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CHARACTERIZATION OF SIMPLISIA AND CLOVE LEAVES EXTRACTS (*Syzigium Aromaticum* Merr. Et Perry L) AS ACTIVE INGREDIENTS FOR HERBAL MOUTHWASH

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ABSTRACT

Clove leaves (*Syzygium Aromaticum* Merr. Et Perry L) have compounds that can prevent the activity of the *Streptococcus mutans* which is the cause of dental caries. This research is non experimental research which focuses on the characterization of clove leaves. Characterization of simplisia and sample extracts include organoleptic observation, microscopy, phytochemical screening using Thin Layer Chromatography, determination of soluble substances in certain solvents (ethanol and water), total ash content, water soluble ash content, insoluble ash content in acid, then drying shrinkage test. The results of the organoleptic test for simplisia and extract of clove leaves showed that they have a dark brown color, specific aromatic odor, spicy and bitter taste. Clove leaves extract has a brownish black color, aromatic odor and has a bitter and pungent taste. Microscopic test of simplisia of clove leaves showed that there was a mesophyll includes palisade tissue, with calcium oxalate crystal, anomistic type stomata, identification fragments are fragments of the lower epidermis with stomata. The results of the percentage of soluble substances of simplisia and ethanol extract of clove leaf were 50% and 40%, the percentage of soluble substances of simplisia and ethanol extract of clove leaf were 1.73% and 4.33%. The percentage of total ash content of simplisia and ethanol extract of clove leaf 11.4% and 22%, the percentage of water soluble ash content of simplisia and extract was 16.9%, 17.5%. Percentage of insoluble ash content of simplisia acid and extract 9.5% and 10.5%. Shrinkage of simplisia and extracts were 7.6% and 11.46%.

Keywords: Cloves leaves, *Syzygium Aromaticum* Merr. Et Perry L, Caracterization, Mouthwash

INTRODUCTION

Dental caries is a disease that affects many people. This disease is very disturbing patient comfort because it makes bad breath. The increase in the prevalence of dental caries from 2007 in Indonesia was 43.4% until 2013 to 53.2%,and there were approximately 93.998.727 people suffering from dental caries, this high prevalence rate will continue to increase if the public is still unaware of the importance to treat dental hygiene[1].

The main cause of dental caries is a group of bacteria that is bound in an organic matrix and is tightly attached to the tooth surface known as plaque and the bacteria Streptococcus mutant serotype c and serotype d are the main bacteria forming dental plaque that ends in dental caries[2,3]. As the development of science and technology causes many producers to focus on the preparation for the treatment and prevention of cavities or dental caries using herbal

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medicines that come from nature, and it is preferred by the community because it minimizes the use of chemicals and tends to be safer and more effective.

Clove is a spice plant native to North Maluku / Maluku Island[4]. The results showed that clove leaves extract (*Syzygium Aromaticum* Merr. Et Perry L) had antibacterial activity against *Streptococcus mutans* serotype c and serotype d. Clove leaves contain eugenol which are anesthetic, antimicrobial and antiseptic, phenolic and flavonoid[5]. Cloves have many benefits, including as an antibacterial, antiviral, antifungal, antiplatelet, anticancer, antihistamine, and antioxidant[6]. Clove oil is widely used as a toothache medicine because it contains beta caryophilene which has the efficacy of local anesthetics, as well as aromatic, carminative and stimulatory. Clove leaf phenolic compounds are also responsible for the antioxidant activity and flavonoid compounds which act as antidotes to free radicals [7,8].

Making preparations that are useful to prevent dental caries using natural raw materials must pass a standardization test, so researchers conducted a test of the characteristics of clove leaves simplicia as a raw material for dental caries drugs.

MATERIAL AND METHOD

Materials that used in this study were clove leaves, filter paper, ethanol, chloroform, methanol, n-hexan, ethyl acetate, phosphoric acid, acetate, hydrogen peroxide, boric acid, citric acid, dragendroff reagent, chloride acid, aquadest, Liebermann Burchard reagent, Meyer reagent, beaker glass, blender, porcelain cup, krusible cup, cover glass, desiccator, measuring cup, erlenmeyer flask, Rotary Evaporator, water bath, drying cabinet, microscope, volumetric flask, glass stirrer, analytic balance, dropper, ultraviolet lamp 254 and 366 nm, maceration jars, capillary pipets, volume pipettes, TLC GF 254 Plate, chamber, Whatman 42.

Sample preparation

Samples in the form of clove leaves (*Syzygium aromaticum* Merr. Et Perry L) that have been aged, wet sorted, washed, dried, cut into small pieces, then sorted dry.

Sample extraction

Five grams of clove leaves (*Syzygium aromaticum* Merr. Et Perry L) were macerated using 80% ethanol. The sample was macerated for 5 days until a saturated solution was obtained, the sample was filtered, the residue was added with ethanol solvent, carried out repeatedly until a colorless ethanol solution was obtained. Liquid ethanol extract was concentrated using a rotary evaporator at a temperature of 30-50^{O}C. After that, it is dried to get thick ethanol extract using water bath. The extract is then stored in a tightly closed container and protected from light.

Sample caracterization

Organoleptic

Organoleptic test using the senses to describe the shape, color, smell and taste[9]

Microscopic test

Microscopic tests were carried out on powder simplisia. Simplisia powder is placed on an object glass, dropped with explain the solvent and then covered with a glass cover then observed under a microscope. The solvent are ethanol solvent 96% and HCl 37% with ratio 3:1 to see the shape of stomata and water to see another fragments.

Received :2020-05-08 Revised : 2020-05-16 Acceptance : 2020-05-19 Publish : 2020-06-05

Determination of Water Soluble Substance Content
The powder (4/18) is dried in the air, then weighed 5 g of the powder macerated for 24 hours with 100 mL of chloroform water using a clogged pumpkin while repeatedly shaken for 6 hours then allowed to stand for 8 hours Filtered, evaporated 20 mL of filtrate to dry in deep shallow flat base dishes that have been tamed, the rest is heated at a temperature of 105 ^{o}C until it reaches a fixed weight[10]

Determination of Ethanol Soluble Substance Levels
Examination of ethanol extract characterization includes: determination of water content, determination of water soluble extracts, determination of ethanol soluble extracts, determination of total ash content and determination of acid insoluble ash content[11].

The powder (4/18) was dried in the air, then weighed 5 g of powder was macerated for 24 hours with 100 mL ethanol (80%) using a clogged pumpkin while repeatedly shaken for the first 6 hours and then left for 18 hours. Fast filtering by avoiding evaporation of ethanol (80%), evaporating 20 mL of the filtrate to dry in shallow flat dishes that have been tamed, remaining heated at 105^{o}C until a fixed weight[10].

Determination of Total Ash Content
Approximately 2-3 g of simplisia that has been crushed and weighed carefully, put into a platinum or silicate crucible that has been flattened and tamed, and flattened. Flatten slowly until the charcoal runs out, cooled, and weighed. If in this way the charcoal cannot be removed, hot water is added, filtered through ash-free filter paper. Flattened leftover and filter paper in the same crucible. Put the filtrate into the crucible, evaporated, flattened to a fixed weight, and weighed. Calculated ash content of materials that have been dried in the air[10].

Determination of Water Soluble Ash Content
Containers containing total ash, added 25 mL of water and boiled for 5 minutes. Material that does not dissolve is collected into a glass cup or ash-free filter paper. Wash with hot water and burn in a cup for 1 hour at a temperature of 600^{o}C and until the weight remains. Reducing the weight of this residue in mg of total ash weight[13].

Determination of Acid Soluble Ash Content
Ash obtained at the determination of total ash content, boiled with 25 mL HCl 3N for 5 minutes, collected parts that are not soluble in acid, filtered through ash-free filter paper, washed with hot water, sprinkled for 1 hour at 600^{o}C and up to weight fixed, and weighed. Calculated levels of ash which is not soluble in acid to the material that has been dried in the air[13].

Determination of Drying Shrinkage
One or Two gram of simplisia in a shallow, closed weighing bottle that has been preheated at 105^{o}C for 30 minutes and has been tapped. If the substance in the form of large crystal, before weighing crushed quickly until the size of the simplisia is approximately 2 mm. Simplified in a weighing bottle is spread by shaking the weighing bottle until the layer is approximately 5-10 mm thick, put into the drying chamber, opened the lid, dried at 105^{o}C until the weight remains. Before each drying, leave the bottle closed to cool in an excavator to room temperature. If the melting temperature is lower than 105^{o}C, drying is carried out

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at a temperature between 5-10^{o}C under the melting temperature for 1-2 hours, then at 105 oC for a specified time or until the weight remains[10].

Secondary Metabolite Test
Terpenoid and Steroid Test
Using the Liebermann Burchard reagent, which is a mixture of anhydrous acetic acid and concentrated sulfuric acid, then heated at a temperature of 100^{o}C and seen at UV 366 nm.

Steroid Test
Using an 85% phosphoric acid solution reagent mixed with a methanol solvent, then heated at 120^{o}C and seen at UV 366 nm.

Flavonoid Test
Using 25% lead acetate reagents, then seen at UV 366 nm.

Phenolic Test
Using citro boric reagent, which is a mixture of citric acid and boric acid (1: 1), then heated at a temperature of 100^{o}C.

Alkaloid Test
Using Dragendroff reagents and heated at 100^{o}C.

Aromatic Acid Test
Using 3% H_2O_2 reagent and seen at UV 366 nm.

RESULTS AND DISCUSSION

Organoleptic Result of Simplisia and Clove Leaves Ethanol Extract
Organoleptic testing using the five senses to determine the smell, taste and color of simplisia, the five human senses in general are very sensitive to smell, taste and color. This organoleptic identity determination is the first step in identification. The result of organoleptic test shows in Table 1.

Simplisia Microslopic Clove Leaves Results
Microscopic testing is only performed on clove leaves simplisia, because in the form of simplisia the fragments or tissues still appear, using an optical instrument, namely a microscope. Microscopic test results of simplisia mesophyll clove leaves include palisade tissue, with calcium oxalate crystal, anomistic type stomata identification fragments are lower epidermal fragments with stomata

Received :2020-05-08 Revised : 2020-05-16 Acceptance : 2020-05-19 Publish : 2020-06-05

Table 1 Organoleptic Test Results for Simplisia and Extract of Clove Leaves (*Syzygium Aromaticum* Merr. Et Perry L)

Organoleptic Parameters	Result
Simplisia	
Color	Dark brown
Odor	Aromatic typical of clove leaves
Taste	Spicy and bitter
Extract	
Color	Brownish black
Odor	Aromatic typical of clove leaves
Taste	Bitter, sharp and pungent taste

Determination of Content of Soluble Substances for in Simplisia and extract of Clove Leaves

The ability of each plant to dissolve in water or organic solvents is different, which aims to facilitate the handling of material when it is to be used in its dissolved form. The solvents used in this test are water and ethanol, because in pharmaceutical preparations both of these solvents are commonly used mainly for making herbal preparations.

Table 2. Results of Determination of Soluble Substance Content in Simplisia and Clove Leaves Extract using water and ethanol solvent

Dissolved Substance Parameters	Dissolved Content (%)
Simplisia	
Ethanol	50
Water	1,73
Extract	
Ethanol	40
Water	4,33

Result of Determination of Total Ash Content, Water Soluble Ash Content, Acid Insoluble Ash Content for Simplisia and Clove Leaves Extract

Ash is an inorganic substance left over from the combustion of an organic material. Ignition process that is all organic substances will burn into black charcoal, with continuous heating, all organic substances (charcoal) will be burned out and ash will be obtained in the form of the remaining substances consisting of inorganic substances in the form of metal oxides. The growth process of a plant in nature requires nutrients, including those from minerals and other organic compounds.

The parameter to determine the type of nutrient that is predominantly attracted to a plant is the water soluble and insoluble ash content. Water-soluble ash content is obtained by dissolving the ashes in water and which is not dissolved filtered using ash-free filter paper (Whatman 42). This ash-free filter paper has low inorganic content and leaves only a very

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small amount of ash weight when burned, which is ± 0.13 mg. Filter paper and residue are resurfaced at 600°C until the ash weight is constant, while the acid insoluble ash content is the total ash obtained which is dissolved into acid, which is HCl 3N. Insoluble ash is filtered using ash-free filter paper and resumed to a constant ash weight.

Table 3. Test Results for Total Ash Content, Water Soluble Ash Content, Acid Insoluble Ash Content for Simplisia and Clove Leaves Extract

Test Parameter	Result (%)
Simplisia	
Total Ash Content	11.4
Soluble Ash Content	16.9
Acid Insoluble Ash Content	9.5
Extract	
Total Ash Content	22
Soluble Ash Content	17.5
Acid Insoluble Ash Content	10.5

Results of Drying Shrinkage Test for Simplisia and Clove Leaves Extract

The purpose of the drying shrinkage test, knowing this parameter is to determine the amount of material to be used in a dry state. This is useful at the time of making simplisia, so that the simplisia that is formed is durable and not overgrown with fungi, as well as on extracts that want to be used as a basic ingredient in making herbal preparations. In this drying shrinkage test, simplisia is dried in an oven at 10°C so that the solvents still present in the sample, both ethanol and water, can evaporate.

Table 4. Results of Simplisia Drying Shrinkage Test and Leaves Cloves leaves extract (*Syzygium Aromaticum* Merr. Et Perry L)

Test Parameter	Result (%)
Simplisia	7.6
Extract	11.46

Secondary Metabolic Profile of Ethanol Extract of Clove Leaves

The secondary metabolite test uses spray reagents by spraying the reactants onto the TLC plate until it is transparent, then u are seen shining UV 366 nm and 254 nm or both. The secondary metabolites to be tested are steroids, flavonoids, phenolic, alkaloids and aromatic acids. Profile of secondary metabolites of ethanol extract, ethanol extract of Clove Leaves (*Syzygium Aromaticum* Merr. Et Perry L).

Received :2020-05-08 Revised : 2020-05-16 Acceptance : 2020-05-19 Publish : 2020-06-05

Tabel 5. Phytochemical Screening Results

No	Test Parameter	Result
1	Alkaloid	+
2	Steroid	-
3	Flavonoid	-
4	Phenolic	-
5	Aromatic Acid	+

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The results showed that the ethanol extract showed a positive alkaloid reaction characterized by the presence of white precipitate on the addition of Meyer reagents and there was a brick red precipitate on the addition of the Dragendorf reagents[14]. Clove leaves have alkaloids, flavonoids and phenols, in contrast to results that only have alkaloids and aromatic acids. This difference can occur due to temperature and climate and the condition of the soil where the clove leaves grows**.**

CONCLUSION

1. Organoleptic simplisia clove leaves has a dark brown taste rather spicy and bitter and aromatic odor typical of cloves, and clove leaves extract is brownish black to taste a little taste and bitter aromatic odor typical of cloves
2. Clove leaves ethanol extract contains alkaloids compound and aromatic acids
3. Percentage of water soluble ash content of simplisia and clove leaves extract are 16.9 and 17.5%, percentage of water soluble acid content of simplisia and clove leaves extract are 9.5 and 10.5%
4. Percentage of simplisia and clove leaves extract in drying losses, i.e, 7.6 and 11.46%.

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