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RESEARCH ARTICLE

Evaluation of Antibacterial Activity of Cymbopogon Nardus L. on The Growth of Enterococcus Faecalis

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ABSTRACT

Background: *Cymbopogon nardus L. known as citronella grass is traditional medicinal plant that contains saponins, polyphenols, alkaloids, flavonoids, and essential oil. Based on the previous study, it is known that it has antibacterial properties.* ***Objective:*** *The aim of this study was to determine antibacterial activity of Cymbopogon nardus L. in inhibiting the growth of Enterococcus faecalis.* ***Methods:*** *The samples were Cymbopogon nardus L. extracts with concentration of 1%, 3%, 5%,7%, 9%, and 5% NaOCl as positive control. Antibacterial activity of citronella extract against E. faecalis was observed with the formation of inhibitory zone diameter in agar. Data were analyzed using SPSS with one-way ANOVA and LSD test.* ***Result:*** *The result showed that Cymbopogon nardus L. was able to inhibit the growth of E. faecalis at concentration 1%,3%,5%,7% and 9%. There was a significant difference in antibacterial activity among all groups. It showed that concentration of 9% showed the highest zone of inhibition, but it was not as effective as NaOCl 5%. NaOCl showed the highest zone of inhibition.* ***Conclusion:*** *Cymbopogon nardus L. had antibacterial activity in inhibiting the growth of E. faecalis. The higher the concentration of citronella extract, the higher the antibacterial activity possessed.*

Keywords: *Antibacterial, Cymbopogon nardus L., Enterococcus faecalis, inhibition zone.*

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INTRODUCTION

Herbal ingredients have been long used in the health for preventive, curative and rehabilitative purposes. The used of medicinal plants in Indonesia is established, in both fields of medicine and dentistry. So that, the medicinal plants need to be explored, especially Indonesian plants which are known for their rich biodiversity. Since long time ago, people have been believed that herbal ingredients are capable of treating various kinds of diseases and rarely cause adverse side effects compared to synthetic drugs.[1] One of Indonesia's native plants that can be used as herbal medicine is citronella grass (*Cymbopogon nardus L.*).[2]

Cymbopogon nardus L. contains saponins, flavonoids, polyphenols, alkaloids, and essential oils.[3] Saponins work as surfactants form in polar which is able to break down the fat layer of bacterial membrane and causes lysis.[4] Antibacterial flavonoids might be connected with their ability in inactivating microbial adhesins, enzymes, and cell envelope transport proteins. Polyphenols have antibacterial activity by mediating hydrogen peroxide generation in inducing oxidative stress in bacteria.[1] Alkaloids are capable of suppressing oral cavity pathogens by inhibiting ATP-dependent transport of compounds across the cell membrane.[5] Essential oils in *Cymbopogon nardus* L. possess antibacterial activity against *E. coli* and *Salmonella thypimurium.*[6] Previous studies reported that *Cymbopogon* has antibacterial properties against *Streptococcus* and *Staphylococcus*. The results showed that the growth of *S. mutans* bacteria began to be inhibited at a concentration of 6% and *S. aureus* began to be stunted at 1.6% concentration of *Cymbopogon nardus* L. [7,8] Besides the several types of bacteria above, *Enterococcus faecalis* is also found as pathogens that cause failed root canal treatment.

Enterococcus faecalis (E. faecalis) is one type of bacteria often found in root canals with periradicular inflammation. It has a good adaptation in unfavorable conditions and commonly found as resistant bacteria that cause endodontic treatment failed. Irrigation solutions commonly used in root canal treatments include sodium hypochlorite, ethylene diamine tetraacetic acid, chlorhexidine, and iodine potassium iodide. *Enterococcus faecalis* is able to suppress lymphocyte action and contribute to the failure of root canal treatment potentially. Cytoplasmic extracts of *E. faecalis* strain ATCC 29212 can suppress human lymphocyte activation to in vitro mitogenic stimulation, and this suppression is concentration-dependent. The component in the bacterial cultures responsible for this effect, and the SDS-PAGE reveals that the active factor of this extract consists of a 60-kDa polypeptide. When lymphocytes were pre-exposed to this bacterial protein, a decreased proliferative response of the lymphocytes was observed compared with the mitogen-activated lymphocyte proliferation. SEF perturbs the lymphocyte proliferation by blocking cell cycle transition from G0/G1 to S phase. The specific mode and site of action of the SEF in cell cycle progression have yet to be determined; however, it is plausible that immunosuppression mediated by *E. faecalis*, like that mediated by a number of other pathogens, involves pertubation of cell cycle regulatory protein.[9] Antibacterial activity of *Cymbopogon nardus* L. against *E. faecalis* has never been done, so the aim of this research was to determine the antibacterial activity of *Cymbopogon nardus* L. in inhibiting the growth of *Enterococcus faecalis*.

MATERIALS AND METHODS

This study has been approved by the Research Ethics Commission of Mohammad Hoesin General Hospital, Palembang and Medical Faculty of Universitas Sriwijaya with certificate No.0098/kepkrsmhfkunsri/2018. This research is an experimental laboratory study. Thirty samples were divided into 6 groups; Group 1,2,3,4,5 consisted of 1%, 3%, 5%, 7%, and 9% citronella extract respectively, and Group 6 was positive control using 5% NaOCl. Samples were *Cymbopogon nardus* L. with age ranging from

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75-90 days because at this age range, *Cymbopogon nardus* L. reaches maximum contents.[10] Replication was carried out 4 times.

Plant Extraction Preparations

Cymbopogon nardus L. was watery washed, rinsed, cut into small pieces, and dried in indirect sunlight until it got simplisia. Simplisia were mashed into fine powder (30 mesh), put into Erlenmeyer flasks and soaked in 70% ethanol with stirring for 48h. The extract was filtered through Whatmann filter paper no. 41 to separate the filtrate and residue. The filtrates were evaporated (DLAB Scientific Co., Ltd, USA) at 40-50^0 C to obtain pure extract of 100%. The extract was dilute to obtain concentrations of 1%, 3%, 5%, 7%, and 9% respectively.

Preparation of *Enterococcus faecalis*

Enterococcus faecalis ATCC 29212 was put into a test tube containing Brain Heart Infusion (BHI) (Merck, Indonesia), screened at a density of 1 x 10^{-3}, then bacteria were taken using 1 cc micropipette and dripped into a petri dish which contained blood agar media, flattened by shaking the petri dish until the liquid was evenly distributed, allowing 30 minutes to freeze.

Determination of the inhibition zone

Determination of inhibitory zone was performed using *Kirby Bauer* disc method. Petri dishes containing *E. faecalis* in the media completed with six-cylinder filter papers were prepared. Filter papers were set ± 20-25 mm in distance. All discs were cultured overnight for 24 hours at 37^{0}C. The antibacterial assay was evaluated the next day. The observation was repeated 4 times.

Data analysis

Data were analyzed using SPSS 22 vs. (IBM® Inc.pvt ltd.) and Microsoft Excel (Microsoft Inc®). Data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Post Hoc LSD. *P* values less than 0.05 were considered statistically significant.

RESULT

The evaluation of inhibitory zone was shown as Figure 1 and the results of antibacterial assay were shown on Figure 2.

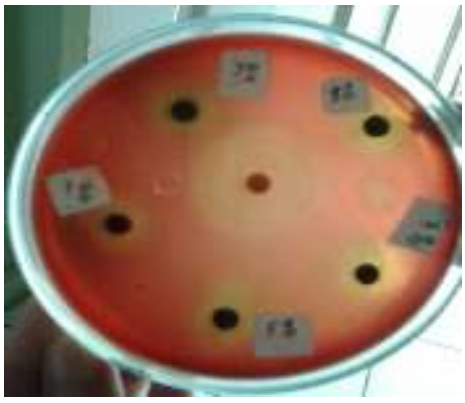

Figure 1. Inhibition zone of 1%, 3%, 5%, 7%, and 9% *Cymbopogon nardus* L. against *E. Faecalis.*

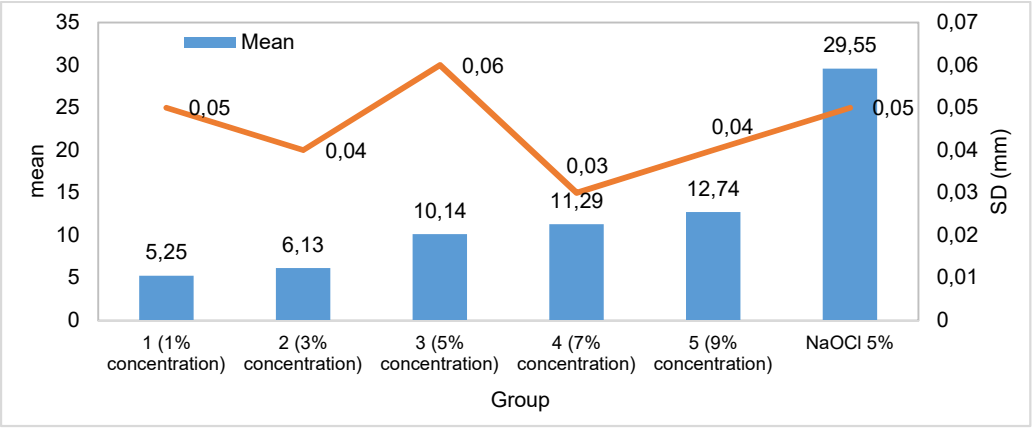

Figure 2. Means of inhibitory zone of *Cymbopogon nardus* L. extract.

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Table 1. Antibacterial effect of *Cymbopogon nardus* L. extracts

	Group 1	Group 2	Group 3	Group 4	Group 5	NaOCl 5%
Group 1		0.00*	0.00*	0.00*	0.00*	0.00*
Group 2			0.00*	0.00*	0.00*	0.00*
Group 3				0.00*	0.00*	0.00*
Group 4					0.00*	0.00*
Group 5						0.00*
NaOCl 5%						

Post Hoc LSD, p<0,05=significant

Figure 2 described that citronella extract with 1% concentration has antibacterial activity. The effect rise by increasing the concentration of *Cymbopogon nardus* L. in which 9% extract had the best effect among other concentrations. NaOCl of 5% had the highest antibacterial effect compared to others.

One-way ANOVA was used to know the difference among all groups, and the result was p<0.00. It meant that there was a significant difference in antibacterial activity of *Cymbopogon nardus* L. against *E. faecalis*. The test was continued with Post Hoc LSD (Table 1).

Table 1 exhibited that there was a significant difference in antibacterial activity among all groups. It showed that concentration of 9% showed the highest zone of inhibition, but it was not as effective as NaOCl 5%.

DISCUSSION

The results showed that *Cymbopogon nardus* L. extract in all concentrations had antibacterial activity. Ukwubile *et al.* [11] reported that Cympogon possessed antibacterial activity with more potency on Gram-positive bacteria than Gram-negative bacteria strains. It was due to the content of the active compounds of *Cymbopogon nardus* L., such as alkaloids, saponins, polyphenols, flavonoids, and essential oils that have antibacterial properties.

Those compounds synergistically lead disruption of membrane integrity, impaired ion balance, membrane leakage, and finally cause cell death.[12] They also have ability to interact with Gram-positive bacterial DNA, damage the double helical structure at the stage replication and transcription, and disrupt bacterial metabolism and growth.[13]

Alkaloids act as antibacterial because of their ability to interact with bacterial DNA, which is to damage the double helix structure at the replication and transcription stages, thereby disrupting metabolism and bacterial growth and finally cell lysis.[14]

Polyphenols are active components that have antibacterial properties. Polyphenols are able to damage bacterial cell walls that have peptidoglycan and inhibit protein synthesis. Flavonoids and essential oils can inhibit bacterial growth by disrupting the permeability of bacterial cell walls.[14]

Manosalva *et al.*[14] found that alkaloids possessed significant antibacterial effects against Gram-positive bacteria strains. Ravi *et al.*[15] reported that saponins were potential as antibacterial activity. Su *et al.*[16] stated that polyphenols contained in some natural plants were capable of killing various types of Gram-positive and Gram-negative bacteria strains, and certain types of bacteria resistant. Alghazeer *et al*.[17] explained that flavonoids were effective as antimicrobials against various microorganisms, because it might have multiple cellular targets, formed complex with proteins, inactivated enzymes and adhesion. Chouhan *et al*.[18] reviewed that essential oil had wide bioactivities as antimicrobial and had been used as alternative medical treatment against multidrug resistance.

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In this study, 9% concentration of *Cymbopogon nardus* L. extract showed the greatest inhibitory zone of *E. faecalis*. The increasing concentration of *Cymbopogon nardus* L. extract showed the higher inhibitory zone of *E. faecalis.* This characteristic is called as dose-dependent effect. The increased concentration resulted in the increased active compounds contained in *Cymbopogon nardus* L. so that the antibacterial activity against *E. faecalis* was also increased.

All concentrations of *Cymbopogon nardus* L. extracts and NaOCl 5% had a significant effect in inhibiting the growth of *E. faecalis*. The difference was because the content of antibacterial substances of 9% was not the same as 5% NaOCl. Zand *et al.*[19] reported that NaOCl was superiorly effective against *E. faecalis* in the root canal system. Sodium hypochlorite (NaOCl) is the most important irrigant in root canal treatment, It is the only presently used solution that can dissolve organic matter in the canal.

Therefore the use of hypochlorite is of utmost importance in removing necrotic tissue remnants as well as biofilm. NaOCl ionises in water into sodium (Na^+) and the hypochlorite ions, OCl^-, and establishes an equilibrium with hypochlorous acid (HOCl). At acidic and neutral pH, most of the chlorine exists as HOCl, whereas at pH of nine and above, OCl^- is most abundant.[20]

Hypochlorous acid has the strongest antibacterial effect while the OCl^- ion is less effective. Hypochloric acid affects directly on the vital functions of the microbial cell, rapidly resulting in cell death.[20] In this study, the concentration of *Cymbopogon nardus* L. extracts used was only 1%, 3%, 5%, 7% and 9%.

Based on the results of this study that the greater the concentration, the antibacterial effect on *E. faecalis* is increasing, so that further research is needed in different concentrations. Further study is needed to find the best concentration in inhibiting the growth of *E. faecalis* effectively.

CONCLUSION

Cymbopogon nardus L. extract had antibacterial activity against *Enterococcus faecalis.* The higher the concentration of *Cymbopogon nardus L*. extract, the higher the antibacterial activity possessed.

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