



Antibacterial Activity of Lemon Basil (*Ocimum citriodorum*) and Key Lime (*Citrus aurantifolia* S.) Extract Combination on *Streptococcus mutants*

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Abstract

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BACKGROUND: Lots of plants that are used in Southeast Asian and Indonesian cuisine have antibacterial properties.

AIM: This study aimed to find out the effectiveness of lemon basil (*Ocimum citriodorum*) and key lime (*Citrus aurantifolia* S.) extract combination on *Streptococcus mutants*' inhibition.

METHODS: Lemon Basil and Key Lime were extracted and combined with several following concentration, respectively, (30–70%, 40–60%, 50–50%, 60–40%, and 70–30%). *S. mutants* were cultured using Brain-heart Infusion Broth overnight. The inhibition range from the extract combination was measured and analyzed.

RESULTS: All combinations except 60–40 showed significant effectivity against *S. mutants* compared to control ($p < 0.05$). *Post hoc* tests revealed that only the combination Lemon Basil-Key Lime 30–70% that has a significantly wider inhibition range compared to L50%-K50% ($p = 0.034$), L60%-K40% ($p = 0.007$), and L70%-K30% ($p = 0.023$).

CONCLUSION: Combination extract of Lemon Basil and Key lime has effectivity against oral micro bacteria, especially *S. mutants*

Introduction

Micro bacteria in dental plaque have long been known as the etiology of oral diseases such as dental caries and periodontal disease [1]. Years of dental education programs to promote good proper dental brushing to eliminate dental plaque still do not show good evidence of reducing the number of oral problems such as dental caries [2].

Most antibacterial agents can promote bacterial-resistant side effects. Therefore, studies regarding the natural therapeutic agent that is safe for humans and specific for dental caries are getting more interest [3]. Expert now seeking many herbal-based types of mouthwash to help in preventing dental plaque growth after dental brushing. A previous study showed that herbal mouthwash was as effective as a chemical commercial mouthwash [4].

Some of the herbal products that are also known for their antibacterial effect are Lemon basil (*Ocimum citriodorum*) and key lime (*C. aurantifolia* S.). Those plants were easily found in Asia. Indonesian people usually called Lemon basil with

“kemangi” and key lime with “jeruk nipis.” Indonesian used to eat lemon basil raw or cooked it with other meals. While key lime is essential to remove the fish odor before cooking it. The unique flavor and aroma of lemon basil are favorable among Asians. Ancient people also use it to relieve the symptoms of stomachache, and bloating, besides as an antiemetic, and aromatherapy [5].

As a family of mint plants, lemon basil is also consumed raw as a vegetable and as leafy spices for protein-based savory meals in Southeast Asian cuisines. Conventionally, it has also medicinal benefits to treat cough and fever, also wound healing [6], [7]. Basil has also proven effective as a natural preservative that has antimicrobial effects against Gram-positive and Gram-negative bacteria [8], [9].

The antibacterial effect of lemon basil essential oil was proven against Gram-positive bacteria such as *Streptococcus mutant*, *Clostridium difficile*, *Bacillus subtilis*, and *Staphylococcus aureus*. It was also effective against Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumonia* [10], [11], [12]. A previous study showed that a 100% concentration of lemon basil extract has

a strong inhibition response on *Streptococcus mutans* growth *in vitro* that is equal to 10.26 mm [11].

Lemon basil is even effective on some species of fungi such as *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans* [12], [13].

Citrus fruit is grown all over the world and is widely used to refresh any dish. Many studies have explored the antioxidant, antibacterial, anti-inflammatory, anticancer, and other therapeutic activities of this fruit [14]. One of the citrus fruit types that most Asian used for cooking is Key Lime (*C. aurantifolia* S.). Key lime is also famous for relieving cough, as a mucolytic, diuretic, and antipyretic in traditional medicine [15].

A previous study showed that key lime also has an antibacterial effect against one of the bacteria that build the oral biofilm, *Streptococcus mutans* [16], [17], [18]. *Streptococcus* is one of the bacterial families that build the biofilm complex on oral surfaces along with around 700 other species. *Streptococcus mutans* is a Gram-positive facultative anaerobic coccus that dominates the oral cavity as part of normal flora. When dysbiosis of the normal flora is established, the bacteria can initiate the pathogenesis of caries and periodontal disease [18], [19].

Considering many benefits of that herbal product, this study aimed to figure out the effectiveness of lemon basil (*O. citriodorum*) and key lime (*C. aurantifolia* S.) extract combination on *Streptococcus mutans*' inhibition.

Methods

This was a quasi-experimental study conducted at the Laboratory of Pharmacy and Laboratory of Analytic in Jambi Health Polytechnic. Lemon basil (*O. citriodorum*) and key lime (*C. aurantifolia* S.) were extracted and divided into several concentrations (30%, 40%, 50%, 60%, and 70%). Mixture of lemon basil-key lime extracts was made with the following combinations: 30–70%, 40–60%, 50–50%, 60–40%, and 70–30%.

Dental plaque was isolated from free caries and free calculus respondents and cultured overnight using Brain-heart Infusion Broth (BHIB). The cultured sample was further cultured in Blood Agar plate, to specifically grow the Gram-positive bacteria colony. A pathology analyzer was performed to identify the *S. mutans*. *S. mutans* were cultured in Brain-heart Infusion Agar (BHIA) for 24 h. The colony of *S. mutans* that were grown after being cultured overnight in BHIA was taken and mixed in NaCl 0,9%. The density of the bacterial cell was prepared to 10^8 cells mL⁻¹ of 0,5 McFarland standards. The aliquot was then homogenized in BHI agar and plated. Four wells were created on agar plates

using a sterile 6 mm punch. Each well was filled with the extract combination. About 10% of Povidone-iodine was used as a control. The formation of clear inhibition zone diameter was measured after all plates were incubated at 36°C for 18 h.

The effectiveness of combination extract on bacterial inhibition was analyzed by comparing the diameter of inhibition using the Mann–Whitney test, followed by the *post hoc* Turkey test to evaluate which of the group has the best effectivity on bacterial inhibition.

Results

Data of the inhibition range of each extract combination are presented in Table 1.

Table 1: Inhibition range antibacterial activity of combination concentration of Lemon Basil (L) and Key Lime (K)

Combination concentration	Mean inhibition range (mm) (± SD)	p-value*
L30-K70	16,75 (± 1.50)	0.011
L40-K60	14,5 (± 1.95)	0.019
L50-K50	12,75 (± 1.70)	0.025
L60-K40	11,75 (± 1.70)	0.053
L70-K30	12,5 (± 1.73)	0.010
Control	10,5 (± 0.50)	-

*Mann–Whitney test compare to control.

Results showed that all other combinations showed significant differences with the control except for the L60-K60. The higher the key lime concentration extract was used, the inhibition range seemed wider. The widest mean inhibition range was shown on the L30-K70 extract combination which is 16.75 mm (±1.50). To analyze the significance of the difference between combination groups, a *post hoc* using the Turkey test was conducted. The result is shown in Table 2.

Table 2: Post hoc analysis between groups of combination concentration

Concentration	p value†
L30-K70	
L40-K60	0,382
L50-K50	0,034*
L60-K40	0,007*
L70-K30	0,023*
L40-K60	
L30-K70	0,382
L50-K50	0,613
L60-K40	0,210
L70-K30	0,493
L50-K50	
L30-K70	0,034*
L40-K60	0,613
L60-K40	0,919
L70-K30	1,000
L60-K40	
L30-K70	0,007*
L40-K60	0,210
L50-K50	0,919
L70-K30	0,970
L70-K30	
L30-K70	0,023*
L40-K60	0,493
L50-K50	1,000
L60-K40	0,970

†Post hoc test using Turkey test. *Significant result.

Analysis showed that only the L30-K70 combination concentration that has a significant difference from all other combinations while other

combinations showed no significant difference between groups.

Discussion

This study showed that a combination of Lime Basil and Key lime significantly has better bacterial activity compared to the control. Basil is one aromatic plant that is widely used to enrich the flavor of cooked food. The richness of volatile organic compound that basil has, attract food and pharmaceutical research and industries [20]. While, key lime has been used as food and for medicinal purposes. It is citrus family fruit that has greenish-yellow colors and tastes sour-bitter. It originally came from East Asia [21]. The richness of vitamin and strong citrus aroma has long been used in cuisine to block the disturbing aroma from other food [22].

We were using the lemon basil or known as "kemangi" in Indonesian since it grows and is used widely by Indonesian people. There are many different species of basil around the world, such as holy basil (*O. sanctum*), sweet or Thai basil (*O. basilicum*), lemon basil (*O. citriodorum*), and tree basil (*O. gratissimum*) [20]. When compared with other species of basil, lemon basil (*O. citriodorum*) has slightly better activity against some bacterial strains, especially Gram-positive bacteria. This antimicrobial activity might be related to the existence of Rosmarinic acid as another major compound of phenolic acid [23].

Our study combines lemon basil and key lime extract. Key lime has many outstanding properties such as antibacterial, anti-inflammatory, and antioxidant. Many studies have proven that it has anticancer or antitumor, anti-cholesterol, anti-larvae, anti-mosquito, antidiabetic, and anticholinesterase [21]. The high content of d-limonene gives key lime a remarkable radical-scavenging activity [24]. The key lime essential oil has many advantages traditionally such as relieving cough, common cold, flu, asthma, arthritis, and bronchitis. Some even use it to help lose weight and treat drug-induced obesity and related diseases [24].

There were no other studies that ever combine lemon basil and key lime before, but the lemon basil itself has been proven in the previous study for its antibacterial activity. About 100% concentration of basil extract can inhibit the growth of *S. mutans* for 10.26 mm [11]. By adding key lemon extract, our finding showed that the inhibition was stronger.

Basil with 100 mg/L concentration was also found strongly inhibit the growth of *A. hydrophila* [6]. About 100% concentration of ethanolic extract of kemangi or lemon basil inhibits the *Staphylococcus aureus*, another positive gram bacteria [13].

Some of the volatile compounds found in basil are phenylpropanoids, such as estragole, eugenol, and methyl eugenol [5], [10], [20], [25]. This compound has antimicrobial and antioxidant properties that have been used for food or pharmacology needs [25]. Especially eugenol that also used in dental remedies in dentistry since it has antimicrobial and analgesic effects [20]. No wonder Basil is also considered a traditional medicine and flavoring agent for food and has also been reported as a dental and oral traditional remedy for its eugenol properties [7]. Another compound that also has an antibacterial effect in lemon basil that has been studied was β - sitosterol ($C_{29}H_{50}O$). Even though it showed antibacterial activity by *in vitro* assay, yet performed its ability inhibiting of peptidoglycan biosynthesis and prevent the formation of the bacteria cell walls [26].

For key lime itself, the phytochemical properties that have been known for this fruit are phenols, flavonoids, steroids, alkaloids, and terpenoids. Saponin glycosides, anthraquinone, tannin, and carbohydrates are also some other components found in key lime [21]. The major constituent found in key lime D-limonene, along with α -pinene, sabinene, and β -pinene. These chemical components contribute to its sharp aroma and antibacterial activity [27]. A previous study showed that the essential oil also exhibits broad-spectrum antibacterial activity [28].

A higher proportion concentration of key lime showed a significantly best performance in inhibiting the *S. mutans* in our study. A previous study that has a similar result showed that key lime peel extract also has antibacterial activity against *S. mutans*. The effectivity of key lime peel extract in their study showed that 100% concentration has a significantly higher killing effect on the bacteria compared to lower concentration and negative control [16].

Citrus peel extract contains flavonoids, one of the polyphenol compounds that have antioxidant and antibacterial activity. This flavonoid acted by denaturing bacterial cell proteins so the bacterial cell wall destructed. The inhibition of glucosyltransferase (GTF) activity by the flavonoid can also help to prevent the formation of dental biofilm which is the main etiology of oral diseases such as caries and periodontitis [18].

Another antibacterial compound that has also been identified in key lime peel was β -pinene ($C_{10}H_{16}$). This compound was effective in inhibiting the growth of *S. mutans* ATCC 25175 at a concentration of 2000, 1000, and 500 ppm by 13,0 mm, 11,9 mm, and 11,6 mm, respectively, on paper disk assay. β -pinene is also identified as linalool a-terpineol related to the antibacterial activity of bacteria responsible for caries and periodontal disease [17].

The pharmacological properties of basil that has also been studied were anti-cancer activity, radioprotective activity, anti-microbial activity, anti-inflammatory effects, immunomodulatory activity,

anti-stress activity, anti-diabetic activity, antipyretic activity, anti-arthritic activity, anti-oxidant activity, as a prophylactic agent, and in cardiovascular disease [7]. The antioxidant components that can be found in basil are caffeic, vanillic, quercetin, rutin, apigenin, chlorogenic, p-hydroxybenzoic, and rosmarinic acids. The main fatty acid composition of basil species are Stearic acid, Oleic acid, Palmitic acid, Linoleic acid, Myristic acid, α -Linolenic acid, Carpic acid, Lauric acid, and Arachidonic acid [29].

The aqueous and hydroethanolic extracts of lemon basil also revealed the magnificent ability of cytotoxicity and anti-inflammatory activity in the mouse macrophage-like cell line. It also showed cytotoxicity against non-tumoral hepatocytes [23].

The antibacterial response in our findings showed a better result as the concentration of key lime was higher. The previous study confirmed our finding of where the linear relationship between concentration and the inhibition of *S. mutans*. The bigger concentration of the extract, the wider inhibition produced. The ability of lime to disrupts the glucosyltransferase enzyme activity that is needed for the *S. mutans* to synthesize sucrose in a glucan medium. So that the formation of the biofilm was also inhibited [30].

Another possible explanation might be based on the bacterial type, Gram-positive bacteria were more susceptible to lime rather than Gram-negative bacteria since it only consists of an outer peptidoglycan layer and a small amount of protein. While Gram-negative bacteria have a more complex layer on their surface with additional phospholipid membrane, proteins, and lipid-based peptidoglycan [31].

Another key lime property that also has a contribution to its antibacterial activity was Tanin since it natures to tie and shrinks protein, it can inhibit the bacterial cell wall synthesis, and the result was be toxic to bacteria [15].

Conclusion

Within our study limitations, it can be concluded that lemon basil (*O. citriodorum*) and key lime (*C. aurantifolia* S.) extract combination has a significant antibacterial effect against *S. mutans*. A combination of 30% lemon basil and 70% key lime extract has significantly higher effectivity on inhibiting bacterial growth.

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