Antioxidation and anti-inflammatory activities of blended essential oil

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Abstract

The mixtures of essential oils are increasing popularly in holistic medicine. The different ratios of oil components in the preparation can result in different health benefits. Therefore, this study aimed to develop a mixture of essential oil formulation having promising bioactivities for pharmaceutical and cosmetic applications. The five popular essential oils including vetiver oil, lavender oil, eucalyptus oil, lemongrass oil, and phlai oil were selected for blended formulation. The selected oils were mixed in a suitable ratio and their antioxidant and anti-inflammatory activities were evaluated using 2,2-Diphenyl-1-picrylhydrazyl radical scavenging and nitric oxide (NO) radical scavenging, respectively. The prepared blended essential oil formulation had clear yellow-pale color and good smell. The blended oil showed good antioxidative activity by acting as a hydrogen donator. However, the blended oil presents mild anti-inflammatory activity via NO quenching action. The results of this study exposed that the developed blended essential oil formulation has promising properties to be used as a natural antioxidant as well as an anti-inflammatory agent in cosmetic and pharmaceutical applications.

Introduction

Inflammation is an adaptatively physiological condition triggered by tissue injury, stress, or infection to recover tissue homeostasis. In the inflammatory process, nitric oxide (NO) produced from activated macrophages plays the role of inflammatory mediator.[1,2] NO can further react with superoxide radicals to form reactive radical peroxynitrite and directly damage the cellular components which can lead to cellular dysfunction. Thus, the overproduction of NO causes cellular oxidative stress and conducts a higher severity level of inflammation as well as may further induce carcinogenesis.[3] Although inflammation is a recovery process of the body, self-damage caused by inflammation is unavoidable. Eventually, prolonged inflammation can lead to pathological conditions.[2] Moreover, at the site of inflammation, an increase in cell and tissue oxidative stress was found. Reactive oxygen species (ROS) are well-known as molecular intermediates for oxidative stress. Moreover, ROS can also cause the severity of inflammation.[4] Thus, the direct elimination of NO and ROS could be considered as potential targets for inflammation remedies.

Essential oil is one of the interesting natural products that have been used for health promotion for a long time.[5] Essential oils have earned great favor in the food, cosmetic, as well as pharmaceutical industries owing to their biological activities such as analgesic, anti-inflammatory, antioxidant, antibacterial, antiviral, and antifungal.[6,7] Recently, the use of essential oil in the form of a mixture is increasing particularly in holistic care because we can select the desired benefit of each essential oil into the mixture. The combination of essential oils may provide more efficacy than sole use. For example, the mixtures of essential oils produced various effects on antioxidant activity due to their free radical scavenging ability. This activity supports their use in food preservation and for the management of many ailments such as cancers, and neurodegenerative, cardiovascular, and immune system diseases.[8] Based on holistic medicine principles, essential oils that have healthy balance property is often selected for blended essential oil preparations.[9] Popular essential oils that have been used in holistic care such as vetiver oil (*Vetiveria zizanioides*), lavender oil (*Lavandula angustifolia*), eucalyptus oil (*Eucalyptus globulus*), lemongrass oil (*Cymbopogon citratus*), and phlai oil (*Zingiber montanum*). These essential oils and their active constituents have previously been reported as anti-inflammatory agents and antioxidants.[2] However, the preparation of blended essential oil formulations by using these in different ratios can result in different health benefits. Therefore, this study aimed to develop a blended essential oil preparation for inflammation management. In addition, the bioactivities of prepared essential oil formulation including antioxidation and anti-inflammatory activities were also evaluated. The obtained formulation can be applied in cosmetic or pharmaceutical applications.

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Materials and Methods

Chemicals

The essential oils including vetiver oil (*Vetiveria zizanioides*), lavender oil (*Lavandula angustifolia*), eucalyptus oil (*Eucalyptus globulus*), lemongrass oil (*Cymbopogon citratus*), and phlai oil (*Zingiber montanum*) were purchased from Royal Lotus**.** DPPH and gallic acid were obtained from Sigma Aldrich. Ascorbic acid was from Chem-supply. Sodium nitroprusside and naphthyl ethylene diamine were bought from Himedia and Loba Chemie, respectively.

Preparation of blended essential oil

The blended essential oil formulations were prepared by using popular essential oils that have been used in holistic medicine. The selected essential oils were vetiver oil (*Vetiveria zizanioides*), lavender oil (*Lavandula angustifolia*), eucalyptus oil (*Eucalyptus globulus*), lemongrass oil (*Cymbopogon citratus*), and phlai oil (*Zingiber montanum*). The mixtures of selected oils in various ratios (by weight) were developed. Briefly, the largest amount of essential oil was pipetted into the vial first, followed by adding the minor components into the same vial. The blended oil was then homogenized and stored at 4°C prior to use. The physical characteristics of suitable obtained oil formulation such as color and odor were observed.

Evaluation of bioactivities

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical quenching ability of blended essential oil was tested following a previous report.[10] The fresh DPPH solution was prepared by dissolving DPPH in methanol to obtain an absorbance unit of 1.1±0.02 at 515 nm. The sample was diluted with methanol into various concentrations (20-200 µg/mL). The sample solutions (80 µL) and DPPH solution (80 µL) were added to 96-well plates and incubated in the dark condition at room temperature for 30 minutes. Then the absorbance of reactions was measured at 515 nm with a microplate reader. Ascorbic acid was used as a positive control. The experiment was done in triplicate. The % inhibition was achieved by using the following equation: %inhibition = $[(OD_{control}-OD_{sample})/OD_{control}]\times100$.

In vitro nitric oxide radical scavenging assay

The NO radical scavenging test was employed to assess the anti-inflammatory activity of blended essential oil based on a previously reported method[10]. The 20 mM SNP solution was mixed with the blended essential oil solution (5-50 mg/mL) and incubated at ambient temperature for 180 min. Then the mixture was reacted with Griess reagent. The absorbance was read at 546 nm using a microplate reader. Gallic acid (1-20 mg/mL) was used as a positive control. The test was performed in triplicates. The % inhibition was achieved by using the following equation: %inhibition = $[(OD_{control}-OD_{sample})/OD_{control}]\times100$.

Statistical analysis

Results were presented as mean±standard deviation. The data were analyzed using the Microsoft Excel program.

Results and Discussion

Blended essential oil formulation

The blended essential oil formulation was prepared by mixing five essential oils. The mixing ratios are presented in Table 1. The physical characteristic of the obtained oil was the clear yellow-pale color. This ratio was the suitable smell from the proportions of the top note, middle note, and base note.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

The antioxidant potency of blended essential oil was evaluated using DPPH proton radical scavenging. The result found that tested oil and ascorbic acid exhibited DPPH radical scavenging activity in a dose-dependent manner. The highest tested concentration of oil (300 mg/mL) and ascorbic acid (10 mg/mL) presented similar scavenging activity up to 82.34±0.58 and 82.47±0.72 % DPPH radical scavenging (Figure 1). This suggested that blended essential oil carried a lower antioxidant potency of 30 times than ascorbic acid when compared with the highest tested concentrations. The DPPH radical scavenging test was based on the reduction of DPPH, a hydrogen acceptor, in the presence of a hydrogen donator.[11] Thus, the antioxidant activity of blended essential oil is due to its hydrogen-donating properties.

Nitric oxide radical scavenging activity

NO is an important inflammatory mediator. NO is over-released from activated

Table 1. Mixing ratios of blended essential oil.

Essential oil	Ratio (by weight)
Vetiver	2
Lavender	2
Eucalyptus	1.5
Lemongrass	1.5

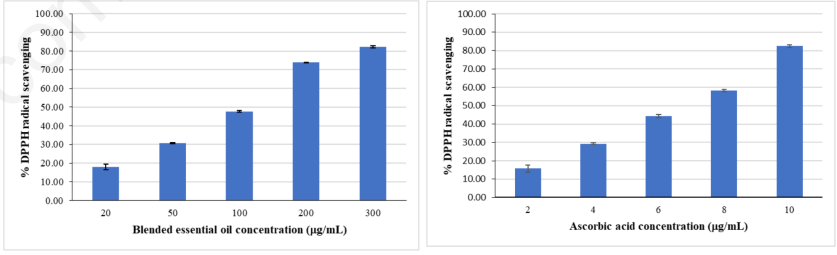

Figure 1. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of blended essential oil and ascorbic acid. DPPH, 2,2-Diphenyl-1-picrylhydrazyl.

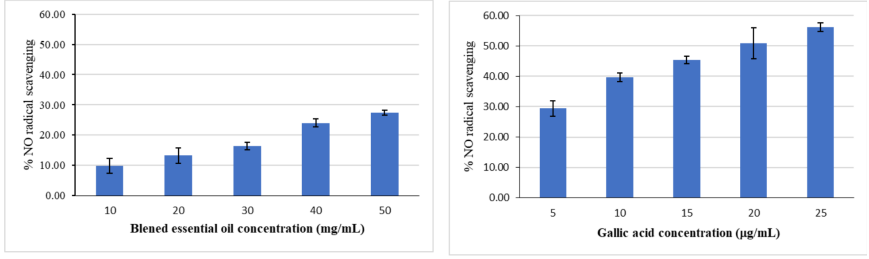

Figure 2. Nitric oxide radical scavenging activity of blended essential oil and gallic acid.

macrophages during inflammation.[12] Thus, NO scavenging is one of the mechanisms of anti-inflammatory agents. The anti-inflammation potency of blended essential oil was evaluated using inflammatory mediator NO radical scavenging. The result found that tested oil and gallic acid presented dose-dependent NO radical scavenging capacity (Figure 2). However, NO radical quenching at the highest tested concentration of essential oil (27.41±0.87%) was approximately 2.1 folds lower than the scavenging efficacy of gallic acid (56.17±1.47%) at the highest tested dosage. This finding revealed that blended essential oil possessed mind anti-inflammatory activity. The increase in the used dosage may improve the anti-inflammatory potency of this essential oil.

Conclusions

The present study has developed a blended essential oil formulation from the popular essential oils used in holistic medicine to use for pharmaceutical and cosmetic applications. The biological activities of prepared oil were evaluated and showed that the oil has a promising potential as an antioxidant by acting as a proton radical scavenger. In addition, the anti-inflammatory property of the oil was expanded via inflammatory mediator NO scavenging.

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