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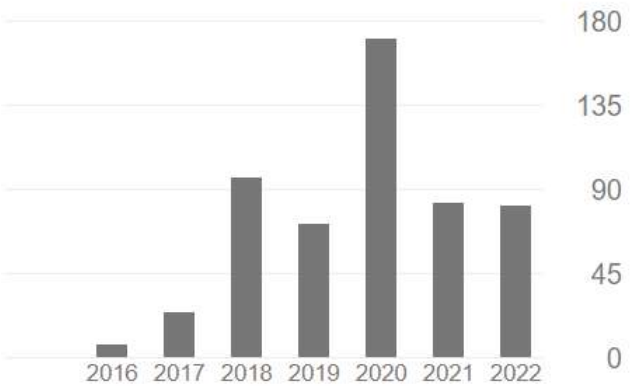

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Antifungal activity of eugenol and clove leaf essential oil (*Syzygium aromaticum L.*) against clinical isolates of *candida* species

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Abstract*---Candida*** species are causative microorganisms of oropharyngeal, vulvovaginal, and skin infections. Clove (*Syzygium aromaticum L.*) and eugenol oil have considerable antifungal activity against human pathogenic fungi, including *Candida* species. The anti*candida*l action of the studied oils is significantly dependent on the concentration of eugenol and further main and minor constituents of the oils with well-known antifungal effects must have additional and synergistic activities. This study was conducted to compare the antifungal activity of clove leaf essential oil (*Syzygium aromaticum L.*) and eugenol to determine the role of the content other than eugenol in clove leaf essential oil as an antifungal against *Candida* species isolates from HIV-AIDS patients. Methods: This study is an in vitro test using the disk diffusion method to determine the zone of inhibition of clove leaf essential oil (*Syzygium aromaticum L.*) concentrations of 5%, 10%, and 15% as well as eugenol which was equivalent to the concentration of eugenol in clove leaf essential oil at concentrations of 5%, 10% and 15% in 40 isolates of *Candida* species. The average inhibition zones of clove leaf essential oil (*Syzygium aromaticum L.*) concentrations of 5%, 10% and 15% for all isolates were 10,18 ± 5,357 mm, 17,97 ± 4,829 mm and 22,52 ± 6,716 mm. The mean inhibition zone of eugenol which was equivalent to the concentration of eugenol in clove leaf essential oil at concentrations of 5%, 10%, and 15% for all isolates was 9,85 ± 5,543 mm, 18,08 ± 7,558 mm, and 22,84 ± 7,278 mm. There was no significant difference between the clove leaf essential oil and eugenol inhibition zone with the same concentration ($p > 0.05$). There was antifungal activity in the form of an inhibition zone in clove leaf essential oil and eugenol against isolated *Candida* species, but no ingredients other than eugenol increase the antifungal activity of clove leaf essential oil.

***Keywords*---**Clove leaf essential oil (Syzygium aromaticum L.), Eugenol, Antifungal, HIV/AIDS.

Introduction

Candida species are causative microorganisms of oropharyngeal, vulvovaginal, and skin infections. They are responsible for the four most common nosocomial bloodstream infections with a high mortality rate. Risk groups are immunocompromised patients including infants, pregnant women, cancer, diabetes, and AIDS (Pauli, 2006). The existence of resistance to the main antifungal drugs urges research to find natural ingredients that can be attractive alternatives to antifungals. Most essential oils obtained from many tropical and subtropical plants contain eugenol as the main antifungal component (Schmidt et

al., 2007). The concentration of eugenol in several plants is different. In the literature it is stated that clove (*Syzygium aromaticum L.*) is considered the highest source of eugenol (45–90%) (Gupta & Prakash, 2021). So far, the clove plant in Indonesia has only been optimally utilized in the flower part, namely as a raw material for cigarettes. Whereas in the stems and leaves, it can use essential oils to add value to the clove plant. The most dominant component in clove leaf waste is eugenol (Nuraini, 2014). Research conducted by Bhuiyan and colleagues in which they compared the essential oil content of clove leaves and flowers, showing that the eugenol content was higher in clove leaves, namely 74.28% while in flowers as much as 49.71% (Bhuiyan, et al., 2010). The most common methods for the isolation of eugenol are steam and water distillation (Khalil et al., 2017).

Variations in components and composition depend on the variety, agro-ecological conditions, pre-treatment, processing, and extraction method (Nurdjannah & N., 2012). Research conducted by Megawati et al. provides an overview of the metabolites in clove oil from areas on the islands of Sumatra, Sulawesi, Maluku and Java related to the quality of clove oil obtained by steam and water distillation. Based on the completeness of the metabolites and the relative levels of its major components, the essential oil of clove flower buds from Maluku (Amboyna) and Java (Tawangmangu) had better quality than clove flower buds originating from Sumatra (Padang) and Sulawesi (Palopo) (Megawati, et al., 2010). Chemically, the main bioactive constituents in clove belong to secondary metabolites such as tannins, alkaloids, and phenols, also responsible for their antimicrobial and antifungal activities (Gupta & Prakash, 2021). It is known that both eugenol and clove essential oil can alter protein properties and react with cell membrane phospholipids changing their permeability and inhibiting a large number of Gram-negative and Gram-positive bacteria and various types of fungi (Gupta & Prakash, 2021; Jafri et al., 2020). In a study conducted by Schimdt et al. (2007), it was found that eugenol alone had weaker antifungal activity than clove leaf essential oil against clinical isolates of *Candida* albicans. It was concluded that the antifungal activity of the essential oil studied against clinical isolates of *Candida* albicans in addition to being significantly dependent on the concentration of eugenol in the essential oil, also depended on other major and minor constituents of some samples with partially known anti*candida*l effects, such as methyl eugenol and linalool. This indicates that the content other than eugenol in essential oils has a synergistic and/or synergistic role in providing antifungal effects (Schmidt et al., 2007). So it is necessary to do further research to compare the antifungal activity between clove leaf essential oil and pure eugenol to determine the effectiveness of other ingredients besides eugenol in clove leaf essential oil used in this study providing an inhibition zone for the growth of *Candida* spp.

Method

This study was an experimental laboratory to evaluate the comparison of the antifungal activity of clove leaf essential oil (*Syzygium aromaticum L.*) and eugenol against 40 isolates stored *Candida* spp. which consisted of 20 *Candida* albicans and 20 non-albicans *Candida* isolated from the oral cavity of HIV/AIDS patients who were hospitalized at the Infectious Disease Intermediate Treatment Unit

(UPIPI) RSUD Dr. Soetomo Surabaya for the period April 2019 – July 2019 which was reactivated.

Based on the former study, the concentration of clove leaf essential oil (*Syzygium aromaticum L.*) was 5%, 10%, and 15%. Pure eugenol was used, which concentration was equal to the eugenol content of clove leaf essential oil. Eugenol 15% is pure eugenol with a concentration of 12.6%, equivalent to a concentration of 15% eugenol in clove leaf essential oil. Eugenol 10% is pure eugenol with an 8.4% concentration equal to 10% eugenol concentration in clove leaf essential oil. Eugenol 5% is pure eugenol with a concentration of 4.2% which is equivalent to a concentration of 5% eugenol in clove leaf essential oil.

The antifungal activity was evaluated with paper disks or blank disks using the disk diffusion method. These data were entered into a data collection sheet and analyzed with SPSS (Statistical Package for Social Sciences). This research has obtained ethical approval from the Ethics Committee of Dr. Soetomo General Academic Teaching Hospital Surabaya (0286/KEPK/X/2021).

Results

The results of steam distillation from 1 kg of clove leaves in this study produced 15 ml of essential oil (% yield = 1.5%). The obtained clove essential oil was analyzed using gas chromatography-Mass Spectroscopy (GCMS) to determine the compound and eugenol concentrations. From the results of qualitative identification, it is known that the clove leaf essential oil used in this study contains several compounds, including Isohexane, Chavicol, Alpha-Cubebene, 1,3,4-Eugenol, 4-Allyl-2-Methoxy-Phenol, 2- Methoxy-4-Formylphenol, Beta-Caryophyllene, (E)-isoeugenol, Alpha-Caryophyllene; %-Oxatricyclo [8.2.0.04,6] Dodecane,4,12,12-Trimethyl-9-Methylene, (1R, 4R, 6R, 10S), Delta-Cadinene. The level of eugenol in clove leaf essential oil in this study was 84% (Figure 1).

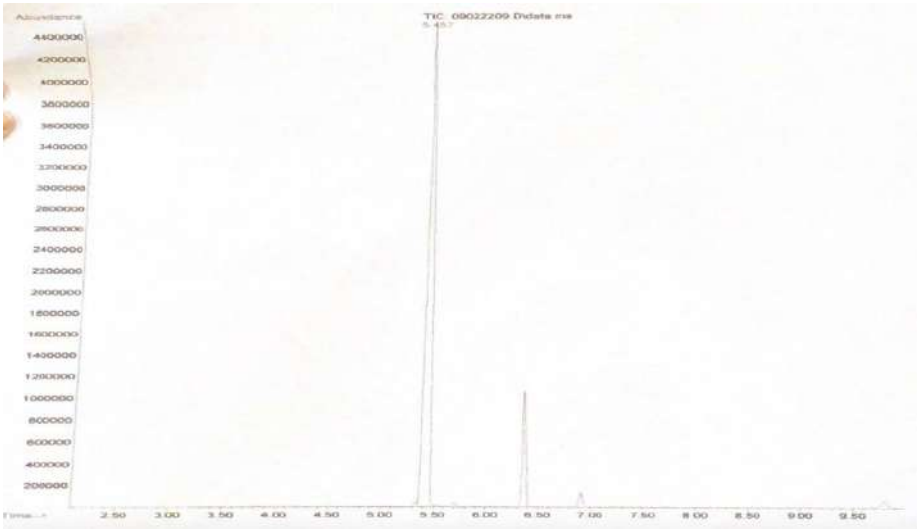

Figure 1. Chromatogram of clove leaf essential oil

An inhibition zone test on clove leaf essential oil (*Syzygium aromaticum L.*) was carried out with concentrations of 5%, 10% and 15%. The mean inhibition zone of clove leaf essential oil concentrations of 5%, 10% and 15% in 40 isolates of *Candida* spp. respectively are 10.18±5.357 mm, 17.97±4.829 mm and 22.52±6.716 mm. The mean inhibition zones of eugenol 5%, 10% and 15% were obtained in 40 isolates of *Candida spp.* respectively are 9.85±5.543 mm, 18.08±7.558 mm and 22.84±7.278 mm (Table 1).

Comparative analysis of clove leaf essential oil (*Syzygium aromaticum L.*) concentrations of 5%, 10%, and 15% with eugenol 5%, 10%, and 15% on the growth of *Candida* species isolates was carried out. It established the analysis by comparing the results of the diameter of the inhibition zone on all 40 isolates of *Candida spp.* Analysis using non-parametric statistical methods (Mann Whitney Test) because the data are not normally distributed and not homogeneous. The comparison is said to be significant if the p-value <0.05. This study showed that there was no significant difference with the p-value sequentially of 0.307; 0.366; 0.773 (Table 1).

Table 1
Comparison of the mean diameter of the inhibition zone of clove leaf essential oil (*Syzygium aromaticum L.*) and eugenol in all isolate species *Candida.*

Material (n=40)	Average inhibition zone (mm) ±Standart Deviation (SD)	p-value
Clove leaf essential oil concentration 15%	22,52±6,716	0,773
Eugenol 15%	22,84±7,278	
Clove leaf essential oil concentration 10%	17,97±4,829	0,366
Eugenol 10%	18,08±7,558	
Clove leaf essential oil concentration 5%	10,18±5,357	0,307
Eugenol 5%	9,85±5,543	

Discussion

Many essential oils are obtained from several tropical and subtropical plants, containing eugenol as the main antifungal component (Schmidt et al., 2007). Clove oil (*Syzygium aromaticum L.*) is considered the highest source of eugenol (45–90%) (Gupta & Prakash, 2021). The results of steam distillation from 1 kg of clove leaves in this study produced 15 ml of essential oil (% yield = 1.5%). The literature states that the product of clove oil from flowers ranges from 10-20%, from stalks 5-10%, and from leaves 1-4% (Setya, et al., 2012). Gas chromatography (GC) examination was carried out on clove leaf essential oil to determine the eugenol concentration contained qualitatively. From the results of these examinations, it is known that the concentration of eugenol in clove leaf essential oil in this study was 84%. The eugenol concentration is under the SNI 06-2387-2006 standard, which states that the minimum concentration of eugenol in an extract is 78% (Sari, et al., 2020).

The concentration of eugenol in this study was higher than in a study conducted by Bhuiyan and colleagues. They compared the concentration of eugenol essential oil from the leaves and flowers of cloves in Bangladesh (74.28% and 49.71%) (Bhuiyan et al., 2010). The quality of essential oils is influenced by origin, variety, agro-ecological conditions, pre-treatment, processing, and extraction method (Nurdjannah, 2012).

A study by Schimdt et al. (2007) concluded that the antifungal activity of essential oils was not only significantly dependent on the concentration of eugenol but also on other major and minor constituents with partially known antifungal effects, such as methyl eugenol and linalool (Schmidt et al., 2007). This study also compared the antifungal activity between clove leaf essential oil and pure eugenol to determine the effectiveness of other ingredients besides eugenol in clove leaf essential oil used in this study in providing an inhibition zone for the growth of *Candida* spp.

The average diameter of the inhibition zone between clove leaf essential oil and eugenol in all isolates showed that clove leaf essential oil had a larger diameter of inhibition zone than eugenol. However, the results of comparative analysis between clove leaf essential oil and pure eugenol showed no significant difference with a p-value > 0.05 for both *Candida* albicans and non-albicans *Candida* isolates. It indicates that statistically pure eugenol with the same concentration as the concentration of eugenol in essential oils has no different antifungal activity. Another eugenol content in clove leaf essential oil was thought to have no significant role in helping provide antifungal activity in *Candida* species isolates. It is different from the results of Schimdt et al. (2007) study, which stated that pure eugenol had significantly weaker antifungal activity against clinical isolates of *Candida* albicans compared to clove leaf oil. Ingredients other than eugenol with known anti*candida*l effects, such as methyl eugenol in clove essential oil, take an additional and synergistic role in providing antifungal effects. It indicates that clove leaf essential oil has better antifungal activity than pure eugenol (Schmidt et al., 2007). In this study, the absence of methyl eugenol content in clove leaf essential oil is thought to be the cause of the lack of content that provides a synergistic effect in providing antifungal effects.

A study conducted by Costa et al. (2017) determined the chemical composition and evaluated the anti-*Candida* activity of essential oils from leaves of Hymenaea courbaril var. courbaril, Myroxylon peruiferum, and Vismia guianensis. It is known that the content of chavicol and caryophyllene plays a significant role in the antifungal activity of the essential oil of the tested plants (Costa et al., 2017). From qualitative identification, it is known that the clove leaf essential oil in this study contains several compounds other than eugenol, including Isohexane, Chavicol, Alpha-Cubebene, 4-Allyl-2-Methoxy-Phenol, 2-Methoxy-4-Formylphenol, Beta-Caryophyllene, (E)-isoeugenol, Alpha-Caryophyllene; %-Oxatricyclo [8.2.0.04,6] Dodecane,4,12,12- Trimethyl-9-Methylene, (1R, 4R, 6R, 10S), Delta-Cadinene. In this study, chavicol and caryophyllene in clove leaf essential oil did not provide additional effects to increase antifungal activity. It is supported by the absence of literature which states that these two ingredients play a role in delivering antifungal activity in clove leaf essential oil.

Conclusion

There was antifungal activity in clove leaf essential oil and eugenol against isolated *Candida* species. In this study, the absence of methyl eugenol content in clove leaf essential oil is thought to be the cause of the lack of content that provides a synergistic effect in providing antifungal effects.

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