ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Antimicrobial Activity of *Syzygium aromaticum L.* Leaves Essential Oil against *Candida albicans* and *Streptococcus mutans*

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ABSTRACT:

Background: *Candida albicans* and *Streptococcus mutans* infection cases are increasingly common diseases giving bad impact on humans. High evidence of microbial included bacterial and fungal resistance because frequently used antibiotics contributes disability and death significantly worldwide. Hence, alternative and safe of antimicrobial agents are required. Clove leaves (*Syzygium aromaticum* L.) are part of the Myrtaceae family containing essential oils that are rich in eugenol as the main component having high antimicrobial activity. Therefore, this study focuses on evaluation of antimicrobial activity of clove leaf essential oil against *Candida albicans* and *Streptococcus mutans*. **Methods*:*** In the current study, the antibacterial efficiency of *Syzygium aromaticum* L. leaf essential oil against *Candida albicans* and *Streptococcus mutans* was determined by the disc diffusion method. Furthermore, the physical characterizations of essential oils that were carried out were colour, odour, solubility, density and total eugenol, respectively. **Results***:* Clove leaf essential oil exhibited antimicrobial activity against pathogenic isolates *Candida albicans* was recorded at 0.5% having inhibition zones of 33.3±0.28 mm, 1% of 34±0.00mm, 1.5% of 35±0.28mm. While against gram-positive bacteria *Streptococcus mutans* at 0.5% presented an inhibition zone of 19.95±1.76mm, 1% of 20.5±2.12mm, 1.5% of 22.1±1.55mm. The physical characterization obtained from Clove leaf essential oil revealed that the essential oil presented yellow and distinctive odour, solubility in ethanol was 70% (1:2 clear), the density was 1.047g/ml, and eugenol total was 80%. **Conclusion*:*** This study indicates that essential oil of *Syzygium aromaticum* L leaves can be considered as potential antimicrobial agents.

KEYWORDS: *Candida albicans*, Essential Oil**,** *Streptococcus mutans*, *Syzygium aromaticum* L.

INTRODUCTION:

Bacteria and fungi are microbial causing infections for many years and is increasing sharply. These microorganisms can effect to different diseases and diverse therapeutical agents can be used. Antifungal drugs are utilized for pathogenic fungi, antibiotics are used for bacterial infections and antiviral drugs for viruses[1–3].

Infectious disorders caused by microbial resistance to medications, on the other hand, are a major cause of death in underdeveloped nations[4]. Candida-infected superficial fungal infections[5] have developed resistance[6] as well as unpleasant side effects such as rashes, itching, inflammation, hypersensitivity, and contact dermatitis. Dental caries is caused by *Streptococcus mutans*, the most common and destructive cariogenic bacteria present in the oral cavity. According to epidemiological longitudinal and cross-sectional studies, *Streptococcus mutans* is the most common cause of human dental caries[7,8]. Hence, Antimicrobial agents from natural sources against emerging and re-emerging microbial diseases must be necessitated for continual searching[9]. Nowdays, there are lots of studies regarding exploration

Received on 25.10.2021 Modified on 08.12.2021
Accepted on 10.01.2022
Research J. Pharm. and Tech 2022; 15(12):5672-5676.
DOI: 10.52711/0974-360X.2022.00956

of bioactive compounds from medicinal plants as alternative solutions to overcome microbial resistant[10]. Prospective sources of medicinal plants as antimicrobial bioactive agents included tannins, flavonoids, phenolic compounds and alkaloids had been reported[11]. Myrtaceae family has one of famous species namely *Syzygium aromaticum*. This species has medical advantages included antimicrobial, antioxidant, anti-cancer, anti-inflammatory and anti-diabetic[12]. It is reported that genus Syzygium is the largest genus of flowering plants that were comprised of approximately 1200 – 1800 species[13].

Syzygium aromaticum (L.) produces clove essential oil that has aromatic and volatile substance characteristics which is used commonly as food and beverage flavoring. Essential oils (EOs) are lipophilic plant derivatives that contain volatile components and are produced from various portions of plants using a variety of processes[14]. Essential oils have been used for therapeutic purposes since ancient times and are still commonly used today[15]. Furthermore, clove essential oil has antimicrobial, anti-inflammatory, and antioxidant properties and is also listed by the US Food and Drug Administration as "Generally Regarded As Safe" chemical[16].

Several studies related to *Syzygium aromaticum* (L.) showed that the essential oil of this plant suppressed the growth of S. aureus and E. coli strains with inhibition zone diameters of 16 and 6 mm respectively[17]. Moreover, *Syzygium aromaticum* (L.) extracts exhibited the minimum bactericidal concentration (MBC) at 2 mg/disc against Methicillin-resistant *Staphylococcus aureus* (MRSA) strain, and 1 mg/disc against *E. coli* and *S. typhi* strains[18].

Clove leaves have not been widely used compared to clove flowers or stalks. Clove leaves contain 1-4% essential oil[19]. The main component of clove EO, Eugenol (4-allyl-2-methoxy phenol), was reported to have ability against antimicrobial both Gram-positive and Gram-negative[20–22]. Clove essential oil showed in vitro inhibitory and bactericidal effect against *S. aureus*, *E. coli*, *L. monocytogenes* and *S. Typhimurium*[23].

This current study is conducted to establish antimicrobial activity of clove leaves essential oil against pathogenic bacterial, *Streptococcus mutans* and pathogenic fungal, *Candida albicans*. Moreover, physical characterizations included colour, odour, solubility, density and total eugenol, respectively were determined.

METHODS:

Instruments and Materials:

Material:

Streptococcus mutans (Laboratory of the Indonesia University), *Candida albicans* (Laboratory of Indonesia University), MgSO4 (Merck®), HCl (Merck®), KOH (Merck®), Aluminum Foil (Klin Pak® (Swalayan Nana Jaya), 70% and 85% Ethanol (Merck®), SDA media (Saboraud Dextrose Agar) (Merck®), MHA media (Mueller Hinton Agar) (Merck®), Ciprofloxacin (Novapharin® (Saranani Pharmacy), Ketoconazole (Hexpharm Jaya® (Saranani Pharmacy)).

Instruments:

Steam-water distillation apparatus (BIOBASE WD-A10®), Erlenmeyer (Pyrex®), Analytical Balance (Precisa®), Measuring Cup (Pyrex®), Beaker (Pyrex®), Incubator (Memmert®), Laminar Air Flow (LAF) (E-Scientific®), Petri dish (Pyrex®), Dropper (Pyrex®), Volume Pipette (Pyrex®), Oven (Gallenkamp CAivilab-Australia®), Pycnometer (Pyrex®), Caliper (Sigmat®), Autoclave (Labtech LAC-5065-SP®), Micropipette (Pyrex®), Stirring Rod (Pyrex®), Round Loop (Pyrex®), Syringe (OneMed®), Magnetic Stirrer (VELP ACEC®).

General procedure:

Preparation of Clove Leaves:

Clove leaves were from Latuo Village, Samaturu District, Kolaka Regency, Southeast Sulawesi, Indonesia. Collecting of Clove leaves was conducted in the morning starting at 09.00 am and determined in the Laboratory of Biology Faculty of Halu Oleo University.

Clove Leaves drying:

Clove leaves were dried for four days by aerating. The drying process was conducted at room temperature without direct sunlight. Essential oil from clove leaves was extracted using steam-water distillation apparatus (BIOBASE WD-A10®)[24,25].

Preparation Essential Oil:

The process of extracting essential oils using the distillation method began with 2 kg of clove leaves was put in steam-water distillation container. The steam-water distillation process was carried out for 4 hours for about 400 grams of clove leaves. The distillate was accommodated in a container. Furthermore, the layer of clove leaves essential oils and the water layer were separated using a separating funnel, then $MgSO_4$ was added to the clove oil to remove the remaining water[24,25].

Detection Method:

Antimicrobial efficiency of the clove extracts:

Antibacterial Activity against *Streptococcus mutans*:

The Antibacterial Activity of Clove Leaf Essential Oil (*Syzygium aromaticum* L.) against *Streptococcus mutans* bacteria was carried out using the well diffusion method.

MHA media that had been inoculated with *Streptococcus mutans* and perforated with a size of 6 cm using a perforator then filled with clove leaf essential oil. The wells made were 5 wells consisting of clove leaf essential oil concentration which had concentration variations of 0.5%, 1%, 1.5%, negative control (tween 80 and) and positive control (ciprofloxacin 2%). The MHA media was then put into a desiccator to be incubated at 37ºC under anaerobic conditions for 24 hours. The diameter of the inhibition zone was measured using caliper (Sigmat®) by measuring the diameter of the transparent area minus the diameter of the well[26,27].

Antifungal Activity against *Candida albicans*:

The antifungal activity test was presented by the well diffusion method. Five Wells in SDA (Sabouraud Dextrose Agar) media that had been inoculated with *Candida albicans was* perforated with 6 cm diameter of each then filled with essential oils of 0.5%, 1%, 1.5%, tween 80 (negative control), and ketoconazole (positive control). Then incubated at 37ºC for 1-3 days. Diameter of the inhibition zone was measured using caliper (Sigmat®). The zone of inhibition was obtained by the overall diameter of transparant area minus diameter of the well. The results were recorded in triplicate[28].

Physical characterization of Essential Oil:

Organoleptic:

Some organoleptic parameters are carried out visually by observing the shape, colour, smell and taste[29].

Specific gravity/ Density of Oil:

Specific gravity (density) was determined using a pycnometer (Pyrex®). The empty pycnometer was weighed first, then the pycnometer was filled with essential oil and weighed again. Specific gravity was calculated as followed[30]:

$$\text{Specific gravity} = \frac{\text{Filled pycnometer (g)} - \text{Empty pycnometer (g)}}{\text{Volume pycnometer (ml)}}$$

Solubility in ethanol:

A total of 1 mL of essential oil was added with 70% ethanol drop by drop to 10mL. Then the mixture was shaken until a clear solution was obtained [31].

Eugenol Total:

A total of 10ml of clove leaves oil was put into a cassia flask, added with a 4% KOH (potassium hydroxide) solution to two-thirds of the volume and shaken for 30 minutes. Added another 4% KOH solution to the mark of the volume scale of the cassia flask. The cassia flask was tapped until the oil droplets rose to the neck of the cassia flask. Then the total volume of the oil layer on the neck of the cassia flask was recorded (v)[29]. The formula for calculating total eugenol is as follows:

$$\text{Eugenol Total} = \frac{10\text{-v}}{10} \times 100\%$$

RESULTS AND DISCUSSION:

Essential oil yield:

The principle of method used to obtain essential oil from clove leaves is through the distillation process. The weight of the sample of dry clove leaves by distillation was 3266g and the essential oil yield was 3,8%, which was 125mL.

Antibacterial assay:

All variation concentrations of Clove Leaves Essential Oil provided potency of antimicrobial against the tested pathogenic microbial strains with different sensitivity patterns. As demonstrated in Fig. 1. A, antibacterial activity of Clove Leaves Essential Oil with variation concentrations of 0.5%; 1%; and 1.5%. against *Streptococcus mutans* had inhibition zone diameters (mm) of 19,95±1.7678; 20,5±2.1213; 21,45±1.0607; and 22,1±1.5556 respectively as listed in Table 1. *Streptococcus mutans* was susceptible in 1%; and 1.5%, while in 0.5%; the bactery had intermediate category inhibition. Antifungal of Clove Leaves Essential Oil with variation concentrations of 0.5%; 1%; and 1.5%. showed inhibition zone diameters (mm) of 33,3± 0.2828; 34±0.0000; 35±0.2828; 35,4±0.6364 against *Candida albicans* as shown in Fig. 1. B and Table 1. *Candida albicans* was more susceptible in all variations of Clove Leaves Essential Oil than *Streptococcus mutans*.

Table 1. Antimicrobial activity of clove leaves Essential Oil against different pathogenic microbial strains.

Concentra-tion of clove leaves Essential Oil (%)	Inhibition Zone Diameter (mm)			
	Candida albicans	Category*	*Streptococcus mutans*	Category*
0,5	33,3 ±0.2828	*susceptible*	19,95 ±1.7678	*intermediate*
1	34 ± 0.0000	*susceptible*	20,5 ± 2.1213	*susceptible*
1,5	35 ± 0.2828	*susceptible*	21,45 ± 1.0607	*susceptible*
Positive Control (Keto-conazole);	35,4 ± 0.6364	*susceptible*	22,1 ± 1.5556	*susceptible*
Normal Control	0	-	0	-

*antimicrobial test categories: susceptible (≥ 20 mm), intermediate (15-19 mm) and weak (≤14 mm)[32].

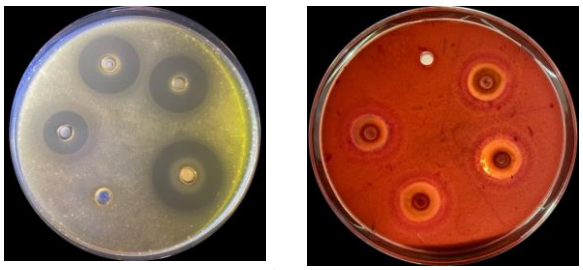

Fig. 1. Antibacterial activity of clove leaves Essential Oil against (A) *Candida albicans* (B) *Streptococcus mutans*. a: 0,5%; b: 1%; c: 1,5%; d: Positive Control (Ketoconazole); e: Normal Control

Physical characterization of Essential Oil

Table 2. Physical properties of clove leave Essential Oil

Physical characterization	Result	Criteria*
Organoleptic (colour dan odour)	Yellow	Yellow to dark brown
Specific gravity	1,047 g/ml	1,0250-1,0609 g/ml
Solubility in ethanol	1:2 clear**	1:2 clear**
Total of Eugenol	Minimum 78% v/v	80% v/v

***Sourced from the Standard Nasional Indonesia Table for Clove Leaf Essential Oil requirements (SNI 06-2387-2006)

****Clear solution formed in ethanol with a volume ratio of essential oil: ethanol (1:2) ml

Multidrug-resistant pathogens in high incidence contribute mortality rate every year significantly[33]. This study exhibited that the *Syzygium aromaticum* leaves *Essential Oil* possessed a potential antifungal activity with suppressive zones of 33,3±0.2828; 34±0.0000; 35± 0.2828, respectively against *Candida albicans*. These results were in accordance with those of [34] who revealed the antifungal efficacy of *Syzygium aromaticum* essential oils against drug-resistant strains of Candida spp isolated from different clinical conditions including vaginitis, urinary tract and blood infections. Our in vitro study also revealed the antibacterial activity of S. aromaticum leaves *Essential Oil* with inhibition zones of 19,95±1.7678; 20,5±2.1213; 21,45±1.0607 against *Streptococcus mutans*. A similar observation was also evident from [21] results that showed antibacterial activity S. aromaticum leaves *Essential Oil* against Porphyromonas gingivalis at a concentration of 31.25 µM. Essential oils of *Syzygium aromaticum* exerted antibacterial potency against Staphylococcus aureus strains isolated from milk of cows with mastitis recording MIC values of 0.392mg/ml[35].

The antimicrobial test categories are divided into susceptible if the inhibition zone diameter is 20mm, intermediate if the inhibition zone diameter is 15-19mm and weak or resistant if the inhibition is 14mm[32]. As stated in Table 1, the inhibitory potential of *Syzygium aromaticum* leaves essential oil against *Candida albicans* at all concentrations in this experiment is susceptible. Meanwhile, against *Streptococcus mutans* at the lowest concentration of 0.5% is intermediate and at other concentrations is susceptible categorized. The inhibition of antibacterial substances is influenced by their concentration. The higher concentration, the more compound bioactive substances have implications for stronger inhibition.

Syzygium aromaticum reported to have antimicrobial potency may be ascribed to its eugenol content which this bioactive compound affects negatively the permeability of cytoplasmic membrane resulting in ions disturbance, ATP transport and results in the initiation of cell death[36]. Clove essential oil contains phenolic as microbicidal action that disrupts the active transport, electron flow and proton motive force which lead to coagulation of pathogens bacterial cell contents[37]. In addition, clove oil disturbs the proteins of the electrons transport system in bacterial cell membrane that can inhibit microbial growth[38].

Physicochemical properties are important to assess the quality of *Syzygium aromaticum* leaves Essential Oil. Organoleptic, solubility in ethanol, density, and eugenol total are parameters observed of the properties[34]. Organoleptic and specific gravity results of *Syzygium aromaticum* leaves Essential Oil were in accordance with the Indonesian National Standardization Agency, which is yellow with a distinctive smell of *Syzygium aromaticum* and not less than one (> 1g/ml)[29]. Solubility in ethanol describes the number of polar compounds in the oil. In general, essential oils containing oxygenated terpenes are more soluble in alcohol. The result of the solubility (Table 2.) of essential oils in ethanol obtained is a ratio of (1:2) ml. The solubility of the essential oil of *Syzygium aromaticum* leaves is due to the high content of eugenol which is an alcohol group compound[29,31]. Eugenol is reactive to strong bases, especially KOH. This property is used to extract eugenol from clove leaf oil. Clove leaf oil is reacted with excess KOH to form sodium eugenolate which is soluble in water. In this reaction only phenol content, namely eugenol reacts with KOH. Eugenol moves from the caryophyllin phase to the aqueous phase as an extraction process and then eugenol in water will react with KOH[39].

CONCLUSION:

The presence of this bioactive compound in the *Syzygium aromaticum* leaves essential oil and its significant activity against *Candida albicans* and *Streptococcus mutans* sufficiently validate the medicinal uses of the plant. To conclude, the investigation of antimicrobial and antifungal activity is required to be further optimized and updated as microbial drug resistance will continue to put global health in danger. Novel antimicrobial compounds can be derived from natural compounds like components of essential oils.

DECLARATION OF COMPETING INTEREST:

The authors state that they have no known competing financial interests or personal relationships that may

result in influence the study reported in this paper.

ACKNOWLEDGMENT:

All authors extend their appreciation to the Universitas Lampung for financial supporting and Universitas Halu Oleo for collaboration and laboratory facilities so that this research can be completed.

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