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PHYTOCHEMICAL PROFILING OF CAYENNE PEPPER (*Capsicum frutescens* L.) STEM EXTRACT

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ABSTRACT

As an agricultural country, Indonesia has great potential in the utilization of biological resources, one of which is the cayenne pepper plant (*Capsicum frutescens* L.) which is commonly used as a cooking spice and also has health benefits. So far, the cayenne pepper, often discarded as waste, is assumed to hold potential as a value-added product due to its rich phytochemical compounds. This research focuses on detection of various secondary metabolite compounds in the extract of cayenne pepper stems through the phytochemical screening method. Extraction was carried out using ethanol solvent for maceration and providing rotary evaporator for purity. Pure extract was tested qualitatively using phytochemical screening method to identify the secondary metabolite compounds. The findings presented alkaloids, saponins, tannins, and indications of terpenoids, while flavonoids and steroids were not detected. These are influenced by the concentration of the compound, the suitability of the solvent, and the stability of the compound during the extraction process. The conclusion of this study showed that cayenne pepper stems have the potential as a source of natural ingredients containing bioactive compounds and can be developed in the pharmaceutical field.

Keywords : Cayenne Pepper (*Capsicum frustescens* L.) Stem, Phytochemical Screening, Secondary Metabolites

INTRODUCTION

Indonesia is known as an agrarian country rich in plant biodiversity, especially in the agricultural sector. One of the agricultural commodities with great potential in Indonesia is the chili plant. Chili is considered a highly important horticultural commodity due to its integral role in daily needs, particularly in household consumption. Indonesian often recognizes and uses mainly as a flavoring ingredient in cooking.

With its tropical climate, Indonesia has natural conditions that are highly supportive of cultivating various types of chili plants. Several chili varieties grow abundantly in different regions of the country. One of the most commonly found types in Indonesian markets is cayenne pepper (*Capsicum frutescens* L.). In 2012, the production of fresh cayenne peppers with stems reached approximately 702.25 thousand tons (Parfiyanti *et al.*, 2016).

The chemical compounds found in cayenne pepper are highly diverse, with capsaicin being the primary component. Capsaicin belongs to the alkaloid group and plays a key role in giving chili its characteristic pungent flavor. In addition to capsaicin, cayenne pepper also contains capsanthin, carotenoids, resins, essential oils and vitamins, all of which contribute to the plant's distinctive aroma and medicinal properties. Capsaicin is known to have antibacterial properties that can help inhibit the growth of various bacteria, making

cayenne pepper useful not only as a food ingredient but also beneficial for human health (Munira *et al.*, 2019).

In this era, many people assume that the most valuable part of chili lies only in its fruit, leading to the common practice of discarding the stem, as it is considered to have no significant value or useful content. Cayenne pepper stems contain multiple secondary metabolites that play a crucial defensive role for the plant, while also potentially yielding benefits for human well being. Some of the compounds found in cayenne pepper stems include flavonoids, tannins, alkaloids, saponins, phenolic compounds, and lignin (Widayoko *et al.*, 2023).

Typically, plants produce primary metabolites (such as sugars and amino acids) vital for their development, and secondary metabolites (such as alkaloids, flavonoids, tannins, and terpenoids), which are of interest in pharmacology because of their possible biological effects. Phytochemical screening serves as a preliminary qualitative procedure to reveal these compound classes in plant extracts, giving an initial assessment of the extract's bioactive promise for further medicinal exploration.

In addition, the taxonomic approach in phytochemical studies is based on the idea that closely related plants tend to contain similar chemical compounds, thus accelerating the search for sources of active compounds. The results of phytochemical screening serve as a crucial foundation for further analysis to determine a plant's pharmacological potential and guide the development of plant-based drugs (Luciana *et al.*, 2025).

According to Fadhli *et al.* (2025), the flavonoid content in ethanol extract of cayenne pepper stems is significantly lower than in the fruit. This difference may be due to the distribution of flavonoids within the plant. Flavonoids are more commonly found in parts that serve a protective function, such as fruits and leaves, compared to the stem, which primarily functions as structural support. Widayoko (2023) also states that cayenne pepper stems do in fact contain several important secondary metabolites with potential benefits for humans, such as flavonoids, tannins, alkaloids, saponins, phenolic compounds, and lignin.

According to Kusnadi *et al.* (2019), the spiciness level of cayenne pepper can be influenced by the content of capsaicinoid compounds, particularly capsaicin and dihydrocapsaicin. *Capsicum frutescens*, commonly known as cayenne pepper, contain higher levels of capsaicinoids compared to other chili varieties. Capsaicinoids are the chemical compounds responsible for the spiciness in chili peppers. Besides imparting a pungent taste, the compound capsaicin exhibits antioxidant and anti-inflammatory effects and is also used for its analgesic (pain relieving) properties.

The purpose of the research titled "Phytochemical Profiling of Cayenne Pepper (*Capsicum frutescens* L.) Stem Extract" is to identify and analyze the secondary metabolite compounds present in the stem extract of cayenne pepper. These compounds, such as alkaloids, flavonoids, tannins, saponins, terpenoids, and others, are known for their potential biological and pharmacological activities. The research used phytochemical screening methods, which involve a series of qualitative chemical tests to detect the presence of various classes of secondary metabolites in the plant extract.

METHODS

Materials and methods

The equipment utilized throughout the study include a stirring rod, blender, bowl, beaker glass (Pyrex), glass funnel (Pyrex) , separatory funnel (Pyrex), watch glass (Pyrex), cuvette (Pyrex), volumetric flask (Pyrex), analytical balance (Shimadzu), volumetric pipette (Pyrex), dropper (Pyrex), rotary evaporator (Shimadzu), spatula (Merk), aluminum foil (Cheffy) and test tubes (Pyrex).The main material used in this study is cayenne pepper stems. The chemical reagents used include Etanol 96%, ethyl acetate (EA), chloroform

($CHCl_3$), n-hexane (C_6H_6), ammonia (NH_3), H_2SO_4 (5N), Liebermann–Burchard reagent, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, distilled water, HCl (5 N), ethanol, magnesium metal (Mg), $FeCl_3$ 1%, and dimethyl sulfoxide (DMSO).

Preparation of Sample

Using leftover kitchen-stem waste, cayenne pepper stalks were cleaned under running water, air-dried, milled, and sieved; exactly 30 g of the powdered material was macerated in 300 mL of 96 % ethanol for three days (with 5-minute stirrings every 2 hours), then filtered and evaporated to dryness via rotary evaporator to produce the extract (Ibrahim, 2014)

Phytochemical Screening

The sample used for phytochemical screening was the 96% ethanol extract of Cayenne pepper stems. Each test used a specific solvent depending on the type of compound being identified.

Alkaloid Identification

A porcelain dish was loaded with 2 mL of extract and acidified with 5 mL of 2 M HCl. After partitioning this solution into three test tubes, the first served as a blank with only HCl; the second received Dragendorff's reagent, where a positive result is marked by an orange precipitate; the third was treated with Mayer's reagent, where a yellow precipitate signals alkaloid presence (Wahid and Safwan, 2020).

Flavonoid Identification

After boiling 2 mL of extract in hot water for five minutes and filtering, 5 mL of the filtrate was treated with 0.05 mg magnesium powder and 1 mL concentrated HCl and agitated; a red, yellow, or orange tint indicates a positive flavonoid result (Harborne, 1987).

Saponin Identification

The extract (2–3 mL) was warmed in a test tube, then mixed with 10 mL of hot water and shaken vigorously for 10 seconds; the presence of a foam column between 1 and 10 cm in height, lasting for at least 10 minutes, confirms the presence of saponins (Depkes RI, 1995).

Tannin Identification

To detect tannins, 1 mL of extract was mixed with several drops of 1 % $FeCl_3$ solution. The sample turning dark blue to greenish black indicated a positive result (Jones & Kinghorn, 2006).

Terpenoid Identification

Upon addition of Liebermann–Burchard reagent to 2 mL of extract, the development of red or violet hues indicated a positive test result, aligning with documented colorimetric responses in standard phytochemical assays (Mukhriani, 2014)

RESULT AND DISCUSSION

The results are shown in Table 1. Maceration using etanol 96% was selected due to its ability to dissolve a wide range of polar and semi-polar secondary metabolites. it revealed no flavonoid or steroid compounds in the ethanol extract of cayenne pepper stems. However, alkaloids, saponins, terpenoids, and tannins were all detected. Alkaloid testing with Mayer's and burchardart's reagents returned positive results, whereas Dragendorff's reagent did not indicate alkaloid presence.

This inconsistency among the three reagents suggests that the alkaloid content in the cayenne pepper stem extract may be low or not sufficiently specific. It could also indicate that the alkaloids present are in small quantities or possess chemical structures that are less reactive to certain reagents. The presence of alkaloids in the sample is confirmed by the formation of a yellowish-white precipitate upon addition of Mayer's reagent, reflecting a

metal–alkaloid complex that forms when heavy mercury ions bind to the nitrogenous bases of alkaloid molecules.

Meanwhile, the brown precipitate formed in the test using Bouchardat's reagent returned a positive result, confirming the presence of alkaloids. In this reaction, a coordinate covalent bond is formed between potassium ions (K^+) and nitrogen groups in the alkaloid structure, leading to the formation of a precipitate. Bouchardat's reagent contains iodine and potassium iodide, which play a crucial role in the complex formation process (Sulistiyartini *et al.*, 2020).

Tabel 1. Phytochemical Screening of Cayenne Pepper Stem Extract

Reagents		Presence	Result	Color
Alkaloid	Mayer	+	Positif	Yellowish-White
	Bouchardart	+	Positive	Brown
	dragendroft	–	Negatif	No color change
Flavonoid	Mg + HCl	–	Negatif	No color change
	$FeCl_3$	–	Negatif	No color change
Steroid	Lieberman	–	Negatif	No color change
	Bouchard	–	Negatif	No color change
	Salkowsky	–	Negatif	No color change
Terpenoid	Lieberman	–	Negatif	No color change
	Bouchard	–	Negatif	No color change
	Salkowsky	+	Positive	No color change
Saponin	Aquadest	+	Positive	Formation of clear foam
Tannin	$FeCl_3$	+	Positive	Dark green

In the Dragendorff assay, the lack of an orange precipitate confirmed a negative result, which is significant because alkaloids normally form orange to red-brown complexes through ligand exchange with nitrogen atoms in this reaction. This reaction involves a ligand exchange process, in which the nitrogen atom in the alkaloid possessing a lone pair of electrons forms a coordinate covalent bond with potassium ions (K^+), resulting in a precipitated potassium-alkaloid complex (Habibi, 2018).

The flavonoid test using Mg + HCl and $FeCl_3$ reagents returned negative results, confirming the absence of flavonoids in the extract. The flavonoid test using 5% $FeCl_3$ reagent did not indicate the presence of flavonoids in the extract, as no color change to pink or dark green was observed after the addition of the reagent. This may be due to the low solubility of flavonoids in the solvent used. Flavonoids are phenolic compounds that are polar in nature, making them more soluble in polar solvents such as ethanol or methanol (Habibi, 2018). However, semi-polar solvents like ethyl acetate also have the ability to dissolve polar compounds, including flavonoids, although not as effective as pure polar solvents. Therefore, the selection of an inappropriate solvent that does not align well with the chemical properties of flavonoids can affect the detection results of these compounds in phytochemical screening tests (Putri and Lubis, 2020).

The identification of steroid using Liebermann–Burchard and Salkowski reagents also showed negative (−) results, suggesting that steroid compounds were not present in the extract. The terpenoid test, which was also conducted using Liebermann–Burchard and Salkowski reagents, showed a positive (+) result with the Salkowski test, but a negative (−) result with the Liebermann–Burchard test. This indicates that terpenoid compounds are present, although not all reagents produced a positive result, possibly due to differences in sensitivity or specificity of the reagents used.

The analyzing of terpenoid on the ethanol extract of cayenne pepper stems showed varying results depending on the reagent used. In contrast to the negative Liebermann–Burchard reaction, the Salkowski test produced a distinctive brick-red coloration, indicating a positive result and affirming the presence of terpenoids that react with strong

acidic reagents. The reaction triggers an oxidation process that forms conjugated double bonds within the terpenoid structure, resulting in the distinctive color change. The appearance of brick-red color serves as a strong visual indicator of the presence of terpenoids. This finding represents an important preliminary step in identifying bioactive compounds that may have pharmacological potential in cayenne pepper plants.

The saponin detection using distilled water (aquadest) showed a positive (+) result, confirming the saponin compounds in the cayenne pepper stem extract. Lastly, the tannin test using $FeCl_3$ reagent also produced a positive (+) result, confirming that tannins were detected in the extract due to formation of foam or froth after the homogenization process. This is due to the physical properties of saponins, which are readily soluble in water (aquadest) and have the ability to reduce surface tension, thereby producing foam when shaken or stirred. The presence of stable foam is a characteristic indicator in saponin testing and confirms that saponin compounds are indeed present in the tested extract.

After the addition of 5% $FeCl_3$ reagent, the tannin test yielded a positive result with a color change of the solution to greenish black. This color change signifies a reaction between the tannin compounds present in the cayenne pepper stem extract and the Fe^{3+} ions from the $FeCl_3$ solution. The reaction occurs because the hydroxyl groups in the tannin structure are able to form complexes with metal ions, resulting in a distinctive color that serves as a positive indicator of tannin presence (Mailuhu *et al*, 2017).

CONCLUSION

Based on the results of the phytochemical profiling, the ethanol extract of cayenne pepper (*Capsicum frutescens* L.) stems was found to contain several secondary metabolites, including alkaloids, saponins, terpenoids, and tannins. Tests for flavonoids and steroids showed negative results, indicating the absence of these compounds in the extract. The presence of stable foam confirmed saponins, while color changes observed in specific reagent tests confirmed the presence of terpenoids and tannins. Alkaloids were detected through positive reactions with Mayer's and Bouchardat's reagents, although not with Dragendorff's, suggesting relatively low or structurally unique alkaloid content. These findings indicate that the stem part of cayenne pepper, which is often considered waste, contains bioactive compounds that may have potential pharmacological value. To obtain more accurate and comprehensive results, it is recommended to complement the phytochemical screening with qualitative analysis using the Thin Layer Chromatography (TLC) method.

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