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Antibacterial activity of essential oil extracts from Curcuma xanthorrhiza roxb. rhizomes against bacteria causing pulp necrosis

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Abstract

Objective: To analyze antibacterial activity of essential oil extracted from Curcuma xanthorrhiza Roxb. rhizomes against bacteria causing pulp necrosis, Enterococcus faecalis, and Fusobacterium nucleatum.

Material and Methods: The method used in this research was disc-diffusion on MH-A media, based on the standard protocol of National Committee for Clinical Laboratory Standards (NCCLS). This method used 4 replications in each of the study group. The study group consists of 4 different concentration groups (25%, 50%, 75% and 100%) of Temulawak rhizomes essential oil extracts, ChKM and Cresophene were used as the positive control group and DMSO (10%) and Tween 80 (0.5%) was used as the negative control group.

Results: The inhibition zones of 25%, 50%, 75%, and 100% of temulawak rhizomes essential oil extracts against E. faecalis were 8.17 mm, 8.54 mm, 9.53 mm and 10.17 mm, respectively. Whereas, the inhibition zones of 25%, 50%, 75% and 100% of temulawak rhizomes essential oil extracts against F. nucleatum were 8.54 mm, 8.95 mm, 9.65 mm and 10.75 mm, respectively. Data analysis using Kruskal-Wallis in both of bacteria showed p=0.00 ($p<0.05$) and therefore, significant levels of inhibition.

Conclusion: Essential oil extracts from C. xanthorrhiza Roxb. rhizomes has medium antibacterial activity against E. faecalis and F. nucleatum.

Keyword: Enterococcus faecalis, Fusobacterium nucleatum, Temulawak rhizomes essential oils, antibacterial activity
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Introduction

Pulp necrosis (98.5%) involves the invasion of intracanal bacterias and its products.[1,2] Failure in root canal treatment is possibly due to the fact that those microorganisms are persistent and recolonize within the canal.[3] Root canal infection is a polymicrobial infection involving both gram-positive and gram-negative anaerobic bacteria.[4] The most common microorganisms found in root canal infections are Enterococcus faecalis and fusobacterium nucleatum, respectively as gram-positive and negative bacteria.[5,6]

Chlorophenol Kamfer Menthol (ChKM) and Cresophene are commonly used for root canal sterilization. However, ChKM and Cresophene are reported to reduce the viability of fibroblast cells.[7,8] Therefore, it suggested to find other environmentally friendly potential drugs, such as a herbal medicine, as an alternative for root canal sterilization with minimal side effects.

One of the herbal medicine that can be used is temulawak rhizomes. Temulawak (Curcuma xanthorrhiza Roxb.) is a plant originating from the Indo-Malaysia region. Temulawak rhizome is reported to contain essential oil, in about 4-6% abundance.[9] The largest active compound that constitutes the essential oil of the temulawak rhizomes is xanthorrhizol. Xanthorrhizol is only found in temulawak and turmeric. Xanthorrhizol is an excellent antibacterial agent at high temperatures (121°C, 15 minutes) and in extreme pH, both acid and base. Xanthorrhizol works by disrupting the formation of biofilms from oral cavity bacteria.[10]

The purpose of this study is to determine the potential antibacterial activity of essential oil extracts of temulawak rhizomes to E. faecalis and F. nucleatum.

Material and Methods

This is a laboratory experimental study with post-test only control group design. This experimental study consist of 4 concentrations of extracts of temulawak rhizomes by diluting the essential oil using 10% DMSO and 0.5% Tween 80 (pure 100%, 75%, 50%, 25%), 2 positive control groups (ChKM and Cresophene) and a negative control group (10% Dimethyl Sulfoxide and 0.5% Tween 80).

The antibacterial test used was disc-diffusion method on the MH-A media (Mueller Hinton Agar). The antibacterial test and the inhibitory zone diameter measurements were performed according to the standard protocol of the National Committee for Clinical Laboratory Standards (NCCLS).[11]

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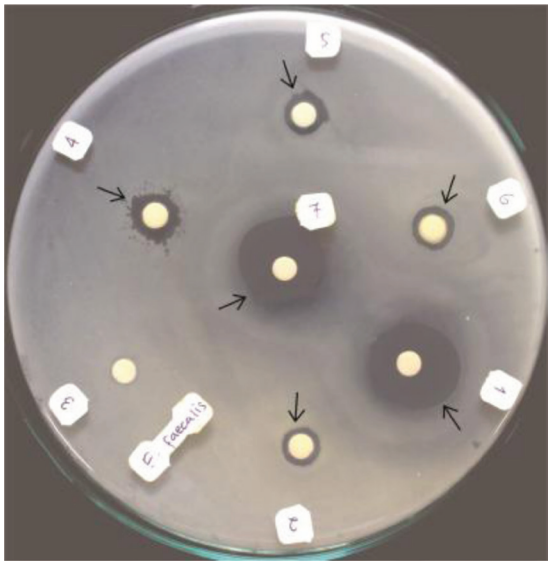
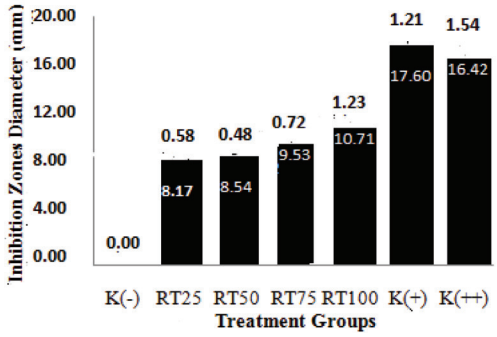


Figure 1 Disc-diffusion test results against E. faecalis showed clear inhibition zones around the disk (arrows)



K(-): Negative control (10% Dimethyl Sulfoxide (DMSO) + 0.5% Tween 80)
RT25: Essential oil extracts of temulawak rhizomes with concentration of 25%
RT50: Essential oil extracts of temulawak rhizomes with concentration of 50%
RT75: Essential oil extracts of temulawak rhizomes with concentration of 75%
RT100: Essential oil extracts of temulawak rhizomes with concentration of 100%
K(+): positive control (ChKM)
K(+): positive control (Cresophene)

Figure 2 The average value of inhibition zone diameter essential oil extracts of temulawak rhizomes against E. faecalisatseveral concentrations: 25% (RT25), 50% (RT50), 75% (RT75), 100% (RT100), 10% Dimethyl Sulfoxide (DMSO) + 0.5% Tween 80 as negative control (K(-)), ChKM as positive control (K(+)) and Cresophene as positive control (K(++))

Data analysis used was non-parametric statistical test Kruskal-Wallis which is then continued with Mann-Whitney test to know the differences among the research groups. p-value used for Kruskal-Wallis and Mann-Whitney test in this study was 0.05.

Results

The disc diffusion test results from the essential oil extracts of temulawak rhizomes (C. xanthorrhiza Roxb.) against E. faecalis showed clear inhibition zones around the disk, which can be seen in figure 1.

The average value of inhibition zone diameter against E. faecalis was presented in figure 2.

The result of data analysis using Kruskal-Wallis test returned a significance value of data p = 0.00 (p <0.05). This data showed that the essential oil extracts of temulawak rhizomes had antibacterial activity against E. faecalis. Data analysis was then continued using Mann-Whitney test to find out which research groups were different. Mann-Whitney test results showed that there were significant differences between all treatment groups with negative control group, 25% concentration group with concentration of 75% and 100%, and 50% concentration group with 100%.

The disc-diffusion test results from the essential oil extracts of temulawak rhizomes (C. xanthorrhiza Roxb.) against F. nucleatum showed clear inhibition zones around the disk, which can be seen in figure 3.

The average value of inhibition zone diameter against F. nucleatum was presented in figure 4.

The result of data analysis using Kruskal-Wallis test returned a significance value of data p = 0.00 (p<0.05). This data showed that the essential oil extracts of temulawak rhizomes had antibacterial activity against F. nucleatum. Data analysis was then continued using Mann-Whitney test to find out which research groups were different. Mann-Whitney test results showed that there were significant differences among all treatment groups 25%, 50%, 75% and 100%.

Discussion

This research used the essential oil of temulawak rhizomes with concentrations of 25%, 50%, 75%, and 100%. Essential oils of temulawak rhizomes in this research were diluted using 10% DMSO and 0.5% Tween 80. The solvent was reported to have no antibacterial activity against gram-positive and gram-negative bacteria, which did not affect the results of the study.[12-14] Tween 80 (0.5%) was used

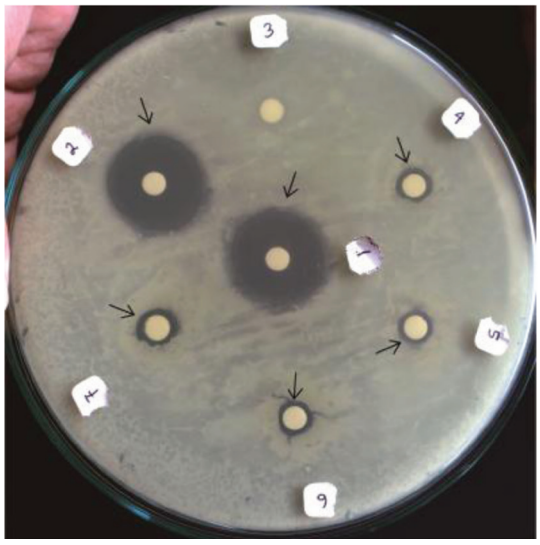
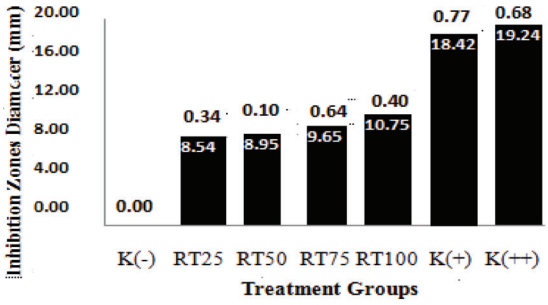


Figure 3 Disc-diffusion test results against F. nucleatum showed clear inhibition zones around the disk (arrows)



K(-): Negative control (10% Dimethyl Sulfoxide (DMSO) + 0.5% Tween 80)
RT25: Essential oil extracts of temulawak rhizomes with concentration of 25%
RT50: Essential oil extracts of temulawak rhizomes with concentration of 50%
RT75: Essential oil extracts of temulawak rhizomes with concentration of 75%
RT100: Essential oil extracts of temulawak rhizomes with concentration of 100%
K(+): Positive control (ChKM)
K(+): Positive control (Cresophene)

Figure 4 The average value of inhibition zone diameter essential oil extracts of temulawak rhizomes against F. nucleatum at several concentrations: 25% (RT25), 50%(RT50), 75% (RT75), 100% (RT100), 10% Dimethyl Sulfoxide (DMSO) + 0.5% Tween 80 as negative control (K(-)), ChKM as positive control (K(+)) and Cresophene as positive control (K(++))

as an emulsifier for water and oil to be homogeneous. Tween 80 is a fatty acid ester of polysorbate 80 or polyoxyethylene 20 sorbitan monooleate ($C_{64}H_{124}O_{26}$). The advantage of Tween 80 as an emulsifier is that it can stabilize the emulsion system and its solubility is high in water, oil, ethanol, and organic solvents.[15]

Antibacterial activity of essential oil extracts of temulawak rhizomes is directly related to the active compounds that constitute them. Xanthorrhizol is the main compound of the essential oil of temulawak rhizomes. The rest, in small amounts, are composed of camphene, curcumin, α-Pinene, β-curcumene, zingiberene, α-thujene, β-pinene, myrcene, linalool, β-bisabolol, and ar-curcumene.[14,16,17] Ninety-two percent of essential oils of temulawak rhizomes are largely composed of terpene classes, particularly sesquiterpenes and derivatives of other oxygenated compounds.[16] Previous studies using other herbals can also inhibit the growth of gram-positive and gram-negative bacteria where the results are consistent with this study. Indriana et al.[18] conducted a study using Hibiscus sabdariffa ethanol extract reportedly proven to inhibit the growth of Porphyromonas gingivalis and Streptococcus sanguis. Another study by Tanumihardja et al.[19] using ethanol root extract of Sida rhombifolia was also shown to inhibit E. faecalis and Actinomyces spp.

Terpene is a hydrocarbon chain formed through the combination of several isoprene units (C_5H_8).[20] Terpene and other oxygenated compounds are hydrophobic or lipophilic, which can typically enter the membrane structure. This leads to expansion of the cell membrane causing in increased fluidity and permeability of membrane that eventually leads to bacterial cell lysis.[20-22] Terpene antibacterial activity also involves the destruction of transmembrane proteins, respiratory inhibition, and changes in ion transport processes.[23] Generally, the main target of the terpene is the bacterial cell membrane.[15]

The antibacterial activity of essential oil of temulawak rhizomes can be classified based on its inhibition zone diameter. Ponce et al.[23] classifies antibacterial activity into 4 levels, those are weak (not sensitive), moderate (sensitive), strong (very sensitive) and very strong (extremely sensitive). Antibacterial activity is said to be weak if the inhibition zone diameter is <8 mm, moderate is 8-14 mm, strong is 15-19 mm, and very strong if >20 mm. The classification of Ponce et al.[23] has been widely used by researchers in comparing the antibacterial activity of an extracts, as in the studies conducted by Celikel et al.[24] Zenati et al.[25] Cherfia et al.[26] and Ed-Dra et al.[27]

The inhibition zone diameter of essential oil extracts of temulawak rhizome against E. faecalis and F. nucleatum at all concentrations was in the range of 8-11 mm. According to the classification of Ponce et al.[23] all four of these concentrations have moderate-level antibacterial properties in both bacteria.

Research on the antibacterial activity of essential oils extracts of temulawak rhizomes against gram-positive and gram-negative bacteria has also been done. A study conducted by Lopez-Romero et al.[15] reported that gram-positive bacteria were more resistant to essential oils than gram-negative bacteria, in line with studies conducted by Prabuseenivasan et al.[14] and Borges et al.[28] This is in contrast to what reported by Sylvester et al.[14] in his journal that gram-negative bacteria are more resistant than gram-positive bacteria, in line with previous studies by Zaidan et al.[29] Gangoué-Piéboji et al.[30] and Hafidh et al.[31]

The antibacterial activity of essential oil extracts of temulawak rhizomes are slightly different in gram-positive and gram-negative bacteria due to different membrane structure and cell walls. Gram-negative bacteria are composed of thin peptidoglycan layers adjacent to the cytoplasmic membrane and outer membrane (OM), whereas gram-positive bacteria have a thicker peptidoglycan layer but do not have an outer membrane.[13,14] The outer membrane of the gram-negative bacteria is constructed by phospholipids and lipopolysaccharides and forms a hydrophilic permeability barrier that provides protection against hydrophobic antibacterial agents.[19] On the other hand, although gram-positive bacteria have no outer membrane, the bacterial cell wall is formed by a thick layer of peptidoglycan, making it difficult to penetrate by a antimicrobial and more resistant to turgor pressure and permeability changes.[13]

The antibacterial activity of essential oil extracts of temulawak rhizomes is not only influenced by the active compound contained in it, but is also influenced by the membrane structure and cell wall of bacteria.[14,18] There are several other things that also affect its antibacterial activity, including the chain construction of the essential oil active compounds, physicochemicals and the bacterial cell surface tension, the releasing of K + ions, and the bacterial cell form.[13] These may be the reasons why essential oil extracts of temulawak rhizomes are effective against gram-negative and gram-positive bacteria.

The concentration of essential oil extracts of temulawak rhizomes used in this study refers to the research done by Prabuseenivasan et al.[12] those are 25%, 50%, 75% and 100%. Essential oil with 100% concentration has greater antibacterial activity than concentrations of 75%, 50% and 25% in both bacteria. This is in line with research conducted by Prabuseenivasan et al.[12] and Jantan et al.[16] that the essential oil extracts of temulawak rhizomes will work better at higher concentrations.

This study used ChKM and cresophene as positive controls. Both materials are selected because they have been commonly used as root canal sterilization. The inhibition zone diameter of ChKM and cresophene on E. faecalis is in the range of 16-17 mm, while on F. nucleatum in the range of 18-19 mm. Referring to the classification of Ponce et al.[21] antibacterial activity of ChKM and Cresophene are strong in both bacteria.

Further research needs to be done to optimize the antibacterial activity of the essential oil extracts of temulawak rhizome in order to be a comparable product to those that already exist on the market. Optimization may including extracting a single active compound from the essential oil of the temulawak rhizomes or increasing the potentiation by a combination of two herbal medicines. Thus, this study can be used as an alternative to root canal sterilization with minimal side effects.

Conclusion

The essential oil extracts of temulawak rhizomes (C. xanthorrhiza Roxb.) has antibacterial activity against bacteria causing pulp necrosis, E. faecalis and F. nucleatum. The four concentrations of essential oils of temulawak rhizomes, 25%, 50%, 75% and 100%, have inhibition zones diameter in the range of 8-11 mm against E. faecalis and F. nucleatum. The antibacterial activity of essential oil extracts of temulawak rhizomes is in the medium level.

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Conflict of Interest

The authors report no conflict of interest.

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