Evaluation of Antibacterial and Antioxidant Effects of Mix Essential Oil for Oral Health Care

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Keywords: Mix essential oil, antioxidant, antibacterial

Abstract: Essential oil have some antioxidant and antimicrobial properties. The aim of this study was to determine the chemical compounds, antioxidant and antimicrobial activities of essential oil. The analysis of the mix essential oil was carried out using gas chromatography mass Spectrometry. The antioxidant activity of the essential oil was also evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antimicrobial properties of the essential oil were assessed against *Enterococcus faecalis, Streptococcus mutans, Streptococcus sanguinis* using the disk diffusion method. Free radical scavenging potentials showed values for IC_{50} in 194 µg/ml for mix essential oil, which are close to the natural antioxidant (ascorbic acid) with an IC_{50} of 2.98 µg/mL. The major of mix essential oil were α-pinene (24.54%), D-limonen (18.00%), cis-1-methyl-4-(1-methylethenyi)-l-cyclohexane (14.95%), 3-carene (8.92%), L-menthone (8.26) and β-pinene (5.72%).

1 INTRODUCTION

In the 21st century, multidrug resistant antibiotic is widely recognized as a serious threat to global health (Martelli and Giacomini, 2018.) According to World Health Organization (WHO) data in 2017, the most dangerous multidrug-resistant to which new antibiotics should be highly discovered (World Health Organization, 2017). The discovery of new antibiotics agents was mainly from natural product (Jackson et al., 2018). Natural products have been a source of medicinal agents and traditional medicine system that have been used for thousands of years in many countries (Dias et al., 2012; Newan and Cragg, 2016). Natural antimicrobial and antioxidant agents can be obtained from different sources including plants, bacteria, algae animals, and fungi, but there has been an increased interest in plant-based active compound as an alternative to the common antibiotics (Rossiter et al., 2017; Helal et al., 2019).

Essential oil of many plant special have become popular in recent years. Essential oils are volatile natural mixtures extracted from different plant parts (seeds, flowers, buds, leaves, twigs, bark, herbs, wood and roots), and are composed of terpenoid structures with broad activities (Seow et al., 2014). Plant essential oils are also well-known to be the rich sources of bioactive compounds. They are use as alternative medicines, particulary as anti-inflammatoty, antimicrobial, analgesic, antipasmodic, anthelmintic, antipruritic and many other theraperutic (Bakkali et al., 2008; Jaradat et al., 2017). Nowadays, essential oils are used broadly in preservatives in food and beverages industry, cosmetics and pharmaceutical products (Seow et al., 2014; Bakkali et al., 2008). Research on the use and efficacy of essential oils significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely knowns. Therefore, in this study the antimicrobial and antioxidant activities of essential oils are the subjects of particular interest. Evaluation of antioxidant properties and antimicrobial activity against different oral bacteria.

Juniarti, ., Abdussalam, M., Permata Yuda, I. and Kusuma, I.
Evaluation of Antibacterial and Antioxidant Effects of Mix Essential Oil for Oral Health Care.
DOI: 10.5220/0009957801190122
In *Proceedings of the 2nd International Conference of Essential Oils (ICEO 2019)*, pages 119-122
ISBN: 978-989-758-456-5

2 MATERIAL AND METHODS

2.1 Material

Essential oil was provided by from WA Japan, Co (Saitama-shi, Saitama-ken, Japan), which was dried with anhydrous sodium sulphate and stored in vial at 4°C before use. Ascorbic acid, methanol (Merck, German), 2,2-diphenyl-1-picrylhydrazyl (DPPH), chlorhexidine (were purchased from Sigma Aldrich) as positive control, and anaerobic jar (for anaerobic condition) for antibacterial assay.

2.2 GC-MS Analysis Conditions

The analysis of the mix essential oil was performed using Agilent 19091S-433, Equipped with HP-5 MS capillary column (30 m x 0.25 mm, i.d., 0.25 µm film thckness) and a HP 5972 mass selective detector. For GC-MS detection an electron ionization with ionization energy of 70 eV was used. Helium was the carrier gas at a flow of 20 mL/min. Injector and MS transfer line temperatures were set at 150 and 250 °C, respectively. Column temperature was initially kept 80°C for 3 min, then gradually increased to 325°C at 3°C/min rate. 2 µL of sample were injected manually and split mode**.**

2.3 Antimicrobial Screening

The antibacterial activity against *Enterococcus faecalis* ATCC 29212, *Streptococcus mutans* ATCC 25175, *S. sanguinis* ATCC 10556 was detected using disk diffusion method. The Kirby-Bauer disk diffusion susceptibility test was used to determine the sensitivity or resistance of bacteria to essential oil. Bacteria was inoculated to nutrient broth (NB), incubated at 37°C for 24 hours. Inoculum was diluted by using physiological solution (NaCl 0.9%) to match 0.5 Mc Farland standard. A paper disk was dropped 50-µl essential oil in certain concentration and put the disk in Mueller Hinton agar plate content bacteria inside. The plates were incubated at 37°C for 24 hours. Chlorhexidine was used as a positive control. Inhibition area diameter (IAD) was recorded as sensitivity by measured the clear zone of growth inhibition on agar surface around the disk.

2.4 Antioxidant Activity

The antioxidant activity of essential oil was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The DPPH method was employed to evaluate the antioxidant activities of essential oil radical-scavenging activity as described by Panda (Panda, 2012). Briefly, 1 mL of the extract at varying concentrations (25–200 μg/mL) was stirred together with 1 mL of DPPH in methanol (0.3 mM) and 1 mL of methanol. The mixture solution was incubated in dark room for 30 minutes and then the absorbance was measured using spectrophotometer at wavelength 517 nm. The percentage of DPPH inhibition was calculated using the following equation: % inhibition = $[(A\text{DPPH} - AS\text{ADPPH})] \times 100$, (1) where ADPPH is the absorbance of DPPH without a sample and AS is the absorbance of DPPH with a sample or the standard. DPPH scavenging activity was presented as the concentration of a sample required to decrease DPPH absorbance by 50% (IC_{50}). This value can be determined by plotting the absorbance (the percentage of inhibition of DPPH radicals) against the concentration of DPPH and fitting the slope of the linear regression.

3 RESULT AND DISCUSSION

The chemical composition of mix essential oil was analysed by employing GC-MS, leading to comparison of the relative retention time and the mass spectra of mix oil component with data library as shown in Table.1.

Table 1: Chemical composition for mix essential oil

No	RT (min)	Component[a]	Composition (%)
1	2.113	α-pinene	24.54
2	2.353	β-pinene	5.72
3	2.498	α-phellandrene	1.56
4	2.669	D-limonen	18.00
5	2.891	γ-terpinene	1.69
6	3.250	3-carene	8.92
7	3.926	L-menthone	8.26
8	4.063	1-menthone	3.14
9	4.191	*cis*-1-methyl-4-(1-methylethenyi)cyclohexane	14.95
10	5.234	D-carvone	4.21
11	5.952	4-methyl-1-(1-methylethyl)cyclohexene	1.34
12	7.294	Eugenol	1.93
13	8.380	Caryophyllene	1.91

[a] major component (> 1%)

Structure analysis resulted in the identification of thirteen compound representing 96.8% of the mix oil. The main component were cyclic monoterpenes and sesquiterpene. The result of bioassay showed that mix essential oil exhibit antimicrobial activity against *Enterococcus faecalis, Streptococcus mutans, S. sanguinis* using the disk diffusion method shown as in Table 2 and Figure. 1. Pinene compounds (α-pinene and β-pinene) have oral antibacterial bioactivity. Mercier (2009) reported that α-pinenes is the largest contribution of active fractions against gram-negative bacteria that seek jaw infections, pardontitis or periodontitis (Mercie *et al*., 2009). D-limonene compound, the main component of citrus essential oil has activity against the bacteria *Porphylomonas gingivalis* with a significant inhibition in the range of 0.33-1.00 mg/mL (Mizrahi *et al*., 2006). Mint leaves (*Mentha piperita*), the main component is the L-menthone compound has antibacterial activity against *Aggregatibacter actinomycetemcomitans*, periodontal disease bacteria (Karicheri and Antony, 2016).

Table 2. Antimicrobial activity from mix essential oil

Concentrations (%)	Inhibition Zone (mm)					
	E. faecalis		*S. mutans*		*S. sanguinis*	
	CHX[1]	EO[2]	CHX	EO	CHX	EO
2.0	18.4	-	28.0	-	13,9	-
12.5	NA	-	-	-	-	-
25.0	-	8.8	-	-	-	-
50.0	-	9.8	-	8.0	-	-
100.0	-	11.8	-	9.5	-	7.8

[1]*Chlorhexidine*
[2]*Essential Oils*

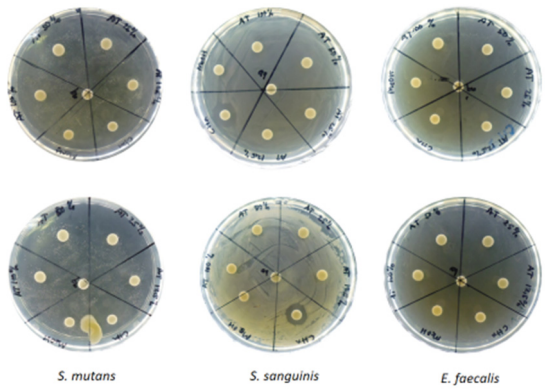

Figure 1: Antibacterial efficacy of essential oils compared to a chlorhexidine

Free radical scavenging activity was measured with DPPH methods. Employing the DPPH methods the reult show in Table 3, antioxidant activity (IC_{50} 194.90 ± 1.36 µg/mL) for the essential oils studied, was lower efficient than ascorbic acid (IC_{50} 2.98 ± 0.06 µg/mL). The absence of antioxidant activity observed for the essential oils in the DPPH reduction can be explained by the reality that they are not capable of donating a proton and the low solubility provided by them in the reaction medium of the assay, because this test utilizes methanol as solvent. Otherwise, ascorbic acid have the ability to donate the hydrogen atoms to DPPH reagent, can also describe this low inhibition concentration oxidizing activity (Gharred et al., 2019; Umaru et al., 2019). Therefore, the reality that the essential oils of this study do not show significant antioxidant activity can be explained, since both oils are composed of monoterpene and sesquiterpene compound.

Table 3. Antioxidant activity of essential oils

Sample	Calibration equation	R^2	IC_{50} (µg/mL)
Essential oils	0,2752x - 4,0685	0,9984	194.90 ± 1.36
Ascorbic acid	14,05x + 9,016	0,9950	2.98 ± 0.06

value IC_{50}±SD, n: 3

4 CONCLUSIONS

The major of mix essential oil were monoterpene and sesquiterpene such as α-Pinene, D-Limonen, cis-1-methyl-4-(1-methylethenyi)-l-cyclohexane, 3-carene, L-menthone and β-pinene. Antimicrobial properties of the essential oil were give less active assessed against *Enterococcus faecalis, Streptococcus mutans, Streptococcus sanguinis* using the disk diffusion method. Free radical scavenging potentials showed values moderate activity for IC_{50} in 194.90 ± 1.36 µg/mL for mix essential oil, which are close to the natural antioxidant (ascorbic acid) with IC_{50} of 2.98 ± 0.06 µg/mL.

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