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Characterization Evaluation of Clove Flower Oil (*Syzygium aromaticum* (L.) Meer) and Pharmacological Properties Of Anthelmintic

Cut Suraiya Wahyuni Utami[1*], Ummu Balqis[2], Farida Athaillah[2], Muhammad Hanafiah[2], and Cut Dahlia Iskandar[2]

[1]*Post Graduate Program of Veterinary Public Health of Syiah Kuala University, Jl. Tgk. H. Hasan Krueng Kale No. 4 Darussalam, Banda Aceh 23111, Indonesia*
[2]*Faculty of Veterinary Medicine of Syiah Kuala University, Jl. Tgk. H. Hasan Krueng Kale No. 4 Darussalam, Banda Aceh 23111, Indonesia.*
[*]*Email:cutsuraiya@gmail.com*

Abstract

Clove oil is distilled from clove flowers (*Syzigium aromaticum*) and characterized by physical properties and GC-MS. Distillation was carried out by steam distillation method for 8 hours. Analysis of clove oil was carried out by GC-MS method. The distillation results obtained 163 mL of clove oil with physical properties that meet the requirements of SNI 06-4267-1996. The results of the analysis using GC-MS showed that clove oil contained 6 components, namely, Eugenol 88.20%, Alpha Copaene 0.49%, Caryophyllene 3.77%, Alpha Humulene 0.48%, Eugenyl acetate 6.76%, and Caryophyllene Oxide 0.30%. Another fact shows that clove flower oil has anthelmintic activity.

Keywords: Syzigium aromaticum, clove flower oil, anthelmintic

Background

Clove oil is included in the family Myrtaceae genus *Syzygium*, the essential oil produced from clove plants (*Syzigium aromaticum*) is found in Indonesia, India, and Madagascar (Alma *et al.,* 2007). Clove oil can be isolated from leaves (1-4%), stems (5-10%), and clove flowers (10-20%). Essential oils from clove flowers have the best quality and are expensive because they contain eugenol reaching 80-90%, clove flower oil has biological activities which are as anti-microbial, anti-pain, antioxidant, anti-cancer, anti-inflammatory, anti-inflammatory, anti-depressant, and antiulcer (Kamataou *et al.,* 2012). Clove oil is highly valued in the medical field as carminative and stimulant and is said to be an anthelmintic. In recent years, it has been recognized as an anesthetic that is effective in calming fish in a number of fisheries management (Mittal *et al.,* 2014). The focus of this review is to provide information about the components of *Syzygium aromaticum*, and pharmacological activities against anthelmintics.

Materials and Methods

Determination of plants

Determination of clove plants is carried out at Herbarium Bogoriense, Botany Field, Bogor Biology Research Center-LIPI. This determination aims to ascertain the truth of the plant species to be used.

Clove flower oil distillation

Clove flowers are distilled using the steam distillation method. The sample used was 4 kg distilled for 8 hours connected to the condenser. During heating the distillation of the oil will evaporate with water vapor through the condenser, then a condensation process occurs that produces essential oils in clove flowers. Destilates are collected and separated from the water contained in them using anhydrous sodium sulfate in a separating funnel (Prianto *et al.,* 2013).

Characterization of the physical properties of clove flower oil

Characterization of physical properties is done by looking at the color,

smell, refractive index, and specific gravity measurements. These parameters have standardized values as a condition of trade quality in Indonesia listed in SNI 06-4267-1996 (Prianto *et al.,* 2013).

Table 1. Characteristics of physical properties of clove oil SNI 06-4267-1996.

Parameters	SNI 06-4267-1996
Color	Not light yellow
Smell	Typical clove oil
Bias Index	1,529-1,537
Species weight	1,040-1,070

Characterization of clove flower oil components using the Gas chromatography-mass spectrometry (GC-MS) method

The characterization of clove flower oil components was carried out by injecting 0.05 µL of clove flower oil into the syringe of GC-MS with a capillary column length of 30 m. The stationary phase used is 5% diphenyl or 95% dimethylpolisiloksan while the mobile phase used is helium gas with a flow rate of 53.0 mL / minute at a pressure of 12.0 kPa. Column temperature is 50.0°C and 300,00°C injector temperature with split ratio of 93.0. Identification of the components of clove flower oil based on the comparison of standard eugenol characterization (Prianto *et al.,* 2013).

Results and Discussion

Determination of plants

Clove plants obtained from Lambaro Seubun Village, Lhoknga Subdistrict, Aceh Besar Regency were conducted at Herbarium Bogoriense, Botany Field, Bogor Biology Research Center-LIPI. The results of the determination showed that the plants used in the study were *Syzygium aromaticum* (L.) Merr. L.M. & Perry from the Myrtaceae tribe.

Clove flower oil distillation

Clove flower oil distillation of 4 kg was carried out using a steam distillation method for 4 days with a time of 8 hours per day. The distillation yields 163mL of clove flower oil. Purification of clove flower oil was carried out using Sodium Sulphate Anhydrous (Na_2SO_4). Anhydrous sodium sulfate aims to separate and bind water bound to clove flower oil (Prianto *et al.,* 2013).

Characterization of the physical properties of clove flower oil

Parameters characterized have values that have been standardized on trade quality requirements in Indonesia listed in SNI 06-4267-1996. The results of the characterization in Table 2. have met the quality requirements of SNI 06-4267-1996, which means meeting the quality standards of essential oil trading in Indonesia. The refractive index of clove oil produced is 1.52 with a specific gravity of 1.05, light yellow and has a distinctive smell of clove oil.

Table 2. Characteristics of physical properties of clove oil SNI 06-4267-1996

Parameters	SNI 06-4267-1996	Research result
Color	Not light yellow	Light yellow
Smell	Typical clove oil	Typical clove oil
Bias Index	1,529-1,537	1.52
Species weight	1,040-1,070	1.05

Characterization of clove flower oil components using the Gas chromatography-mass spectrometry (GC-MS) method

Analysis of clove flower oil using GC-MS produced a chromatogram that showed 6 components of the main compound (Figure 1). The first compound with a retention time of 24,538 minutes with a percentage concentration of 88.20%. The second compound has a retention time of 24.883 minutes with a percentage of 0.49%. The third compound with a retention time of 26.147 minutes with a percentage concentration of 3.77. The fourth compound with a retention time of 27.113 minutes with a concentration concentration of 0.48. The fifth compound with a retention time of 28,656 minutes with a concentration concentration of 6.76. Sixth compound with a retention time of 30.480 minutes with a concentration concentration of 0.30.

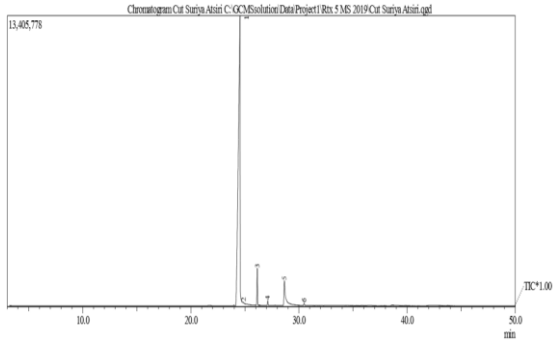
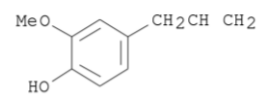
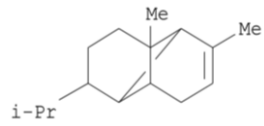


Figure 1. Chromatogram of clove flower oil from steam distillation

The results of the MS spectrum analysis showed that the first compound with a retention time of 24,538 was identical as eugenol from the database with a similarity of 87%. The second compound with a retention time of 24,883 was identified as alpha copaene with a similarity of 82%. The third compound with a retention time of 26.147 minutes was identical as a caryophyllene with a similarity of 94%. The fourth compound retention time of 27.113 minutes is identical as alpha humulene with a similarity of 95%. The fifth compound retention time of 28,656 minutes is identical as eugenyl acetate with a similarity of 96%. The sixth compound retention time of 30.480 minutes is identical as caryophyllene oxide with a similarity of 90%.



(a)



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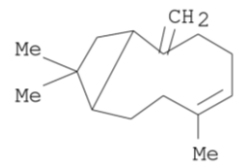
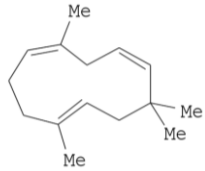
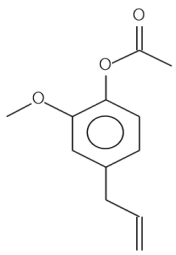


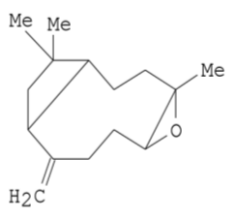
(c)



(d)



(e)



(f)

Information:
(a). Eugenol 88.20%,
(b). Alpha Copaene 0.49%,
(c) Caryophyllene 3.77%,
(d) Alpha Humulene 0.48%,
(e) Eugenyl acetate 6.76%,
(f) Caryophyllene Oxide 0.30%.

Reports of research conducted by (Alma *et al.*, 2007) steam distillation results of clove flowers produced approximately 18 components of compounds including 2-heptanone (0.04%); α-pinene (0.01%); p-cimene (<0.01%); limonene+1.8 cineole (0.01%); 2-heptyl acetate (0.04%); € -β-ocimene (0.33%); 2-nonanone (0.02%); linalool (0.01%); methyl salicylate (0.07%); p-allyl phenol (0.19%); eugenol (87.00%); α-copaene (0.10%); β-cariophyllene (3.56%); α-humulene (0.40%); Cad-cadinene (0.04%); eugenyl acetate (8.01%); caryophyllene oxide (0.10%) and 2 (12), 6 (13) -cariopyllene-dien-5-ol (0.02%). Report (Memmou and Mahboub, 2012) distillation results of fresh clove flower water produce 4 components of the compound namely eugenol (47.57%); β-caryophyllene (35.40%); isoeugenol (3.60%); eugenyl acetate (13.42%). According to the report (Srivastava *et al.*, 2005) there are 10 components of the compounds identified including eugenol (70.0%); β-cariophiylene (19.5%); eugenyl acetate (2.1%); α-humulene (0.54%); caryophyllene oxide (0.22%); α-copaene (0.07%); methyl eugenol (0.02%); iso eugenol (0.08%); chavicole (0.17%) and methyl salisylate (0.14%).

The different types of compounds identified and the percentage of components in clove flower oil are caused by several factors including the differences in the varieties of clove flowers used, preparation of samples before distillation and distillation methods. Geographical location will affect the components of inorganic soil where clove plants grow, so that it will affect the secondary metabolites of clove flower oil produced (Srivastava *et al.*, 2005).

Pharmacological Properties of Anthelmintics

Bhowmik *et al.,* (2012) said that clove essential oil contains around 85-92% eugenol which is very active against microorganisms (classified as bactericidal in nature). Cloves are also said to be anthelmintic, antiviral, antimicrobial, antiseptic, and anti-fungal. Dhanraj *et al.,* (2015) in his research entitled In Vitro study of the effect of *Syzygium aromaticum* ethanol extract on carbohydrate metabolism *Cotylophoron cotylophorum* validates the anthelmintic properties of *Syzygium aromaticum* ethanol extract against *C. cotylophorum* characterized by inhibition of carbohydrate metabolic enzymes, namely pyruvate kinase, phosphoenolpyruvate carboxykinase, lactatdehydrogenase, malatdehydrogenase, fumaratreductase and succinate dehydrogenase which can reduce ATP production may be fatal for parasites. This study uses five concentrations, namely. 0,005; 0.01; 0.05; 0.1 and 0.5 mg/mL are incubated for 2, 4 and 8 hours, enzyme activity is expressed in the form of protein.

Preliminary studies conducted by Charitha *et al.,* (2017) *Syzygium aromaticum* and *Melia dubia* have anthelmintic activity in vitro against *Haemonchus contortus* with the results of *S. aromaticum* at concentrations (10mg/mL) very quickly resulting in anthelmintic activity requiring only 1-time minutes compared to *M. dubia* with 5.33 minutes and Piperazine adepate with 5.0 minutes. The time needed to kill all worms at the lowest concentration is 0.16 mg/mL, *S. aromaticum* takes 36.67 minutes and *M. dubia* takes 79.33 minutes.

Evaluation of the anthelmintic activity of *Eugenia caryophyllus* alcohol extract with concentrations (5; 10; 15 mg/mL) & *Uncaria gambier* with concentrations (50; 100; 150mg/mL) on *Pheretima posthuma* worms in Sonalkar and Sachin (2014) obtained a concentration of 15mg/mL clove extract showed a paralysis response in 1.5 minutes and lysis occurred in the 9th minute. *Uncaria gambier* at a concentration of 150mg/mL paralysis occurred in the 2.5 minute and lysis occurred in the 62 minutes. *Eugenia caryophyllus* and *Uncaria gambier* alcohol extract showed significant anthelmintic activity the greater the concentration the faster the time of death of worms. The comparative evaluation of these two plants shows that *Eugenia caryophyllus* has a strong anthelmintic activity compared to *Uncaria gambier.*

Patil *et al.,* (2013) used ethanol extract of *Syzygium aromaticum* with the aim of investigating the potential of anthelmintic on *Pheritma posthuma* with concentrations (2.5; 5 and 10 mg / mL). Positive control as standard used albendazole 20 mg / mL and distilled water as a negative control. The results obtained by the *S. aromaticum* ethanol extract concentration of 2.5; 5 and 10 mg/mL show paralysis at 4.267±0.252, 2.433±0.306 and 1.033±0.551 minutes while lysis occurs at 45±2.00, 35±4.35 and 2.333±0.351 minutes. Albendazole (20 mg/ml) was shown to have paralysis at 1185.33±79.02 minutes while lysis at 1608.33±78.57 minutes. This study shows that the ethanol extract of *S. aromaticum* showed significant anthelmintic activity at higher concentrations as evidenced by bleeding and necrotic patches observed externally on the worm's body. From this it can be concluded that the ethanol extract of *S. aromaticum* has anthelmintic activity which is useful for fighting worm infections.

Kumar and Singh (2014) in his study said that *Allium sativum*, *Ferula asafoetida* and *Syzygium aromaticum* can be used as anthelmintic. Anthelmintic activity was tested in vitro against the liver worms *Fasciola gigantica*. The results of his research state that ethanol extract is more toxic than other organic extracts. The ethanol extract of *S. aromaticum* (2h LC50 2.95 mg/mL) was more toxic to *F. gigantica* than *A. sativum* (2h LC50 3.48 mg/mL) and *F. asafoetida* (2h LC50 3.94 mg/mL). The in vitro anthelmintic activity of *Pheritima posthuma* worms from *S. aromaticum* (flower buds) and *C. zeylanicum* (bark) water extracts examined by Kumari *et al.,* (2015) stated that activity depends on the concentration observed in the extract. A concentration of 20 mg/mL of *C. zeylanicum* showed significant activity compared to *S. aromaticum*. This study also carried out

phytochemical screening of extracts of *S. aromaticum* and *C. zeylanicum* revealing the presence of alkaloids, tannins, phenolics, phlobatannins, cardiac glycosides, anthraquinone glycosides, hydrocinone hydrakuinone, flavonoids and terpenoids.

Conclusion

The distillation results obtained 163 mL of clove oil with physical properties that meet the requirements of SNI 06-4267-1996. The results of the analysis using GC-MS showed that clove oil contained 6 components, namely, Eugenol 88.20%, Alpha Copaene 0.49%, Caryophyllene 3.77%, Alpha Humulene 0.48%, Eugenyl acetate 6.76%, and Caryophyllene Oxide 0.30%. Another fact shows that clove flower oil has anthelmintic activity.

References

Alma, M. H., Ertas, M. N., Kolimannsberger, H. 2007. *Essential Oil Content*, Turkish Cloves, Bioresources.

Bhowmik Debjit., K.P. Sampath Kumar., Akhilesh Yadav., Shweta Srivastava., Shravan Paswan., Amit Sankar Dutta. 2012. Recent Trends in Indian Traditional Herbs *Syzygium aromaticum* and its Health Benefits. *Journal of Pharmacognosy and Phytochemistry*. 1(1): 13-22.

Charitha V, Gnani., J, Adeppa., P, Malakondaiah. 2017. In vitro anthelmintic activity of Syzygium aromaticum and Melia dubia against Haemonchus contortus of sheep. *Indian Journal of Animal Sciences*. 87 (8): 968–970.

Dhanraj M. K and Veerakumari L. 2015. *In vitro* studies on the effect of ethanol extract of *Syzygium aromaticum* on the carbohydrate metabolism of *Cotylophoron cotylophorum*. *International Journal of Advanced Veterinary Science and Technology*. 4(1): 199–210.

Kamatou G. P., Vermaak I., Vilijoen A. M. 2012. Eugenol-from the remote Maluku islands to the International market place: A review of a remarkable and versatile molecule. *Journal Molecules*: 17: 6953-6981.

Kumar Pradeep dan D. K. Singh. 2014. In vitro anthelmintic activity of *Allium sativum*, *Ferula asafoetida*, *Syzygium aromaticum* and their active components against *Fasciola gigantica*. *Journal of Biology and Earth Sciences*. 4(1): B57-B65.

Kumari P. Lakshmi., A. Sujitha and N. Mahammad Rafi. 2015. In Vitro Anthelmintic Activity of *Syzygium Aromaticum* Floral Buds and *Cinnamomum Zeylanicum* Bark Aqueous Extracts. *International Journal Pharma Bio Sciences*. 6(1): 319-325

Memmou, F., Mahboub, R. 2012. Composition of Essential Oil from Fresh Flower of Clove. *Journal of Scientific Research in Pharmacy*. 1(2): 33-35.

Mittal Monika., Nomita Gupta., Palak Parashar., Varsha Mehra., Manisha Khatri. 2014. Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences.* 6(8): 67-72.

Patil R. N., J. S. Kadam., J. A. Chavan And A. J. Salunkhe. 2013. Anthelmintic Activity of Ethanolic Bud Extract of *Syzygium Aromaticum* Against *Pheretima Posthuma*. *Weekly Science Research Journal*. 1(14): 1-5.

Prianto, H., Retnowati, R. dan Juswono, U. P. 2013. Isolasi dan Karakterisasi dari Minyak Bunga Cengkeh (*Syzygium aromaticum*) Kering Hasil Destilasi Uap. *Jurnal Kimia*. Universitas Brawijaya Malang. 1 (2): 269-275.

Sonalkar Manisha Yogesh and Sachin Annasaheb Nitave. 2014. Comparative anthelmintic activity of *Eugenia caryophyllus* & *Uncaria gambier*. *World journal of pharmacy and pharmaceutical sciences.* 3(7): 1409-1414.

Srivastava, A. K., S K Srivastava, K. V., Syamsundar. 2005. Bud and Leaf Essential Oil Composition of *Syzygium aromaticum* from India and Madagascar, Flavour Fragr Journal. 20: 51-53.