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Characterization and Screening Phytochemicals Orange Purut Leaves (*Citrus hystrix*) Extract from Kampar, Riau

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Abstract

Orange purut leaves (*Citrus hystrix*) are one of the shrubs that have high economic value because they contain vitamin C and are often used as a food flavoring. The aim of this research is to determine whether orange purut leaf extract contains phytochemicals and to characterize the properties of orange purut leaf extract. Phytochemical screening of orange purut leaf extract (*Citrus hystrix*) included the examination of flavonoids, tannins, saponins, steroids/triterpenoids, alkaloids, and glycosides. Phytochemical screening was conducted on both simplicia powder and simplicia extract, and characterization of the simplicia was carried out. The water content of orange purut leaves was found to be 8%, the water-soluble juice content was 37.9%, the ethanol-soluble juice content was 19%, the total ash content was 7.6%, and the acid-insoluble ash content was 0.6%. Orange purut leaf extract positively contains alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides.

Abstrak

Daun jeruk purut (Citrus hystrix) adalah salah satu tanaman semak yang memiliki nilai ekonomi tinggi karena mengandung vitamin C dan sering digunakan sebagai bahan perasa makanan. Tujuan penelitian ini adalah untuk mengetahui apakah ekstrak daun jeruk purut mengandung fitokimia dan untuk menentukan karakteristik ekstrak daun jeruk purut. Skrining fitokimia ekstrak daun jeruk purut (Citrus hystrix) mencakup pemeriksaan flavonoid, tanin, saponin, steroid/triterpenoid, alkaloid, dan glikosida. Skrining fitokimia dilakukan pada serbuk simplisia dan ekstrak simplisia, serta karakterisasi simplisia dilakukan. Kandungan air pada daun jeruk purut adalah 8%, kandungan jus larut air 37,9%, kandungan jus larut etanol 19%, kandungan abu total 7,6%, dan kandungan abu yang tidak larut asam 0,6%. Ekstrak daun jeruk purut secara positif mengandung alkaloid, flavonoid, saponin, tanin, steroid/triterpenoid, dan glikosida.

Keywords: Orange purut leaves, ethanol extract, characterization, phytochemical screening

Kata kunci: *Daun jeruk purut, ekstrak etanol, karakterisasi, skrining fitokimia.*

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INTRODUCTION

Orange purut leaves (*Citrus hystrix*) are one of the plants that have high economic value because they contain vitamin C and are often used as a food seasoning. The chemical compounds contained in orange purut leaves (*Citrus hystrix*), such as tannins, steroids / triterpenoids, and essential oils, have significant health benefits. These leaves have been widely recognized for their versatile uses in culinary dishes and traditional medicine. Besides their culinary applications, the health benefits of orange purut leaves extend to their use in boosting immunity, aiding in digestion, and promoting skin health due to their rich content of antioxidants and essential nutrients[1].

Orange purut leaves (*Citrus hystrix*) are shrubs that offer many benefits, especially the fruit and leaves. The leaves are often utilized for cooking seasoning, while the fruit also serves as a valuable ingredient in many local recipes. In addition to being a flavorful addition to food, *Citrus hystrix* provides numerous health benefits, including its ability to support immune function, improve respiratory health, and act as a natural remedy for skin conditions. Furthermore, orange purut leaves are a key component in traditional beauty treatments, contributing to healthy and radiant skin[2].

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One of the herbal ingredients rich in benefits is orange purut leaves, which are valued economically due to their high vitamin C content. Vitamin C is known for its antioxidant properties, which help protect the body from oxidative stress and support the immune system[3]. Beyond vitamin C, orange purut leaves contain a range of secondary metabolites such as flavonoids, saponins, and essential oils, all of which contribute to their medicinal properties. These compounds not only provide therapeutic benefits but also make the leaves an attractive option for the herbal medicine market[4].

Phytochemistry is the branch of science that deals with organic compounds, including their chemical structure, biosynthesis, transformations, biological functions, isolation, and the comparison of chemical compositions across different plant types[5]. Phytochemical analysis is crucial for identifying secondary metabolites in plants, as these compounds may have toxic or pharmacological effects. Environmental factors, such as temperature, light, soil, and climate, significantly influence the quantity and quality of secondary metabolites present in plants[6]. In the case of orange purut leaves, these environmental factors affect the levels of key compounds, such as flavonoids and essential oils, which contribute to the plant's medicinal and economic value[7,8].

Determination of Ash Content No Late Sour

The ash content was determined by heating the sample and cooling it with 25 mL of dilute hydrochloric acid. The mixture was stirred for 5 minutes. The insoluble portion was then collected, filtered through an ash-free filter paper, and washed with hot water. The residue and filter paper were heated at 600°C for 3 hours, then cooled and weighed until a constant weight was achieved. The ash content, insoluble in acid, was calculated based on the materials that had been air-dried[15].

Making Orange Leaves Purut Ethanol Extract (Citrus hystrix)

The ethanol extract of orange purut leaves (*Citrus hystrix*) is prepared using the maceration method with 96% ethanol as the solvent. Ten parts (500 g) of the powdered sample are placed in a vessel, then 75 parts (3750 mL) of ethanol are added. The vessel is closed and stirred occasionally, and left undisturbed for 5 days, protected from sunlight. After 5 days, the mixture is filtered, and the dregs are squeezed. The dregs are then washed with enough ethanol to obtain 100 parts (5 liters) of macerate. The macerate is then transferred to a closed vessel and left in a cool, light-protected place for 2 days before being filtered. The macerate is then concentrated using a rotary evaporator, and the final weight is recorded[16].

Phytochemical Screening

Phytochemical screening of the orange purut leaves extract (*Citrus hystrix*) includes the examination of compound groups such as flavonoids, tannins, saponins, steroids/triterpenoids, alkaloids, and glycosides. The phytochemical screening was conducted on both the powdered sample and the extract sample.

Flavonoid Examination

Ten grams of both the powdered sample and the extract were weighed. Then, 100 mL of hot water was added, and

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the mixture was brought to a boil for 5 minutes and filtered while hot. The filtrate obtained was then taken, and 5 mL was added to 0.1 grams of magnesium powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol. The mixture was shaken and left to separate. The presence of flavonoids is indicated by a red or yellow-orange color on the amyl alcohol layer[17].

Tannin Examination

0.5 grams of both the powdered sample and the extract were weighed and mixed with 10 mL of distilled water. The mixture was then filtered, and the filtrate was diluted with distilled water until it was colorless. Next, 2 mL of the solution was taken, and 1 to 2 drops of iron (III) chloride reagent were added. The presence of tannins is indicated by the formation of a blue or green-black color[18].

Saponin Examination

0.5 grams of both the powdered sample and the extract were weighed and placed into a reaction tube. Then, 10 mL of hot distilled water was added. The mixture was cooled and shaken vigorously for 10 seconds. If a solid foam forms and remains at a height of 1-10 cm for at least 10 minutes, 1 drop of 2N hydrochloric acid solution was added. If the foam does not disappear, it indicates the presence of saponins[19].

Steroid/Triterpenoid Examination

1 gram of both the powdered sample and the extract were weighed and macerated with 20 mL of n-hexane for 2 hours, then filtered. The filtrate was evaporated using a rotary evaporator. To the remaining residue, 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid were added. A purple color indicates the presence of triterpenoids, while a green color indicates the presence of steroids[20].

Alkaloid Examination

For alkaloids, weigh 0.5 grams of the sample, then add 1 mL of hydrochloric acid and 9 mL of distilled water. Heat the mixture in a water bath for 2 minutes, then cool it down and filter the mixture. The filtrate is then tested using three reagents: For Mayer's reagent, add 2 drops of the reagent to 3 drops of the filtrate. A white or yellow precipitate indicates a positive result. For Bouchardat's reagent, a positive result is indicated by the formation of a chocolate to black precipitate. For Dragendorff's reagent, a red-colored precipitate indicates a positive result[21].

Inspection Glycosides

Three grams of both the powdered sample and the extract were weighed, then mixed with 30 mL of a solution consisting of 7 parts ethanol (96%) and 3 parts distilled water, to which 10 mL of 2N HCl was added. The mixture was refluxed for 30 minutes, cooled, and filtered. Twenty mL of the filtrate was taken and mixed with 25 mL of distilled water and 25 mL of 0.4 M lead (II) acetate, shaken, and left to stand for 5 minutes, then filtered. The filtrate was then mixed with 20 mL of a 3:2 mixture of chloroform and isopropanol and this process was repeated three times. The evaporated water essence was collected at room temperature, not exceeding 50°C. The remaining residue was dissolved in 2 mL of methanol. Then, 0.1 mL of the solution was taken, placed in a reaction tube, and evaporated in a water bath. To the remaining residue, 2 mL of water and 5

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drops of Molisch's reagent were added. Slowly, 2 mL of concentrated sulfuric acid was added along the wall of the tube. The appearance of a purple ring at the interface of the two liquids indicates the presence of glycosides[22].

RESULT AND DISCUSSION

The sample used in this study is orange purut leaves. A total of 5000 g of fresh orange purut leaves were obtained, and after drying, 1065 g of powdered sample was produced. The extraction method used was maceration with 96% ethanol as the solvent, resulting in 96 g of thick extract, which is green-black in color and has a distinctive smell.

Inspection Characterization Sample

The characterization results of the powdered orange purut leaf can be seen in Table 1.

Table 1. Examination Characterization Sample Orange Leaves Powder Purut

No	Parameter	Results (%)	Condition (%)
1	Water content	8	≤10
2	Soluble juice content in water	37.9	≥7
3	Ethanol content	19	≥3
4	Total ash content	7.65	≤15
5	Contents ash No late sour	0.6	≤1

The water content of the powder sample was tested based on the standard table to determine the water content in powdered drugs. In general, the water content of a sample should not exceed 10%. The test results for the water content of the orange purut leaf powder showed 8%, which meets the required condition. This is because if the water content exceeds 10%, fungi and bacteria can easily develop.

Next, the water-soluble essence content in the powder sample was examined to determine how much of the active components can be extracted using water as a solvent. The soluble essence content in the powdered orange purut leaf sample was found to be 37.9%, which meets the required standard. The standard for orange purut leaves is typically less than 6%. On examination, the ethanol extract yielded 19%, which also meets the required condition, as the standard for ethanol extraction is greater than 6.5%.

The total ash content in the sample was found to be 7.6%, which satisfies the required condition. Additionally, the acid-insoluble ash content was 0.6%, which is within the target range. These inspection results and characterizations are conducted to ensure the uniformity and quality of the sample, ensuring that it meets the standard conditions for both the sample and the extract.

Phytochemical Screening

Phytochemical screening was conducted to identify the secondary metabolites present in the plant leaves. The screening was performed on both the powdered and extract forms of the leaves, focusing on detecting the presence of alkaloids, saponins, tannins, flavonoids, steroids/triterpenoids, and glycosides. The results, which confirm the presence of all these compounds, are summarized in Table 2.

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The secondary metabolites contained in the powders and extracts of orange purut leaves include alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides, as shown in Table 2. When Bouchardat's reagent was added, a chocolate-black precipitate formed, and when Dragendorff's reagent was added, an orange-colored precipitate formed, indicating the presence of alkaloid compounds. The orange color on the amyl alcohol layer indicates the presence of flavonoid compounds, confirming that both the powder and extract of orange purut leaves contain flavonoid compounds. The presence of saponins was observed by the height of the foam, which was 2 cm, obtained from both the powder and extract. The presence of tannins was confirmed by the green-black color formed upon adding FeCl3 reagent, marking both the powder and extract of orange purut leaves as positive for tannin compounds. Additionally, the formation of a green color indicates the presence of steroid/triterpenoid compounds, confirming that the powder and extract of orange purut leaves are positive for steroids. The formation of a purple ring with the addition of Molisch's reagent indicates the presence of carbohydrate compounds in the powder and extract of orange purut leaves. The Molisch test was conducted to confirm the presence of carbohydrates in general, as this compound reacts with α-naphthol to form a purple-colored complex or ring.

Table 2. Phytochemical Screening

No	Test	Powder	Extract
1	Flavonoid	+	+
2	Alkaloid	+	+
3	Tannin	+	+
4	Saponins	+	+
5	Steroids/triterpenoids	+	+
6	Glycosides	+	+

CONCLUSION

Based on results research conducted can concluded that extract leaf orange purut (*Citrus hystric* DC) in testing characterization fulfil standards and on testing screening phytochemicals got positive results . Testing leaf water content orange purut of 8%, the water - soluble content of 37.9%, the water-soluble content of 37.9 % ethanol obtained 19% and the level total ash obtained was 7.6% and content ash No late sour obtained 0.6%. Extract leaf orange purut positive contains alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides .

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