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ANALYSIS OF GRINGSINGAN (Hyptis suaveolens L. Poit.) SECONDARY METABOLITE COMPOUNDS AND THEIR POTENTIAL AS LOCAL TIMOR TRADITIONAL MEDICINE

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ABSTRACT

The weed Hyptis suaveolens L. Poit.in East Nusa Tenggara can be easily found, it can grow anywhere, such as on the side of the road or even on dry land. In Kupang City, is known as a weed and nuisance plant that grows wild on roadsides or former fields. The main aim of this research is to determine the content of secondary metabolite compounds in the roots, stems, and leaves of H. suaveolens. The method used was phytochemical screening by testing the content of secondary metabolite compounds in the roots, stems, and leaves of H. suaveolens. The research found that the roots, stems, and leaves of H. suaveolens positively contain secondary metabolite compounds, namely alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, and quinones. Therefore, this local species was potentially to be used as basic ingredients for a medicinal plant as well as as a natural insecticide. However, further research was needed to test the antibacterial activity, and its effectiveness as a medicinal plant, and to confirm the efficacy and determine safe doses as natural medicines.

Keywords: Hyptis suaveolens, phytochemical screening, metabolite compounds, medicinal plant

INTRODUCTION

Plants are one of the biological resources that can be used as food, clothing, shelter, medicine, cosmetics, and so on. Various types of plants can be found around us, so they can be used to meet our needs, one of which is a type of plant that has the potential to be used as medicine. The use of plants as medicine is based on the daily experiences of people in a place, which is part of the cultural system in developing public health, which is supported by new thinking about back to nature. Plants that have the potential to be used as medicine are easily obtained in nature without spending much money and can also be cultivated both on large areas of land and in home gardens. Apart from that, plants that have the potential to be used as medicine are popular with the public because they rarely cause side effects, can be used as first aid, and cure diseases without medical assistance or modern medicines, and the way they are used is simple based on daily habits and experiences that can be passed down from generation to generation.

Plants used as medicinal raw materials are closely related to the chemical content contained in them, especially bioactive compounds. Bioactive compounds found in plants are generally secondary metabolite compounds. Secondary metabolites are biosynthetic compounds derived from primary metabolites produced by organisms through secondary metabolism. Examples of secondary metabolites are flavonoids, alkaloids, terpenoids, steroids, and others. One species of plant that has potential as medicine, which was used by the community was *Hyptis suaveolens* L. Poit. (Gringsingan). *H. suaveolens* (Lamiaceae) is a flowering herb that grows wild, lives annually, has an upright stature, and can reach 1.5 m. This species was able to grow in the lowlands up to an altitude of 1,000 meters above sea level. *H. suaveolens* is an aromatic annual herb distributed in tropical and subtropical areas. This plant is generally considered a weed that grow in disturbed habitats. *H. suaveolens* originates from Tropical America but is now widespread throughout the world because it has a very fast spreading ability (Barbosa et al. 2013). *H. suaveolens* is a weed that is very easy to find; it can grow anywhere, such as on the side of the road, even on dry land. This plant is a weed on roadsides and cultivated, grasslands, open forests, riverbanks, flood plains, coastal areas, disturbed locations, and waste areas. Due to its unpalatability, *H. suaveolens* has the ability to dominate good, native grasslands, especially when these grasslands are overgrazed.

Despite its detrimental impacts, *H. suaveolens* has been considered a beneficial plant due to its fungicidal activity (Cowie 2012), suppression of bacterial growth, and inhibition of weed seed germination (Schwarzkopf et al. 2009). This species has potential economic value for small farmers. In Southern Benin, both aqueous extracts and live specimens of *H. suaveolens* have been shown to significantly reduce population densities of the stem borer (*Sesamia calamistis*, a problematic pest that inhibits maize production (Adda et al. 2011). In several regions of Asia, farmers use this species to protect livestock from pests (Handayani 2003). The leaves can be used as a bed bug repellent. In many areas of Central America, *H. suaveolens* seeds are made into a drink (Standley and Williams 1973). In Mexico, this species is also used in the treatment of cataracts (Irigoyen-Rascón 2015), and as poultry feed by the Maya of Yucatán. In addition, antimicrobial, antifungal, hypoglycemic, anti-inflammatory and antioxidant activities have been found in this species (Rojas Chavez and Vibrans 2011). In Benin, this species is used externally in combination with other medicinal plants to treat jaundice, hyperthermia, hemorrhoids, breast abscesses, perianal edema and candidiasis (Adjanohoun et al. 1989), and in the treatment of sexually transmitted diseases. In Nigeria, this plant is used in the treatment of coughs, fever, and anemia (Odugbemi 2008).

Handayani (2003) has mentioned some of the uses of this species in Asia. In some parts of this continent, the leaves and stems are used to treat wounds, eczema, bruises, and other skin diseases. In the Philippines, the leaves are used externally to treat rheumatism, and internally as an antispasmodic. The leaves and roots are used as an insecticide and against rheumatism, respectively. In Papua New Guinea, the leaves are used internally to treat catarrh and fever. In Thailand, crushed leaves and branches are used as a flea repellent for chickens. The entire plant is sometimes used as animal feed (Handayani 2003).

H. suaveolens is included in the vegetable pesticides, because it contains antimicrobial compounds, both antibacterial and anti-fungal, and is known as a plant that is efficacious as an antiseptic, cancer and skin disease. Apart from that, it is also used as a cooking spice and can increase milk flow (Heyne 1986). In India, the leaves of *H. suaveolens* twigs are used as an antiseptic for burns, antirheumatism, and as a treatment for skin diseases. The smoke from burning dry leaves can repel mosquitoes. Almost all parts of *H. suaveolens* can be used as traditional medicine to treat diseases (Shenoy 2009). Meanwhile, in Brazil it is popular as a treatment for respiratory infections, digestive disorders, colds, pain, fever, and skin diseases and the leaves are used as an anticancer and antifertility drug (Moreira et al. 2010). Chatterjee and Pakrashi (1997) reported that *H. suaveolens* leaves have anti-inflammatory activity and are also applied as an antiseptic on burns, wounds and various skin complaints. This plant is also used to treat respiratory tract infections, colds, pain, fever and skin diseases (Mabberley 1990; Iwu 1993). Ethanol extract from the leaves shows healing properties that support antioxidant enzymes (Annie et al. 2003). In the past, phytochemical screening of this plant has revealed the presence of di- and triterpenoids and steroids (Manchand et al. 1973; Misra 1983).

The weed *H. suaveolens* in East Nusa Tenggara can be easily found. This weed can grow anywhere, such as on the side of the road or even on dry land. *H. suaveolens*, especially in Kupang City, is known as a weed and nuisance plant that grows wild on roadsides or former fields. The use of this plant, especially its potential for medicinal ingredient, is not yet widely known. Therefore, research was carried out regarding the potential of these local plants to be used as medicinal plants. The main aim of this research was to determine the content of secondary metabolite compounds found in the roots, stems and leaves of *H. suaveolens*. This information is needed as a basis for developing the potential of this local plant in the future.

MATERIALS AND METHODS

The research was conducted in the biology laboratory of the Faculty of Science and Engineering (FST) Nusa Cendana University in 2023. The method used in this research was analytical descriptive. Samples of *H. suaveolens* were taken from plants growing in abandoned areas around Kupang, NTT. Meanwhile, information on the potential use of the *H. suaveolens* as a medicinal ingredient by the community in Kupang, NTT was carried out by interviewing several local communities. Analysis of the secondary metabolite compound of the roots, stems and leaves of *H. suaveolens* was carried out by taking samples of the roots, stems, and leaves as needed. The plant samples taken were placed in a plastic bag, then taken to the biology laboratory of the Faculty of Science and Engineering (FST) Nusa Cendana University for analysis of the content of secondary metabolite compounds using the following steps:

Extraction

Roots, stems and leaves of *H. suaveolens* collected from the field, washed thoroughly, drained. The roots were dried in the sun for 5 days, while the leaves were air-dried at room temperature. The dried roots and leaves were crushed using a mortar, sifted using a sieve. Once finely ground, 230 gr of gringsingan root, stem and leaf powder was weighed, then macerated with 850 ml of 75% ethanol solvent for three days at room temperature, stirring once every 24 hours (Yuda et al. 2017). The filtrate was filtered using filter paper to separate the filtrate and macerate. The filtrate obtained was concentrated using a rotary evaporator at room temperature until a thick extract was obtained. This thick extract was used in the analysis of secondary metabolite compounds.

Analysis of secondary metabolite content (Phytochemical screening)

- Alkaloid Test. About 2 ml of the test solution was evaporated on a porcelain cup to obtain residue. The residue was then dissolved in 5 mL of HCl_2N. After cooling, the solution was filtered. The solution obtained was divided into 3 test tubes. Tube 1 is used as a control, tube 2 is added with 3 drops of Dragendroff's reagent, if a red or orange precipitate forms, it indicates the presence of alkaloid compounds. In the third tube, 3 drops of Mayer's reagent were added (through the tube wall). If a yellow/white precipitate forms in the third tube, it indicates the presence of alkaloids (Djamil et al. 2020).
- Flavonoid Test. A total of 1 ml of each test solution was put into 3 test tubes. Tube 1 is a control, tube 2 is added with 1 mL of 10% Pb acetate (lead acetate) solution, positive for flavonoids if there is a yellow precipitate. Tube 3 was added with several drops of 20% NaOH to form a yellow color if it contained flavonoids (Ugochukwu et al. 2013).
- Tannin Test. A total of 2 ml of each test solution was put into 2 test tubes, tube 1 as a control and tube 2 with a few drops of 5% $FeCl_3$ solution added, a positive sign of tannin if there is a green violet/blue color.
- Triterpenoid/steroid test. About 2 ml of the test solution was evaporated in an evaporator cup until a residue was obtained. To the residue were added 2 drops of anhydrous acetic acid and 1 drop of sulfuric acid (concentrated) (Liebermann-Burchard reagent). The formation of a green or red color indicates the presence of steroid or triterpenoid compounds (Djamil et al. 2020).
- Saponin Test. A total of 4 ml of the test solution was put into a test tube, 5 ml of distilled water was added, then shaken vertically for 10 seconds, then left for 10 minutes. The formation of stable foam in the test tube indicates the presence of saponin group compounds. When added 1 drop of 1% HCL (dilute) the foam remains stable. About 4 ml of the test solution was placed in a test tube as a control (Djamil et al. 2020).

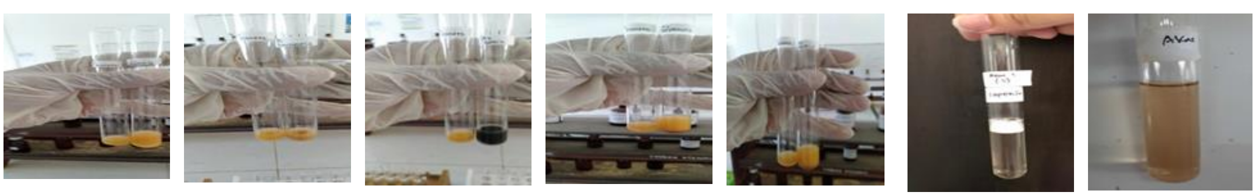
- Anthraquinone/Quinone Test. A total of 50 mg of extract plus 10 ml of water is then heated for 5 minutes and filtered. A total of 3 ml of solution was put into 2 test tubes, tube 1 was added with a few drops of 1 N NaOH solution to form an intense red color indicating the presence of quinone group compounds and tube 2 was used as a control (Djamil et al. 2020).

The results of the metabolite content analysis obtained were analyzed descriptively and tabulated in the form of tables and figures, then a literature review was carried out relating to the potential for *H. suaveolens* to be used as a medicinal plant.

RESULTS AND DISCUSSION

Based on the results of tests for secondary metabolite compounds (phytochemicals), it was found that the roots (Figure 1), stems (Figure 2), and leaves (Figure 3), of *H. suaveolens* all contain secondary metabolite compounds: Alkaloids, Flavonoids, Tannins, Terpenoids, Steroids, Saponins, and Quinones. Phytochemical tests of the roots, stems and leaves of *H. suaveolens* showed positive results, namely white precipitate was formed, color changed, foam formed when the reagent was given/dropped. This result was in line with research conducted by Edeoga et al. (2006) which stated that *H. suaveolens* was rich in chemical bases such as alkaloids, flavonoids, saponins, and tannins. Each test for secondary metabolite compounds produces a different color. Differences in phytochemical screening results can be caused by differences in the sensitivity of the test method used to the amount of chemical content of the natural ingredients tested. Extracts of various parts of *H. suaveolens* have been obtained with solvents such as petroleum ether, chloroform, methanol, ethanol, n-hexane and water using the Soxhlet extraction method, cold maceration and steam distillation and phytochemical screening using standard methods has been carried out by several researchers. The results obtained showed that secondary metabolite compounds such as alkaloids, flavonoids, terpenoids, tannins, were always present in extracts from all parts of the plant, while saponins were only found in the stems and leaves, while they were not found in the roots (Ngozi et al. 2014).

Alkaloids	Flavonoids	Tannins	Terpenoids	Steroids	Saponins	Quinones



Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
A white and cloudy precipitate forms when Mayer's reagent is dropped on it	A yellow precipitate was formed when Pb Acetate solution was added	A dark green color is formed when $FeCl_3$ is added	A brick red color is formed when anhydrous acetic acid and H_2SO_4 are dropped	A brick red color is formed when anhydrous acetic acid and H_2SO_4 are dropped	After adding 1% HCL (dilute) and shaking vigorously, a stable foam (+) is formed.	After adding a few drops of 1N NaOH, a red (+) solution was formed.

Figure 1. Qualitative test of secondary metabolite compounds of *H. suaveolens*: Root extract

Alkaloids	Flavonoids	Tannins	Terpenoids	Steroids	Saponins	Quinones
Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
A white and cloudy precipitate forms when Mayer's reagent is dropped on it	A yellow precipitate was formed when Pb Acetate solution was added	A dark green color is formed when $FeCl_3$ is added	A brick red color is formed when anhydrous acetic acid and H_2SO_4 are dropped	A brick red color is formed when anhydrous acetic acid and H_2SO_4 are dropped	After adding 1% HCL (dilute) and shaking vigorously, a stable foam (+) is formed.	After adding a few drops of 1N NaOH, a red (+) solution was formed.

Figure 2. Qualitative test of secondary metabolite compounds of *H. suaveolens*: Stem extract

Alkaloids	Flavonoids	Tannins	Terpenoids	Steroids	Saponins	Quinones
Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
A white and cloudy precipitate forms when Mayer's reagent is dropped on it	A yellow precipitate was formed when Pb Acetate solution was added	A dark green color is formed when $FeCl_3$ is added	A brick red color is formed when anhydrous acetic acid and H_2SO_4 are dropped	A brick red color is formed when anhydrous acetic acid and H_2SO_4 are dropped	After adding 1% HCL (dilute) and shaking vigorously, a stable foam (+) is formed.	After adding a few drops of 1N NaOH, a red (+) solution was formed.

Figure 3. Qualitative test of secondary metabolite compounds of H. *suaveolens*: Leaf extract

A summary of the results of the analysis of secondary metabolite compounds in the roots, stems and leaves of *H. suaveolens* taken from habitats in the Kupang area, NTT was shown in Table 1.

Table 1. Summary of test for secondary metabolite compounds (phytochemicals) of roots, stems and leaves of *H. suavelolens* of local plants on Timor Island

Secondary metabolite compounds	Parts of plants		
	Roots	Stems	Leaves
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannin	+	+	+
Terpenoids	+	+	+
Steroids	+	+	+
Saponins	+	+	+
Quinones	+	+	+

Note: + = contains secondary metabolite compounds

- **Alkaloids.** Alkaloid testing on the roots, stems, and leaves of *H. suavelolens* was carried out by dropping Mayer's reagent and a white and cloudy precipitate was formed. This is in line with research conducted by Wardhani and Supartono (2015), who tested the alkaloid group by applying Mayer's reagent to rambutan fruit peel powder, and showed a positive response, namely white precipitate appeared. The presence of alkaloid compounds was indicated by the formation of a white precipitate with the addition of Mayer's reagent. It is estimated that the deposit is a potassium-alkaloid complex. In the alkaloid test with Mayer's reagent, it is estimated that the nitrogen in the alkaloid will react with the K+ metal ion from potassium tetraiodo-mercurate (II) to form a potassium alkaloid complex which precipitates. Alkaloids are secondary metabolite compounds that have the most nitrogen atoms, these compounds are often found in plant and animal tissues. Alkaloid compounds mostly come from plants. Alkaloids are alkaline, so they can replace mineral bases in maintaining ion balance in plants. Alkaloids in plants function as poisons that can protect plants from herbivores and insects, growth regulatory factors and storage compounds that can supply nitrogen and other elements that plants need. Alkaloids can be found in various parts of plants, such as roots, stems, bark, twigs, leaves, flowers, fruit, seeds (Andika et al. 2020). Alkaloid compounds are efficacious as anti-cancer, anti-inflammatory, antitoxin, aphrodisiac, heart medicine, burn medicine, and others. Alkaloids are used as anti-diabetic, anti-diarrhea, anti-malarial and anti-microbial, however, alkaloid compounds need to be identified because some alkaloid compounds are toxic (Andika et al. 2020). Alkaloids are of great importance in medicine and constitute some of the most valuable medicines. They have marked physiological effects on animals (Edeoga and Eriata 2001). Alkaloids such as solasodine have been indicated as starting materials in the manufacture of steroid drugs (Maxwell et al. 1995). Phenolic compounds are widespread in the plant kingdom and the presence of phenol is considered potentially toxic to the growth and development of pathogens. Saponins are glycosides of triterpenes and sterols and have been detected in more than seventy plant families (Basu and Rastogi 1967). In medicine, to some extent it is used as an expectorant and emulsifying agent.

- **Flavonoids.** Testing for flavonoid compounds in the roots, stems, and leaves of *H. suavelolens* was carried out by adding Pb Acetate solution and showed positive results with the formation of a yellow precipitate. Flavonoids are compounds that contain two aromatic rings with more than one hydroxyl group. Phenolic compounds with more hydroxyl groups have greater levels of solubility in water or are polar, so they can be extracted in polar solvents (Robinson 1995). Flavonoids are found in all vascular plants. Flavonoids include anthocyanins, flavonols, and flavones. Plants generally contain good flavonoids in both vegetative and generative parts. The function of flavonoids is that they can absorb ultraviolet light to direct insects, regulate plants, regulate photosynthesis, work anti-microbial and anti-virus and work against insects. Robinson (1995) stated that the active components of flavonoids can inhibit bleeding and have antibacterial capabilities which can directly interfere with the function of microorganism cells and inhibit the life cycle of microbial cells. Flavonoids are deliberately produced by plant tissue in response to infection or injury which then functions to inhibit the function of attack. Flavonoid compounds function as antioxidants, anti-microbial, anti-viral for plants, anti-HIV, anti-tumor, anti-fungal, analgesic, anti-diarrheal, anti-hepatotoxic, anti-inflammatory, immuno-stimulant, anti-hyperglycemic, as a vasolidator, medicine for wound infections, anti-viral, anti-fungal, anti-bacterial, cytotoxic, antihypertensive, anticancer, antiallergic, antioxidant and anti-inflammatory (Andika et al. 2020). The flavonoids found in fresh fruit and vegetables can be useful as medicine, one of which is to reduce the risk of stroke and heart disease. Flavonoid compounds can inhibit the growth of cancer cells (Andika et al. 2020). Flavonoids are 15 carbon compounds that are generally distributed throughout the plant world (Harborne 1988). Some isoflavones act as allelochemicals that are widely used in insecticides. They may also play a role in disease resistance (Salisbury and Ross 1992).
- **Tannin.** Testing for tannin compounds in the roots, stems and leaves of *H. suavelolens* was carried out by adding $FeCl_3$ and a dark green color was formed. A phytochemical test using $FeCl_3$ is used to determine whether the sample contains phenol groups. The presence of a phenol group is indicated by a blackish green or dark blue color after adding $FeCl_3$ so that if the phytochemical test with $FeCl_3$ gives a positive result, it is possible that the sample contains phenolic compounds, and it is possible that one of them is tannin because tannin is a polyphenolic compound. Harborne (1987) stated that the classic way to detect simple phenolic compounds is adding the extract with a 1% $FeCl_3$ solution in water, which produces strong green, red, purple, blue and black colors. The formation of a blackish green or inky blue color in the extract after adding $FeCl_3$ is because tannin will form a complex compound with $Fe3^+$ ions. This is in line with research conducted by Ergina et al. (2014), with phytochemical tests of water and ethanol extracts of palado leaves with $FeCl_3$ producing a brownish green color, because the reaction between tannin and $FeCl_3$ forms a complex compound. The formation of a complex compound between tannin and $FeCl_3$ is due to the presence of $Fe3^+$ ions as the central

atom and tannin has an O atom which has a lone pair of electrons that can coordinate with the central atom as a ligand. Tannin is a secondary metabolite compound produced by plants and synthesized by plants. Tannin has a molecular weight of 500-3000 and contains a large number of phenolic hydroxy groups which enable the formation of effective cross-links with proteins and other molecules such as amino acids, polysaccharides, nucleic acids and fatty acids (Andika et al. 2020). Tannin is also a polyphenolic organic compound which, when reacted with iron, will produce a dark color (Andika et al. 2020). Tannin compounds function as personal protection from attacks by plant-eating animals and as an antibacterial (Andika et al. 2020). Tannins are quite often found in food products of plant origin such as tea and fruit. The inhibitory activity of tannin oxidation has been known for a long time and is thought to be caused by the presence of gallic acid and diagallate (Ihekoronye and Ngoddy 1985).

- **Terpenoids.** Terpenoid testing on *H. suavelolens* leaves by dropping anhydrous acetic acid and H_2SO_4 showed positive results by showing a purple color change, while the stems produced a brick red color. The difference in color changes is due to the different chemical content in the leaves and stems of Gringsingan. Terpenoids are plant components that have an odor and can be isolated from vegetable matter by distillation, which are called essential oils. Essential oils derived from flowers were initially known from a simple structure determination, namely by the ratio of hydrogen atoms and carbon atoms of terpenoid compounds, namely 8:5 and with this ratio it could be said that the compound was in the terpenoid group (Minarno 2015).
- **Steroids.** Steroid testing on *H. suavelolens* leaves by dropping acetic acid and H_2SO_4 showed positive results, producing a purple color, while on the stems it produced a brick red color. Steroids are terpenoids whose basic framework is formed from the cyclopentane prehydrophenanthrene ring system. Steroids are a class of secondary metabolic compounds that are widely used as medicines. Steroid hormones are generally obtained from natural steroid compounds, especially in plants (Minarno 2015). Steroids are compounds derived from the hydrocarbon 1,2-Cyclopentenoperhydrophenanthrene. Steroids are found in nature in animals and plants. Steroid compounds in animals are closely related to several hormones and other biological activities, whereas in plants steroids are found in many both higher and lower plants. Steroids in plants are generally found in the form of sterols. Higher plants usually contain phytosterols such as: sitosterol (ß-sitosterol), stigmasterol, and compesterol (Harborne 1987). The content of steroid compounds in medicinal plants has been widely studied by experts. Papaya leaves (*Carica papaya* L.) contain steroid compounds in the sterol group in the form of the compound ergost-5en-3ß-ol. The sidawayah plant (*Woodfordia floribunda* Salisb.) has been known to contain the steroid type stigmasta-7,22- dien-3-ol. Mamahit (2019) isolated a steroid compound in the form of ß-sitosterol from the leaves of the gedih plant (*Abelmoschus manihot* L. Mendik). Steroids are a class of compounds that are quite important in the medical field. More than 150 types of steroids have been registered as drugs. Steroids in the medical world are used as drugs and contraceptives, for example: androgens are steroid hormones that can stimulate male sexual organs, estrogen can stimulate female sexual organs, adrenocorticonoids can prevent inflammation and rheumatism (Nogrady 1992). The stigmasterol compound can lower blood cholesterol, inhibit intestinal cholesterol absorption so that it can inhibit the development of

colon cancer and suppress liver cholesterol (Jones et al. 2000). Apart from these steroid compounds, there are many other compounds in the steroid class that are used in the medical world.

- **Saponins.** Saponins are complex glycoside compounds with high molecular weights produced by plants, lower marine animals and some bacteria. Typical properties of saponins include bitter taste, foaming in water and poisonous to cold-blooded animals. Saponin is a secondary metabolite compound contained in plants. Saponin is a phytochemical compound which has the characteristic of being able to form foam and containing polycyclic aglycones bound to one or more sugars (Majinda 2012). Root and stem extracts of *H. suavelolens* after adding Mg powder and 1 mL of concentrated HCL and shaking formed 2 layers of solution which showed the presence of saponin compounds, while in the leaves after adding 1% dilute HCL and shaking vigorously, a stable foam was formed, this shows the presence of saponin compounds. Saponins are polar because they contain sugar groups, especially glucose, galactose, xylose, which are bound to a hydrophobic aglycone (sapogenin) in the form of triterpenoids or steroid alkaloids. Based on research conducted, saponins have the second highest levels after flavonoids. More saponin extract will be produced if extracted using a polar solvent, because saponin is hydrophilic. Saponins contain polyphenols, especially saponins in high doses, causing very high antimicrobial properties. In plants, saponins are evenly distributed in their parts such as roots, stems, tubers, leaves, seeds and fruit. The chemical structure of saponin is a glycoside composed of glycon and aglycone. Saponin is a complex glycoside compound with a high molecular weight produced mainly by plants, lower marine animals and some bacteria. Saponin can reduce the surface tension of water, which will result in the formation of foam on the surface of the water after shaking. This property has similarities with surfactants. The taste of saponins is very extreme, from very bitter to very sweet and is an amphiphilic compound. Saponin can be used as a shrimp pest repellent, as a detergent, as a foam maker in shampoo, and can inhibit fungi and protect plants from insect attacks. Saponins can be antifungal by reducing the surface tension of cell walls and damaging membrane permeability. Saponin diffuses through the outer membrane and vulnerable cell walls and then binds to the cytoplasmic membrane, disrupting and reducing the stability of the cell membrane.
- **Quinones.** Quinones are colored compounds and have a basic chromophore such as the chromophore in benzoquinone, which consists of two conjugated carbonyl groups. with two carbon-carbon double bonds. The colors of quinone pigments in nature vary, ranging from pale yellow to almost black, and a number of known structures more than 450. For identification purposes quinones can be divided into four groups: benzoquinones, naphthoquinones, anthraquinones, and isoprenoid quinones. Quinone compounds occur as a slightly water-soluble glycoside, but generally quinones are more readily soluble. It is fat soluble and will be extracted from crude plant extracts together with carotenoids and chlorophyll. Anthraquinone and quinone compounds have ability as an antibiotic and pain reliever as well as stimulating growth new cells in the skin (Noer and Pratiwi 2016). Quinones in the roots, stems and leaves of Gringsingan, after adding a few drops of 1 N NaOH, a red solution is formed, which indicates the presence of quinone compounds. Quinones are a derivative of phenolic compounds which show biological and pharmacological activities including antifungal, antimalarial, antibacterial, anticancer and antioxidant (Mutrikah et al. 2018; Ulfah et al. 2018). Quinones are divided into 4

groups, namely benzoquinones, naphthoquinones, anthraquinones, and isoprenoid quinones. To identify quinone compounds in plants, a component separation process is carried out based on polarity or fractionation. One method of fractionation is chromatography, there is flat liquid chromatography (KLT, KKt) and column chromatography (KKG, KCV). Various types of medicinal plants are thought to contain quinone compounds or their derivatives such as anthraquinones or naphthoquinones. Research conducted by Luo et al. (2016) shows that Hedyotis contains quinone derivatives, namely anthraquinones. Apart from Hedyotis, *Aloe vera* also contains anthraquinones based on research conducted (Kang et al. 2017). Other medicinal plants that also contain anthraquinones are *Morinda citrifolia* L (Rudiyansyah et al. 2012) and *Ceratotheca triloba* (Mohanlall et al. 2011). Meanwhile, another group of quinones, namely naphthoquinones, belong to the Bisbul plant (*Diospyros anisandra*) (Gabriela et al. 2020).

H. suaveolens contains essential oils, which cause all parts of the plant to smell good when squeezed, and also cause a sticky feeling. The presence of essential oils in *H. suaveolens* from Timor has been researched by Mali et al (2020), who stated that the components of the essential oil of *H. suaveolens* from the island of Timor are trans-caryophyllene, 1,8-cineole, caryophyllene oxide, 3-cyclohexene-1 ol , 4-methyl-1-1 and 2,7,111-cyclotetradecatrien-1ol with the main component being trans-caryophyllene (16.4%). *H. suaveolens* leaf oil from Timor provides new chemotype compounds that are not found in plants from other countries or regions. The essential oil obtained is yellow in color and has a distinctive smell (typical oil).

From the discussion above, it can be seen that *H. suaveolens* contains chemical compounds that have the potential to be used as medicines. Further research to prove and support the results of this research is necessary to develop *H. suaveolens* as a safe and effective medicinal ingredient. Based on the results of field observations, *H. suaveolens* has been used by the community as a medicinal plant to cure various types of diseases, although still on a small scale. Its use is based on community experience obtained from generation to generation, as local knowledge that is passed down and conveyed only orally. The results of interviews with several people showed that *H. suaveolens* can be used to cure diseases, such as diarrhea, inflammation, influenza, sinusitis, coughs and cancer. This plant can also be used as a natural herbicide to repel insects (mosquitoes). All parts of *H. suaveolens* can be used, but the most widely used are leaves, twigs and stems. In its use, the *H. suaveolens* part used is done by boiling all the parts, the boiled product is drunk, apart from that, it is also blended and the results are filtered, drunk, and can also be eaten directly by adding a little salt, for the treatment of internal diseases. Meanwhile, for external treatment, hyptis leaves are crushed, the water squeezed out is drunk, placed on the wound, and also dripped on the nose to treat flu or sinusitis. As a mosquito repellent, dry leaves and fresh leaves are burned which produces fragrant smoke, which mosquitoes don't like.

The potential of *H. suaveolens* as a drug in the prevention and treatment of disease has also been widely used in various parts of the world.

- **Antioxidant activity**. The antioxidant activity of hyptis leaf methanol extract has been evaluated in vitro by scavenging 1,1 –diphenyl-2-picrylhydrazyl (DPPH) radicals using gallic acid; powerful free radical scavenger butylated hydroxyanisole (BHA); antioxidants known as reference standards (Ngozi et al. 2014). Antioxidant activity is expressed as the IC50 value, which is the sample concentration required to inhibit 50% of DPPH free radicals. The results obtained show that *H. suaveolens* has strong antioxidant radical scavenging activity with an IC50 value of 14.04ugmL-1. The antioxidant activity of methanol extract can be attributed to the presence of flavonoids which are known as strong antioxidants with an IC50 of 3.75mgmL-1 (Ngozi et al. 2014).
- **Antimicrobial activity**. *H. suaveolens* has phytochemicals that are effective against certain fungi such as *Aspergillus niger, Candida albicans, Rhizopus stolonifera, Cryptococcus* and Fusarium. The research results showed that plant bioactive agents were more effective in inhibiting the growth of griseofulvin isolates, an antifungal drug. Extracts of all parts of the plant provide the highest antimicrobial activity compared to stems and roots in chloroform and methanol extracts (Ngozi et al. 2014).
- **Antidiarrheal activity**. Diarrhea is one of the main causes of high mortality rates in developing countries, where more than five million children under the age of five die every year from severe diarrheal diseases. Three to five billion cases occur each year (Ngozi et al. 2014), and approximately five million deaths are caused by diarrhea. The antidiarrheal activity of the ethanol extract of *H. suaveolens* leaves showed good results. Oral administration of the extract (250 and 500) mg/Kg showed significant inhibitory activity depending on the dose used. The antidiarrheal activity of the plant extract at a higher dose (500 mg/Kg) was comparable to the antimotility drug, loperamide at a dose of 50 mg/Kg (Ngozi et al. 2014).
- **Anthelmintic activity**. The in vitro anthelmintic activity of the whole plant extract of *H. suaveolens* has been investigated (Ngozi et al. 2014). The results of the study showed that ethanol and aqueous extracts of the plant were investigated for activity against adult Indian earthworms; *Pheretima posthuma* and *Ascardia galli*, based on the time of paralysis and time of death of the worm, by recording no movement, and death when the worm loses its motility followed by fading of body color. *H. suaveolens* extract showed significant anthelmintic activity at the highest concentration of 100 mg/ml.
- **Antidiabetic activity**. Aqueous extracts, methanol and ethanol of the plant, have been used to monitor their effects on alloxan-induced diabetic rats. Results showed a significant reduction in blood glucose concentration indicating that *H. suaveolens* has antidiabetic activity (Ngozi et al. 2014), which may be related to the presence of tannins, terpenoids, and flavonoids.
- **Anti-inflammatory activity**. Anti-inflammatory activity of two diterpenes, suaveolol and methyl suaveolate isolated from *H suaveolens* leaves by colon chromatigraphy and thin layer chromatography. The anti-inflammatory activity of the compound was tested as an inhibitor of croton oil-induced mouse ear dermatitis. The dose given was compared with the non-steroidal anti-inflammatory drug indomethacin. Anti-inflammatory activity is expressed as a reduction in edema in mice treated with the tested substance compared to control mice. The results showed that suaveolol and methyl suaveolate were only two to three times less active than indomethacin. The anti-inflammatory

properties of diterpenes are considered to be contributors to the antiphlogistic activity of *H suaveolens* extract, thus confirming the use of *H. suaveolens* in dermatological diseases (Ngozi et al. 2014).

- Natural insecticides have long been touted as an alternative to synthetic chemical insecticides for pest control because they do not pose a threat to the environment and human health (Ngozi, et al. 2014). *H. suaveolens* has been reported to be effective against infestations of the young stem borer *Sesbania calamistis*, in maize; has been used to control *Trogoderma granarium*, in stored peanuts (Ngozi, et al. 2014). Also plant methanol extract is effective in biological control of *Sitophilus oryzae*, *Sitophilus zeamays* and *Callosobruchus maculatus* which are serious stored product pests that attack various economically important crops. Essential oils are effective against adult granary *Sitophillis beetles* (Ngozi et al. 2014). Protease inhibitors isolated from *H. suaveolens* seeds have high activity against intestinal trypsin-like proteases of various insect pests, especially against *Prostephanus truncatus*, an important insect pest of corn. Research on its use for protection against mosquito bites shows it to be as effective as DEET, a well-known arthropod repellent (Ngozi et al. 2014). The ability of *H. suaveolens* as an effective insecticide or pesticide is associated with its essential oil.

Based on the research results, it can be concluded that: the roots, stems and leaves of *H. suaveolens* contain secondary metabolite compounds, namely alkaloids, flavonoids, tannins, terpenoids, steroids, saponins and quinones. Root, stem, and leaf extracts of *H. suaveolens* positively contain secondary metabolite compounds which have the potential to be used as basic ingredients for medicines. *H. suaveolens* has potential as a medicinal plant in treating diarrhea, anti-inflammatory diseases, coughs, colds, sinusitis, asthma, diabetes, and cancer, as well as as a natural insecticide. Further research is needed to test the antibacterial activity of *H. suaveolens* extract; to test the effectiveness of *H. suaveolens* extract as a medicinal plant, and to confirm the efficacy and determine safe doses as natural medicines.

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