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CHEMICAL COMPONENTS ANALYSIS OF CLOVE (*Syzygium aromaticum* (L). Merr. & Perry)

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ABSTRACT

Cloves (*Syzygium aromaticum (L.) Merry & Perry*) is one of the plant species in the genus Syzygium which has a lot of content of secondary metabolites which have the potential as a medicinal ingredient . Chemical component composition and special identification for clove varieties of afo, gorontalo and tuni buru selatan has not been reported. This research aims to identify the chemical components of the flowers, flower stalks and leaves from three varieties of cloves. Extraction and maceration of clove samples were carried out in the organic chemistry laboratory of the Chemistry Department IPB. The characterization of chemical components by the GC-MS method was carried out in the analytical laboratory of the UPI Bandung The analysis showed that the main component of clove is eugenol (phenolic) contained in all varieties of clove (gorontalo (81.16%), afo (80.32%) and tuni buru selatan (72.18%). The compound dominant in flowers, flower stalks and clove leaves are components of karyophilene (sesquiterpen) non-phenolic compounds.

Key Words**:** *Clove, identification, chemical components*

preliminary

Secondary metabolism is a part of plant metabolic processes which aims to prepare metabolites as the body's defense against pests and diseases. According to Li et al. (2016), secondary metabolisme defined as organic compounds that are not directly involved in the growth, development, and reproduction of an organism, but is responsible for the interaction between the organism that producesthe environment to avoid environmental stress and disturbing organisms. *Syzygium aromaticum* is one of the plant species in the genus *Syzygium* which has a lot of content of secondary metabolites which have the potential as medicinal ingredients.

The state of a region greatly affects the composition chemical component of a plant. According to Figueiredo *et al* . (2008), The composition of secondary metabolites in plants containing volatile compounds such as essential oils are influenced by several factors including physiological variations, environmental conditions, geographical variations, genetic factors and evolution . The distribution of metabolites in inflorescences and root parts is mainly influenced by variations in some soil nutrients such as Ca, Mg, P, K and Cu (Sampaio *et al* . 2015).

Afo cloves growth on location the hillside of Gamalama mount (800 - 1000 m asl) in Ternate Island, North Maluku. North Maluku is a strip of origin clove, its center located at Ternate, Tidore, Halmahera Makean and Bacan. Afo clove is one of the oldest clove species in the world including clove zanzibar elders. Characteristics of clove afo var. is oval shaped leaves, dark green for old leaves, yellowish red for young leaves, untidy branch form and reddish yellow flowers, 18–27 flowers for each bunch (Suparman *et al* . 2017).

Clove of gorontalo var. is included in the clove zanzibar category . This clove has the advantage of high production and its aroma is widely favored by many clove cigarette manufacturers. Cloves of gorontalo varieties have wet flower production higher than Afo clove and composite zanzibar karo (Supriyadi *et al.* 2012).

Clove of tuni buru selatan vaieties have a morphology that can be distinguished by the variety of zanzibar (afo and gorontalo) with a single main stem, high branching, largest flower size, flower color picking ripe cream or beige with a slight redness on the base of the crown, the sharp scent of flowers. Clove of tuni buru selatan varieties are selected from a population of cloves sashes, has habistus upright, with a plant height of 12-15 m and form a single plant and main stem. The stem size is 88-180 cm with cylindrical canopy shape to a somewhat cylindrical shape. The age of the plant can reach 30-40 years.

Chemical component composition and special identification of clove especially for varieties of afo, gorontalo and tuni buru selatan is not been reported. Therefore, this study aims to identify the chemical components of the flowers, flower stalks and leaves from three varieties of cloves.

Research purposes

This study was conducted to knowing the chemical components from methanol extracts of flowers, the flower stalk and leaf of clovers from afo, gorontalo and tuni buru selatan variety

Research methods

Place and time of research

This research was conducted in June 2016 until July 2017. Extraction, maceration and phytochemical tests of clove samples were carried out in the organic chemistry laboratory of the Bogor Agricultural University. The

characterization of chemical components by the GC-MS method was carried out in the analytical laboratory of the UPI Bandung.

***GC-MS* analysis of clove methanol extract**

Analysis was carried out using GC - MS Shimadzu , temperature column 60°C, injection temperature 280°C, pressure 80.2 kPa, total flow: 264.7 mL/min, column flow: 1.31 mL/min, linear velocity: 41.7 cm/sec. The clove ingredients used are methanol extract of flowers, flower stalks and clove leaf from vaieties of afo, gorontalo and tuni buru selatan.

Results

Results of Chromatogram Analysis

The results of the chromatogram analysis from GC-MS showed that in each of the methanol extracts of flowers, flower stalks and clove leaves from three varieties contained groups of compounds with different components and percentages.

Chromatogram of clove flower extract from afo ,gorontalo and tuni buru selatan varieties shows several peaks with a retention time of 10.479-15.453 (Figure 1). The retention time of eugenol (phenolic) as the main component is (Rt 10.468-11.930) . The group of non-phenolic compounds (sesquiterpen) has a retention time from 11.471-15.453.

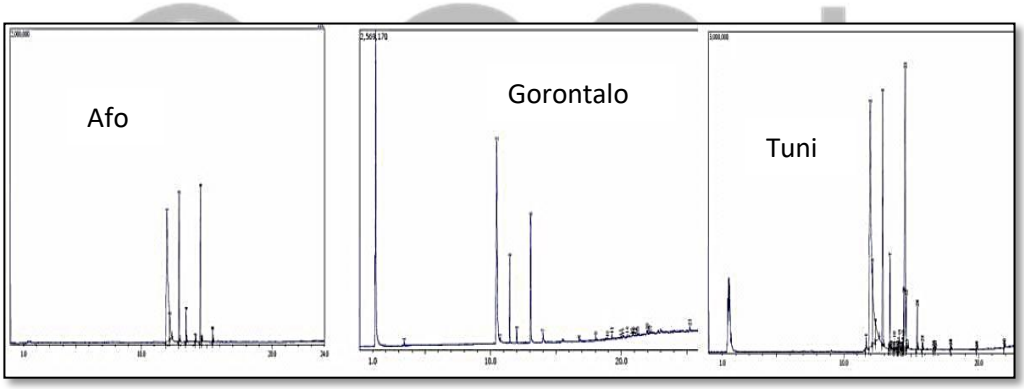

Figure 1 Chromatogram of methanol extract for clove flowers from varieties of afo, gorontalo and tuni buru selatan

The eugenol component is the main component in all parts of the plant. The methanol extract of clove flowers is dominated by a group of non-phenolic compounds consisting of karyophilene, trans-karyophilene and karyophylene oxide. The percentage of the phenolic component compounds , acetisoeugenol and Aceteugenol (64.21%), were higher than the non-phenolic compounds (42.68%) which consisted of karyophilene, trans-karyophilene and karyophylene oxide ((Table 1).

In addition there are major components of cloves and components of the dominant compound, several other minor compounds are also detected in each variety. Methanol extract of clove flower from afo varieties is detected to contain compounds α- deculena and α- cubebena, gorontalo vareties contains α-

humulena, tetrapentacontana and copaena , varieties of tuni buru selatan detected α-deculena, α-copaena, delta-cadinena and α-cubebena.

Table 1 The original chromatogram analysis of *GC-MS* methanol extract of clove flowers

No.	Afo		Gorontalo		uni buru selatan		Compound prediction
	Retention time (minute)	Area (%)	Retention time (minute)	Area (%)	Retention time (minute)	Area (%)	
1	11,956	56.57	10.479	62.02	11,968	36.43	Eugenol (phenolic)
2	12,180	4.62	1370	18.28	14,559	22.61	Acetisoeugenol (phenolic)
3	14,523	18.70					Aceteugenol (phenolic)
4			11,471	10.46			Karyofilen (non phenolic, sesquiterpen)
5	12,887	15.42			12,896	13.09	trans-karyofilen(non phenolic, sesquiterpen)
6	15.453	1.08	14,004	0.81	15.451	1.82	(-) - karyofilen oxide (non phenolic, sesquiterpen)

Clove flower extract was dominated by groups of non-phenolic compounds compared to groups of phenolic compounds. In addition there are major components of cloves and components of the dominant compound, several other minor compounds are also detected in each variety. Methanol extract of clove flower stalk from afo varieties detected compounds containing α-Humulene, Humulene oxida and copaene, varieties of gorontalo contains α- deculene and copaene compounds, and tuni buru selatan varieties contains α- deculene compounds.

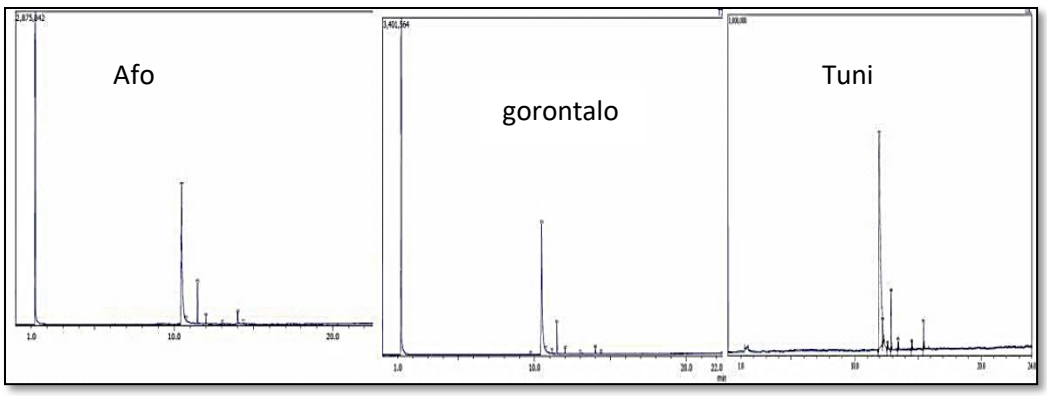

Figure 2 Chromatogram of methanol extract of clove var flower stalk from varieties of afo, gorontalo and tuni buru selatan

Chromatogram of clove flower stalk extract from varieties of afo, gorontalo and tuni buru selatan showed several peaks with retention times from 10.468 to 15.447 (Figure 2). The main component in clove flower extract is eugenol (phenolic) (Rt 10.468-11.930). Sesquiterpenic non phenolic component is the dominant component consisting of aryophilene (Rt 11.471-11.472), and (-)-karyophylene oxide (Rt 14.009-15.447) (Table 2).

Table 2 Results analysis GC-MS chromatogram of the methanol extract of the flower stalk of cloves

No.	Afo		Gorontalo		Tuni hunt south		Compound prediction
	Retention time (minute)	Area (%)	Retention time (minute)	Area (%)	Retention time (minute)	Area (%)	
1	10.468	85.50	10.468	88.96	11.930	88.93	Eugenol (Phenolic)
2	11.472	9.68	11,471	7.50			Karyofilen (non phenolic, sesquiterpen)
3	14.009	2.68			15.447	2.01	(-) - karyofilen oxide (non phenolic, sesquiterpen)

Clove leaf extract is dominated by groups of non-phenolic compounds compared to groups of phenolic compounds. In addition there are major components of cloves and components of the dominant compound, several other minor compounds are also detected in each variety. Minor compounds detected in the methanol extract of clove leaves from three varieties contain the same compound, namely α- deculene .

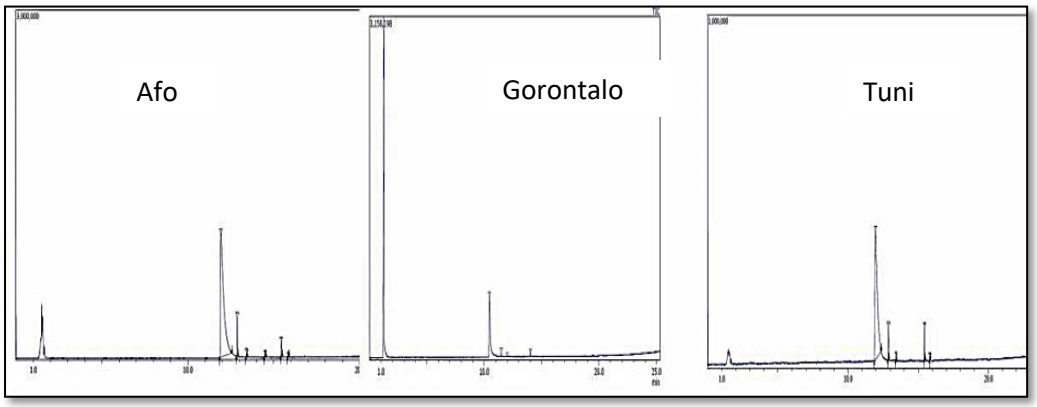

Figure 3 Chromatogram of methanol extract of clove leaves from varieties of afo, gorontalo and tuni buru selatan

Chromatogram of methanol extract of clove leaves from varieties of afo, gorontalo and tuni buru selatan showed several peaks with retention time from 10.448 to 11.955 (Figure 3). The main component in clove leaf extract is eugenol (phenolic) (Rt 10.468-11.930) . Clove leaf extract is dominated by sesquiterpenic

non phenolic components namely (-)-karyofilen oxide (Rt 14.007-15.448), and trans karyophilene (Rt 12.871) (Table 3).

Table 3 Result analysis *GC-MS* chromatogram of the methanol extract of clove

No.	Afo		Gorontalo		Tuni BS		Compound prediction
	Retention time (minute)	Area (%)	Retention time (minute)	Area (%)	Retention time (minute)	Area (%)	
1	11,921-14,498	92.51	10,448	94.73	11,955	91.18	Eugenol (fenolic)
2			11,470	2.60			Karyofilen (non f enolik, sesquiterpen)
3	15,445	1.78	14,007	2.29	15,448	4.01	(-) - karyophylene oxide (non fenolik, sesquiterpen)
4	12,871	4.23					Trans karyofilen (non f enolik, sesquiterpen)

Eugenol and Karyophilene in Methanol Extract of Flowers, Flower Stalks and Leaves colves from varietie of Afo, Gorontalo and Tuni buru selatan

The test results show there are differences the percentage of eugenol in the three clove varieties in each part of the plant was flowers, flower stalks and clove leaves (Table 4).

The highest percentage of eugenol levels is found in the leaves (91-94%) compared to flower stalks (88.93-91.89%) and clove leaf (36.43-62.02%). The highest percentage of eugenol content on clove leaves from gorontalo varieties (94.73%) compared to eugenol of clove leaves afo varieties(92.51%) and varieties of tuni buru selatan (91.18%).

Table 4 Percentage eugenol in the three clove varieties in each part of the plant was flowers, flower stalks and clove leaves

Clove varieties	Levels of Eugenol (%)			
	Flower	Flower stalk	Leaf	Average
Afo	56.57	85.50	92.51	80.32
Gorontalo	62.02	88.96	92.51	81.16
Tuni Buru Selatan	36.43	88.93	91.18	72.18

The percentage of karyophilene as a compound that dominates especially in clove flower stalks is also different (Table 5). Components of karyophilene compounds were detected in the clove flower stalk of afo varieties and all parts of clove gorontalo varieties. The components of the trans karyophilene and (-)-karyophilenic oxide compounds are detected in clove flowers and leaves of afo varieties . The components of the trans karyophilene compound are found in the part of flowers from varieties of tuni buru selatan. Karyophylene oxide is found in the clove flower stalk of afo varieties, on the flowers and clove flower

stalks of gorontalo varieties and all parts of the clove from tuni buru selatan varieties.

Table 5 The content of karyophilene from methanol extract of flowers, flower stalks and clove leaves

Clove varieties	Karyofilen compounds (%)				Total (%)
	Kr *	trans-kr	(-) – kr oxide	kr oxide	
Afo					
Flower	-	15.42	1.08	-	16.5
Flower stalk	9.68	-	-	2.68	12.36
Leaf	-	4.23	1.78	-	6.01
Gorontalo					
Flower	10.6	-	-	0.81	11.27
Flower stalk	7.50	-	-	1.26	8.76
Leaf	2.60	-	-	-	2.6
Tuni Buru selatan					
Flower	-	13.09	-	1.82	14.91
Flower stalk	-	-	-	2.01	2.01
Leaf	-	-	-	4.01	4.01

* Cr: Karyofilen

Discussion

Samples of flowers and clove flower stems are obtained from the harvest of healthy and productive clove trees at the location of each variety. The clove leaves used are clove tree litter at the clove harvest location that is dark brown, in a clean and intact condition. Before use, the leaves are washed first to remove dust marks that are still attached. Flower samples and flower stalks are directly used without needing to be washed first because there is almost no contact with the soil and also have passed the sorting stage first. Washed leaves are dried immediately to avoid chemical changes. All clove samples were immediately smoothed to expand the contact area with the solvent during the extraction process so that the yield obtained was also optimal.

The use of methanol solvents from the clove extraction process in this study is because the methanol solvent has a high dielectric constant and its molecular structure is small so that it can penetrate plant tissues and extract organic compounds. The results of extraction using methanol solvents provide better results than other solvents, as has been reported by several previous researchers.

According to Saeed *et al*. (2013) , The minimum inhibitory concentration of clove methanol extract is better than water extract. According to Chowdhury *et al*. (2016) , the use of methanol solvents compared to aqua and chloroform in clove flower extraction showed that methanol solvents absorb more alkaloids, phenols and flavonoids than other solvents. Abera *et al*. (2018) suggested a comparison of the use of solvents for extraction n-heksana, diklorometana:metanol (1:1) and methanol showed that the use of methanol in this genus is able to attract a number of compounds that are more than the other solvents.

From the results of the study it can be seen that there are differences in the percentage of the main composition of cloves, namely eugenol and compounds that are predominantly part of the flower, flower stalks and clove leaves. Clove flower extract is dominated by phenolic components compared to non-phenolic components. Conversely, the methanol extract of clove flower and clove leaves contains the dominant compound, which is a non-phenolic component compared to the phenolic component. The difference in chemical components in each part of the clove plant, is thought to be influenced also by differences in the location of growth and environmental factors.

Overview of Environmental Factors, Clove Morphology and its Relation to Secondary Metabolite Products

Environmental factors play an important role in determining the mechanism of an organism's adaptation to the environment. Different environmental conditions affect plant biology, especially aspects of plant morphology. The influence of the same environmental factors was also stated by Yuan *et al* . (2016), who suggested that most of *Leymus chinensis* plant morphology such as shoot height, leaf area, leaf weight, and seed weight per area significantly correlated with average annual rainfall, annual temperature and soil water content, rarely correlating with pH soil and soil nutrients. Guo *et al*. (2017), plants can respond to climate change by changing the nature of their leaves, morphological, physiological and anatomical properties. The thickness of leaves in plants is also related to environmental conditions that experience drought.

Clove of afo varieties, gorontalo and tuni buru selatan came from three different provinces, namely afo varieties from North Maluku Province, gorontalo varieties from Gorontalo Province and varieties of tuni buru selatan of the province of Maluku. Ternate City is an archipelago whose territory is surrounded by sea with its geographical location at the position of 0°- 2° North Latitude and 126° - 128° East Longitude. The land area of Ternate City is 250.85 km², and the sea area is 5,547.55 km². In general, the City of Ternate and other regions in North Maluku Province have a tropical climate type, so it is very influenced by the sea climate which is usually heterogeneous in accordance with the general indication of the tropical climate. During 2016 the climate conditions of Ternate City were an average temperature of 28°C with the lowest temperature of 24 ° C and the highest temperature of 33°C , to relative humidity on average 82 %, average rainfall 187 mm^3 , average wind speed 4.08 Km/hour with an absolute maximum speed of 18 knots in December and January (BPS Ternate 2017).

Gorontalo City is located between 00°28'17 "- 00°35'56" North latitude and 122°59'44"-123°05'59" East longitude. In 2016, BPS noted this city was a lowland with height 0-500 masl with an average rainfall of 293 mm^3 per month and an average temperature of 27.3°C. The land surface in Gorontalo Province is mostly hilly. This region lies near the equator, thus affecting the air temperature is hot enough. The minimum temperature occurs in August, which is 23.3°C. While the maximum temperature occurs in April with a temperature of 34.7°C. Relative humadity is relatively high, reaching an average of 81.7 percent. Highest rainfall is 323 mm^3, but the number of rainy days an average of 19 days (BPS Gorontalo 2017).

Buru Selatan Regency is located at 2º30' - 5º50' South Latitude and between 125º00' and 127º00' East Longitude. In 2016, the air temperature ranges from 24-31.6 ºC. Relatively humidity with an average range of 87 percent. Rainfall varies according to the month, the average rainfall ranges from 8 mm (August) to 368 mm (February). The topography of the southern Buru region on land consists of hilly and mountainous regions with a slope of between 15-40%. Common types of soil are alluvial, podzolic, organozol, and grumazol species (BPS Buru Selatan 2017).

The clove superior varieties that exist today, such as clove of zanzibar karo, afo, gorontalo and tuni buru selatan, a specific high yielding varieties (BALITTRO 2015). Clove of tuni buru selatan varieties obtained from the undertaken to select the best clove trees in selected populations that have high productivity. The selected trees are then selected again to get the selected parent tree which is more uniform with higher yield and quality potential than average . The selection results showed that there were 24 selected parent tree which were subsequently submitted to be released as varieties (PUSLITBANGBUN 2017).

Clove from varieties of afo, gorontalo and tuni buru selatan have different morphological forms. This difference is thought to be influenced by different environmental conditions, so that it correlates with the conditions of the three compound compounds of different components and percentages.

Afo Gorontalo Tuni Buru Selatan

Figure 4 Morphology of anthers of clove flowers (shown by arrow) in the varieties of afo, gorontalo and tuni buru selatan at 40 x magnification

The morphology of the flowers, flower stalks and clove leaves of the three varieties observed under 40x magnification also showed differences. In clove flowers, there is a difference in the spread of anthers on flower petals. anthers clove afo var. looks a lot and is in a centered position in the middle, clove gorontalo var. tends to spread but a little and the southern hunting tunnels look a lot and spread out evenly over all parts (four corners) of the flower petals (Figure 4).

According to Kaur and Chandrul (2017), stamens are found in the corola section of clove flower petals. In this section, there is clove essential oil which

spreads in the calyx section, stored in channels that extend in all parts of the flower bud. Production of clove oil in clove flowers ranges from 15-20%, while flower stalks only range from 10-15%.

The results of the analysis showed that there were differences in the compound content, especially in the group of non-phenolic compounds in flower extracts and clove flower stalks, allegedly related to differences in morphological forms on flower buds (different stamen locations) and flower stalks in all three varieties.

Figure 5 Morfologi indentation of cloves flower stalk (circle) varieties of Afo, Gorontalo and Tuni Buru Selatan at 40 x magnification

Morphology of clove flower stalks from varieties of afo, gorontalo and tuni buru selatan also have differences, especially in the flower stem protrusion. Flower stalks of afo has a slightly pointed protrusion slightly elongated in the grooves, gorontalo varieties has a type of indentation in short and not too pointed protrusions, while varieties of tuni buru selatan has a flowery bulge that tends to be more pointed than the clove flower stem of afo varieties (Figure 5).

Morphology of clove leaf bone for varieties of afo, gorontalo and tuni buru selatan have differences, especially in the streaks of leaf bones. Clove leaf afo varieties not clear streaks of leaf bones, in contrast to the leaf bone of the tuni buru selatan, seen to have streaks of thin leaves though the bones. Scars of clove leaf bone gorontalo looks very clear with pinnate type (Figure 6).

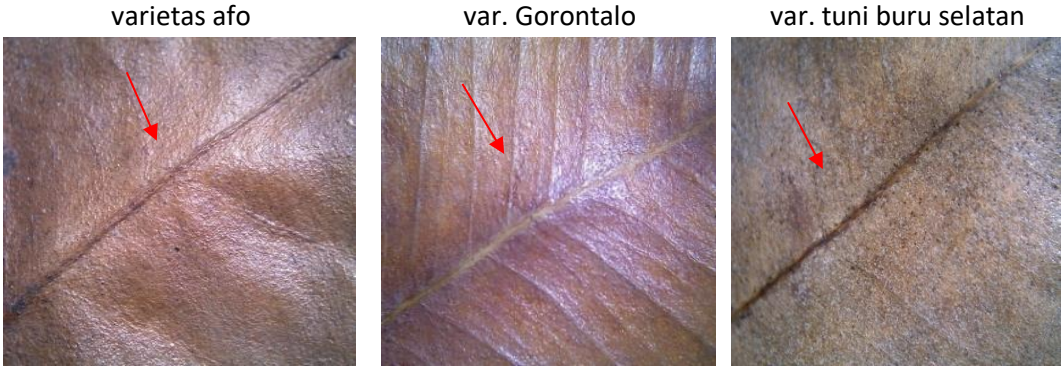

Figure 6 Morphology of venation of leaf bone (shown as arrow) clove varieties of afo, gorontalo and tuni buru selatan at 40 x magnification

Leaf bone Venation in plants is related to environmental factors that play a role in the mechanism of leaf formation. Formation of leaf venation is an important process in the development of plant leaves. Auxin fitohormones are known as the most important molecules for controlling venation patterns and canalization models. In this process, cells undergo higher auxin fluxes differentiate into specific cells for the transport of auxin. The specifications of leaf primordium occur on the side of the shoot apical meristem and depend on the distribution of the auxin hormone gradient . The distribution of auxin hormones is related to the proper transport of microtubules to determine the primordial pattern of leaves to be formed. Distribution of auxin hormones and transport of microtubules is influenced by environmental stress factors on plants Lee *et al*. 2014).

The content content of secondary metabolites in cloves of all varieties in all parts of the plant, namely flowers, flower stalks and leaves is thought to be related to the ability of the clove plant's resistance vrieties of afo, gorontalo and tuni buru selatan to stress environment and pest and disease attacks.

Secondary metabolites such as terpene, phenolic, nitrogen (N) and sulfur (S) contain compounds that function as plant defense media from various herbivores and pathogenic microorganisms and various types of environmental stresses. Pagare *et al*. (2015), secondary metabolic is a compound that is not needed cell (organism) for life, but berrole in cell (organism) with its environment. These compounds are of tenin volved as plan protection materials from environmental stresses both biotic and abiotic environments.

Afo clove plants have been proven to be able to live and are resistant to environmental stress including pests and diseases. Age of clove life afo varieties more than 100 years, even reaching the oldest age in the world, 430 years. Clove from varieties of gorontalo and tuni buru selatan have a lifespan of 30-40 years. High resistance to environmental stress, high production, weight of fruit produced are some of the factors considered in categorizing clove from varieties of afo, gorontalo and tuni buru selatan as a superior variety in Indonesia.

Conclusion

1. The main component of clove is eugenol (components of phenolic) contained in all varieties of cloves. The dominant compounds in flowers, flower stalks and clove leaves are components of non-phenolic compounds karyophilene (sesquiterpen).
2. The highest percentage of eugenol in clove is gorontalo varieties (81.16%) followed by successive afo varieties (80.32%) and varieties of tuni buru selatan (72.18%). The highest percentage of karyophyllen content in afo cloves, followed by varieties of tuni buru selatani and clove of gorontalo varieties.

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