	Formation of red, orange, or yellow color
Tannin	$FeCl_3$	Greenish-black or dark blue color
Alkaloid	Dragendorff	Formation of orange precipitate
Saponin	Distilled water (Aquadest)	Formation of stable foam ±10 cm
Steroid	Chloroform + acetic acid + H_2SO_4	Green/blue color
Terpenoid		Red or purple color
Betacyanin	HCl 2N + NaOH 2M	Color change from red (acidic) to yellow/brownish (basic)

RESULTS AND DISCUSSION

The conducted study resulted in the yield of ethanol extract of beetroot used as the sample. The dried simplicia underwent dry sorting to select materials that met the criteria, namely being completely dry and intact. Based on this process, an ethanol extract of beetroot was obtained from 200 grams of simplicia, yielding a brownish-colored thick extract with a percentage yield of 25%. The obtained extract was subsequently subjected to phytochemical screening to determine the presence of secondary metabolite compounds in beetroot. The extraction yield results are presented in Table 2.

Table 2. Yield of Ethanol Extract of Beetroot

Powder Weight	**Thick Extract Weight**	**Yield**
200 grams	50 grams	25%

Based on the results presented in Table 2, the extraction yield is considered relatively high, indicating that beetroot contains a substantial amount of soluble compounds. The high yield may be influenced by the type of solvent used (Wijaya & Satriawan, 2023), as 96% ethanol is capable of dissolving a wide range of secondary metabolites, both

polar and semi-polar, such as flavonoids, tannins, and saponins. In addition, the maceration method carried out for three days with periodic stirring allows optimal contact between the solvent and the simplicia, thereby enhancing extraction efficiency. Extraction yield is also affected by several other factors, including the particle size of the plant material, solvent-to-sample ratio, extraction duration, temperature, moisture content of the raw material, and the chemical composition of the plant matrix. Smaller particle sizes provide a larger surface area for solvent penetration, while an adequate solvent volume and longer extraction time can improve the diffusion of bioactive compounds into the solvent. Therefore, the relatively high yield obtained in this study may result from the combined effects of the extraction conditions and the intrinsic characteristics of beetroot.

The results of the qualitative identification of secondary metabolites in beetroot (Beta vulgaris L.) extract are presented in Table 3.

Table 3. Qualitative Identification of Secondary Metabolites in Beetroot Extract

Secondary Metabolite	Reagent	Result	Description
Flavonoid	HCl	+	Formation of brick-red color
Tannin	$FeCl_3$	+	Color change to blue or greenish-black
Saponin	Distilled water (Aquadest)	+	Formation of stable foam ±1–10 cm
Alkaloid	Dragendorff	+	Formation of orange precipitate
Steroid	Chloroform + acetic anhydride + H_2SO_4	-	—
Terpenoid		+	Red/purple color formation
Betacyanin	HCl + NaOH	+	Color change from red to yellow-orange

Note:
(+) Positive
(−) Negative

Based on the results of flavonoid identification presented in Table 3, a color change to brick red was observed after the addition of the reagent, indicating a positive result (Indah et al., 2020). Flavonoids are phenolic compounds widely found in plants and are known to exhibit antioxidant, anti-inflammatory, and antibacterial activities (Wardani et al., 2020). The presence of flavonoids in beetroot extract suggests its potential application in the pharmaceutical field, particularly as a protective agent against oxidative stress that may lead to various degenerative diseases. The formation of the brick-red color is shown in Figure 1.

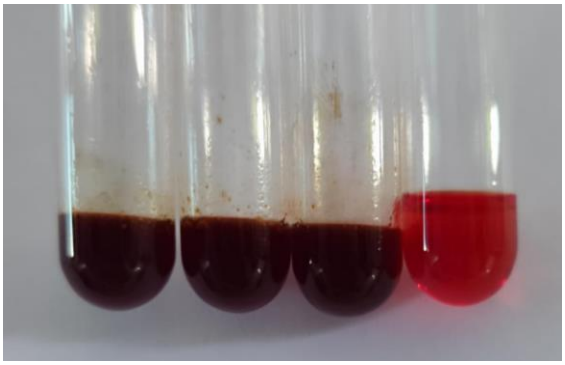

Figure 1. Flavonoid Identification Result

Based on the identification results shown in Figure 1, the addition of concentrated HCl produced a color change to brick red, indicating a positive result. The reaction mechanism is related to the nature of flavonoids as phenolic compounds that can undergo protonation under strongly acidic conditions (Nainggolan et al., 2024). he addition of concentrated HCl leads to the formation of flavilium salts, which produce characteristic red or orange coloration (Mustariani & Hidayanti, 2021). In some methods, this reaction is enhanced by the addition of metals such as magnesium (Shinoda test); however, in this study, HCl acts as an acidifying agent that induces structural changes in flavonoids, resulting in the observed characteristic color indicator.

In the tannin identification, the formation of a blue or greenish-black color after the addition of $FeCl_3$ solution indicates a positive result for the presence of tannins, as shown in Figure 2. Tannins are a group of polyphenolic compounds known for their ability to bind proteins and act as astringents (Kasih et al., 2022). In the health field, tannins are recognized for their antibacterial activity and their role in supporting wound healing processes as well as providing protection against microbial infections. The presence of tannins in beetroot extract further enhances the biological potential of this plant.

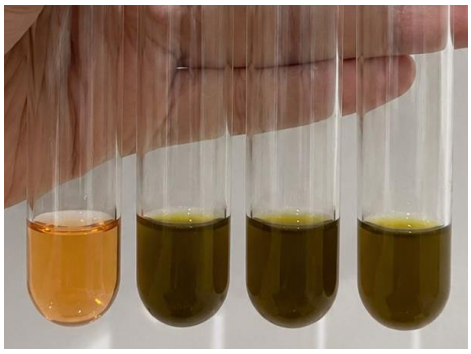

Figure 2. Tannin Identification Result

Based on Figure 2, a positive result is indicated by a color change to blue or greenish-black. The mechanism involved is the formation of a complex between Fe^{3+} ions from $FeCl_3$ and

the phenolic (-OH) groups present in the tannin structure (Rorong, 2015). This complex produces a characteristic dark color, which may vary depending on the type of tannin present, whether hydrolyzable or condensed tannins. Therefore, $FeCl_3$ functions as a complexing agent that is sensitive to the presence of phenolic groups in tannins.

Saponin identification showed a positive result, indicated by the formation of stable foam after shaking with distilled water, as presented in Figure 3. Saponins are compounds with natural surfactant properties, enabling them to produce foam when shaken in aqueous solutions (Nadir et al., 2021). Pharmacologically, saponins are known to exhibit immunostimulant and antimicrobial activities, as well as the potential to reduce cholesterol levels. The presence of saponins in beetroot extract indicates its potential application in the health and pharmaceutical industries.

Figure 3. Saponin Identification Result

Based on Figure 3, the formation of stable foam after shaking with distilled water indicates a positive result. This mechanism does not involve a specific chemical reaction but is based on the physicochemical properties of saponins as natural surfactants (Hawa et al., 2023). Saponins possess an amphiphilic structure, consisting of both hydrophilic and hydrophobic parts, which enables them to reduce the surface tension of water and form stable foam upon agitation. Therefore, distilled water in this test functions as a medium to observe foam formation as an indicator of saponin presence.

In the alkaloid identification, the formation of an orange precipitate after the addition of Dragendorff's reagent indicates a positive result, as shown in Figure 4. Alkaloids are nitrogen-containing compounds known for various biological activities, including analgesic, antimicrobial, and effects on the nervous system (Zahrani et al., 2025). The presence of alkaloids in beetroot extract suggests that this plant may possess broader pharmacological potential, although further studies are required to identify the specific types of alkaloids present.

Figure 4. Alkaloid Identification Result

Based on Figure 4, Dragendorff's reagent contains potassium bismuth iodide, which reacts with alkaloid compounds that are basic in nature and contain nitrogen atoms (Ergina et al., 2024). The mechanism involves the formation of an ionic complex between the alkaloids and the metal ions in the reagent, which subsequently precipitates as an orange-colored compound. Therefore, Dragendorff's reagent functions as a specific precipitating agent for detecting the presence of alkaloids in the extract.

The results of steroid-terpenoid identification showed a color change to red or purple for terpenoids, as presented in Figure 5. This indicates that the beetroot extract contains terpenoid compounds, while steroid compounds were not detected. Terpenoids are known to exhibit various biological activities, such as antibacterial and antioxidant effects. The presence of these compounds further strengthens the potential of beetroot as a natural source that can be developed in the pharmaceutical and health fields.

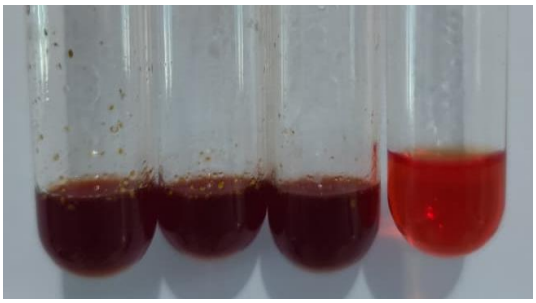

Figure 5. Steroid and Terpenoid Identification Result

Based on Figure 5, the use of a mixture of chloroform, acetic anhydride, and concentrated sulfuric acid resulted in a color change to blue or green for steroids and red or purple for terpenoids. This reaction is known as the Liebermann–Burchard test. The mechanism involves oxidation reactions and the formation of colored complexes due to the interaction between sterol or terpenoid compounds and a strong acid (H_2SO_4) in an organic medium (chloroform) (Lailatusholihah et al., 2023). Acetic acid functions as both a solvent and a catalyst, while H_2SO_4 induces the formation of carbocations that lead to characteristic color changes (Kusumawati et al., 2015). Therefore, this combination of reagents is effective in differentiating steroid and terpenoid groups based on the resulting color.

The identification of betacyanin in beetroot extract showed a positive result, indicated by a color change from red to yellow-orange after the addition of a basic solution, as presented in Figure 6. This finding confirms the presence of betalain pigments, particularly betacyanin, which is a characteristic compound of beetroot. Betacyanin is known for its strong antioxidant activity and its role in scavenging free radicals (Farika et al., 2024). Moreover, the pH-dependent stability of betacyanin serves as a key feature in its identification, where acidic conditions maintain the red color, while alkaline conditions induce a color shift.

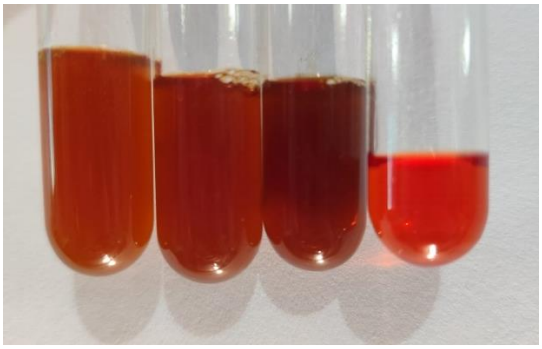

Figure 6. Betacyanin Identification Result

Based on Figure 6, the identification of betacyanin showed a color change from red to yellow-orange after the addition of 2N HCl and 2M NaOH, indicating a positive result. Mechanistically, betacyanin is a pigment that is highly sensitive to pH changes. Under acidic conditions (addition of HCl), the structure of betacyanin remains stable, thereby maintaining its red color (Yulianti et al., 2008). However, under alkaline conditions (addition of NaOH), degradation of the betacyanin chromophore structure occurs, leading to a color change to yellow or brownish tones (Fatjria et al., 2023). Therefore, the use of both acidic and basic reagents in this test serves to confirm the presence of betalain pigments based on their color stability in response to pH variations.

Overall, the results of this study have important implications for the development of pharmaceutical science based on natural products, particularly regarding the role of secondary metabolites as bioactive compounds. The presence of flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and betacyanin in beetroot extract indicates that these secondary metabolites function as key components responsible for various pharmacological activities, including antioxidant, antibacterial, anti-inflammatory, and immunomodulatory effects. These compounds act through multiple mechanisms, such as scavenging free radicals, inhibiting the growth of microorganisms, and modulating biological responses in the body. Therefore, the identification of secondary metabolites through phytochemical screening not only provides information about the chemical composition of plants but also serves as a fundamental basis for further development in the formulation of herbal medicines, dietary supplements, and natural cosmetic products. Thus,

this study represents an initial step in exploring the potential of beetroot as a source of therapeutically valuable bioactive compounds.

CONCLUSION

Based on the results of this study, it can be concluded that the ethanol extract of beetroot (*Beta vulgaris L.*) contains various secondary metabolites, including flavonoids, tannins, saponins, alkaloids, steroids, and betacyanin, as identified through qualitative phytochemical screening. In addition, the extraction process using the maceration method with 96% ethanol yielded a 25% extract, indicating its effectiveness in extracting active compounds from beetroot simplicia. The presence of these secondary metabolites suggests that beetroot has strong potential as a natural source for further development in the pharmaceutical field, including its application in herbal medicines, dietary supplements, and natural cosmetic products.

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