
Research Article

Differences of Basilicum Leaf (*Ocimum Basilicum*) Essential Oil's Inhibition Zones and Parachlorophenol (Chkm) Against *Enterococcus Faecalis*

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Abstract:

The essential oils of basil leaf (*Ocimum basilicum*) have several active components namely linalool, eugenol, methyl chavicol, methyl eugenol, geraniol, geranial and neral. These components have been proven possessing an antibacterial effect on anaerobic bacteria. *Enterococcus faecalis* is anaerobic bacteria which is commonly found in root canals that are difficult to be eradicated because it has a high resistance to some antiseptics. The purpose of this study was to determine the differences of inhibitory effect of basil leaf essential oil (*Ocimum basilicum*) with parachlorophenol campher mentol (ChKm). The type of study was a pure experimental used disc diffusing testing technique. Basil leaf essential oil was made by distillation process to obtain its essential oils with concentration of 40.000 ppm and the test bacteria used was *Enterococcus faecalis* ATCC 29212. The test was performed by measuring the inhibition's zones formed around the discus conducted for 5 consecutive days. The result shows that the mean size of the inhibition's zone around the 40,000 ppm basil essential oil disc is 11.29 mm, while the average inhibition's zone around ChKm is 24.27 mm. Data were analyzed by using ANNOVA test and Tukey test demonstrated that there is no difference in inhibition's zone between basil essential oil with concentration of 40.000 ppm and ChKm. It can be concluded that the essential oil of basil leaf (*Ocimum basilicum*) has the same inhibitory effect as ChKm toward *Enterococcus faecalis*.

Keywords: Basil leaf, Essential oil, ChKm, *Enterococcus faecalis*

Introduction

Basil leaf (*Ocimum basilicum*) essential oil is a food product that has high antimicrobial potential which consists of phenyl propanoid (methyl chavicol, methyl cinamate, eugenol and methyl eugenol) and Terpenes (linalool, geraniol, geranial, camphorandneral). The content variety of basil leaf essential oil are differentiated based on the geographical location plant origin.[1,2] Major compound of basil leaf essential oil that consist of carvacrol, eugenol, linalool and thymol has a wide spectrum antibacterial effect but the major compound consist of linalool has a weak antimicrobial effect.[3,4] Basil leaf essential oil has bacteriostatic effect against gram positive and negative bacteria with minimum inhibition concentration of 36 - 18 µg/ml against gram positive bacteria and 18-9 µg/ml against gram negative bacteria.[5] One of gram positive bacteria that sensitive to basil leaf essential oil is *Enterococcus faecalis* (*E.faecalis*).[6] Basil leaf ethanolic extract antibacterial effect to *E. faecalis* with minimum inhibition concentration 100 µL shown 4 mm inhibition zone it means that basil leaf ethanolic extract has broad inhibitory effect to pathogen mikroorganisme.[7] Basil leaf essential oil with MIC 5000 ppm, 10.000 ppm, and 20.000 ppm has inhibition zone 8,9 mm, 9,25 mm and 11,70 mm towards *E. faecalis*. [8] *E. faecalis* is a persistent microorganism that can survive in the root canal without the absence of other bacteria that

appears in it due to the failure of endodontic treatment and it also may present in the pulp tissue necrosis.[9,10] Several studies had proven that *E. faecalis* is commonly found in root canal of treated teeth without periapical tissue abnormalities.[11] This bacteria may invade dentinal tubules and it can make itself being resistant and difficult to remove from the root canal with some root canal medicament.[10,12] One of root canal medicament is Parachlorophenol Campher Menthol (ChKM) is that a phenolic-type used as root canal medicament that has antibacterial properties. ChKM can be used as a potential antiseptic and disinfectant for root canals compared to other phenol groups.[13,14] ChKM has been proven of having larger ability in inhibiting the growth of *E. faecalis* in root canal compare to povidone iodine.[15] Inhibition zone of ChKM towards *E. faecalis* for 48 hours is 10,53 mm.[16] These studies had shown that ChKM has a good effectiveness against *E. Faecalis* in the root canal because it has a strong anti-bacterial effect.[11] However, ChKM has disadvantages that is toxic, irritating, can cause soft tissue necrosis, may cause infection of periapical tissue, has a very strong odor, and can cause allergic reaction.[17] According to this, the researchers need to find other alternative material that can be used as a root canal medicament which has effectiveness as same as ChKM.

Material and Methods

The research method was true experimental with the data obtained is quantitative. Basil leaf essential oil was gained by using distillation method and was made in various concentrations ie 10.000 ppm, 20.000 ppm, and 40.000 ppm rejuvenated in liquid medium Muller Hinton Broth for 24-48 hours with shuffle speed 150 rpm at 37° C. Standard turbidity of basil leaf essential oil is as same as standard of 0.5 Mc Farland, if bacteria turbidity concentration equal to 108 CFU / ml the testing can be done. Bacteria was isolated and stricted on Muller hinton agar and incubated on incubator with 5% CO2 for 24-48 hours.

Inhibitory zone of basil leaf essential oil and ChKM were determined by disc diffusion method. The test were repeated twice and performed for 5 days. Inhibition zone was shown as a clean area around the discs and the diameter of the zone can be measured by using a caliper. The results of the measurements were analyzed by ANOVA statistical test and continued with Turkey statistical test.

Result and Discussion

Inhibitory zones of basil leaf essential oil (*Ocinum basilicum*) and ChKM are shown in table 1. The data obtained by measuring the clear zone diameter was seen as the area surrounding the disc, this suggests the presence of antibacterial activity.

Basil leaves essential oil with concentration of 10.000, 20.000, 40.000 ppm and parachlorophenol (Chkm) provide inhibitory zone to *E. faecalis* ATCC 29212 bacteria, meanwhile the sample of solvent (ethanol) does not give any inhibition zone against *E. faecalis* ATCC 29212 bacteria. The result of ANAVA statistic analysis with F test on the mean of sample had shown that F count (4,399) > Ftable (3,239), and p value <0,05, this mean there is difference of mean inhibition zone which is very significant at least pair of treatment group. To see which treatment caused different effects on the sample, a further test was done with Tukey's advanced test.

Table 1. Inhibitory zone of basil leaf essential oil compared with ChKM.

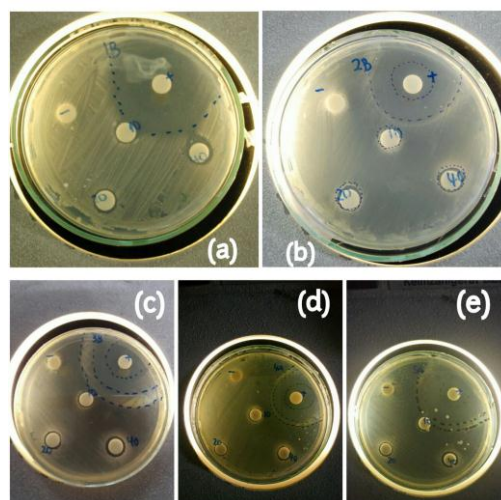
Sampel	Hari ke				
	1	2	3	4	5
Negative control ethanol (-)	0	0	0	0	0
Paramonoclorofenol (+)	50.1	16.35	22.1	15.9	16.9
Basil leaves essential oil 10.000 ppm	9.65	9.5	10.05	9.55	10.45
Basil leaves essential oil 20.000 ppm	10.65	10.15	10.85	10.5	10.6
Basil leaves essential oil 40.000 ppm	12.05	11.45	11.1	10.75	11.1

10.000, 20.000 and 40.000 is the concentration of basil leaf essential oil

Inhibitory zone marked in centimeter (cm)

Based on the results of further tests Tukey, it can be seen that samples given with Paramonoclorofenol (+) is significantly different with the sample with basil leaves essential oil of 10.000 ppm and 20.000, but it has no significantly different with basil leaves essential oil of 40.000 ppm.

Figure 1. Inhibition zone of Basil leaves essential oil in different concentration and ChKM, (a) day 1 (b) day 2 (c) day 3 (d) day 4 and (e) day 5, (+) is parachlorophenol camphor menthol/ ChKM (-) is solvent ethanol 10, 20, 40 is basil leaves essential oil with concentration 10.000, 20.000 and 40.000 ppm



Basil leaf essential oil has the main ingredients of cineole, eugenol, methyl cinnamate, camphor, methyl eugenol, methyl chavicol, geranial, linalool. This content is a group of phenols that are known as an antiseptic and disinfectant against gram positive and negative bacteria [18]. The results shows that there is no differences between the basil leaves essential oil of 40.000 ppm with ChKM. It is happens because both of these materials have the same properties as a strong antiseptic because these materials are group of phenol which is proved to inhibit *E. faecalis* bacterial growth.

Conclusion

Based on the researchs' result, it can be concluded that there is no difference between inhibition zone around the discs of basil leaves essential oil and around its of ChKM to *E. faecalis* bacteria at concentration of 40.000 ppm

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References

[1] Gaio, A.G Saggiorato, H. Treichel, A.J. Cichoski, V. Astolfi, R.I. Cardoso, et.al. 2015. Antibacterial activity of basil essential oil (*Ocimum basilicum* L.) in Italian-type

- sausage. *J. Verbr. Lebensm*, 10: 323-329
- [2] A. Nasser, A. Ali, W.N. Setzerb. 2014. Pharmacological activities of basil oil: a review. *RPMP*, 37: 285-307
- [3] Vanin AB, Orlando T, Piazza SP, Puton BMS, Cansian RL, Oliveira D, Paroul N (2014) Antimicrobial and antioxidant activities of clove essential oil and eugenyl acetate produced by enzymatic esterification. *Appl Biochem Biotechnol* 174:1286–1298
- [4] Hussain Ail, Anwar F, Sherazi STH, Przybylskmi R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*. 108: 986-995 (2008)
- [5] A.M. Moghaddam, J.Shayegh, P.Mikaili, and J.D. Jalil Sharaf. Antimicrobial activity of essential oil extract of *Ocimum basilicum* L. leaves on a variety of pathogenic bacteria. *J. Medicinal. Plants Res.*, 5: 3453–3456 (2011)
- [6] A.K.Anand, M.Mohan, S.Z. Haider and A.Sharma. Essential oil composition and antimicrobial activity of three *Ocimum* species from Uttarakhand. *Int. J. Pharmacy and Pharmaceutical Sci.*, 3: 223–225 (2011)
- [7] Z.M. Jassim. Antimicrobial activity of ethanolic leaf extracts of *Ocimum basilicum* on some bacteria. *J. of al-Qadisiyah for pure science*. 3(20): 41-49 (2015)
- [8] Fatriadi. F, A. Suciati, D. Prisinda. 2016. The difference in anti bacterial activity between basil leaf (*Ocimum Sanctum*) essential oil and chlorhexidine gluconate towards *Enterococcus faecalis*. *FDI Proceeding*: 183-186
- [9] C. Pirani, A. Bertacci, F. Cavrini, F. Foschi, G.L. Acquaviva, C. Prati, V. Sambri. Recovery of *Enterococcus faecalis* in root canal lumen of patients with primary and secondary endodontic lesions. *New Microbiologica*, 31: 235-240 (2008)
- [10].A. Mozayeni, A. Haeri, O. Dianat, A.R. Jafari. Antimicrobial effect of four intracanal medicaments on *Enterococcus Faecalis*; An in Vitro Study. *Iranian Endodontic Journal*, 9(3):195-198 (2014)
- [11] G.O. Zoletti, J.F. Siqueira, K.R.N. Santos. Identification of *Enterococcus faecalis* in root filled teeth with or without periradicular lesion by culture-dependent and independent approaches. *J. Endod*, 32: 722-726 (2006)
- [12] G. Kayaoglu, O. Dag. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. *Crit. rev. Oral Biol. Med*, 15(5): 308-320 (2004)
- [13] G.N. Da Silva, E.A. De Camargo, D.M. Salvadori, D.A. Ribeiro. Genetic damage in human peripheral lymphocytes exposed to antimicrobial endodontic agents. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 104: 58-61 (2007)
- [14] D.R. Violoch, N.P. Chandler. The smear layer in endodontics: A review. *Int Endod J*, 43: 2-15 (2010)
- [15] T. Dammaschke, N. Jung. The effect of different root canal medicaments on the elimination of *Enterococcus faecalis* ex vivo. *European J. of Dentistry*, 7: 442-448 (2013)
- [16] F.L. Charyadie, S. Adi, R.P. Sari. Daya hambat ekstrak daun alpukat (*Persea americana*, Mill) terhadap pertumbuhan *E. Faecalis*. *Denta Jurnal Kedokteran Gigi*, 8 (2014)
- [17] Fauzia, A. Larasati. Uji efek ekstrak air daun avokad (*Persea gratissima*) terhadap *Streptococcus mutans* dari saliva dengan kromatografi lapisan tipis (TLC) dan konsentrasi hambat minimum (MIC). *Majalah Kedokteran Nusantara*, 41(3):173-178 (2008)
- [18] K. Poonkodi. Chemical composition of essential oil of *Ocimum basilicum* L. (basil) and its biological activities-an overview. *J. Critical reviews*, 3: 56-62 (2016)