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Dual Bioactivities of *Laurus nobilis* Essential Oil

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ABSTRACT

The evolution of cosmetic products results in the growing demands for cosmetics that are preservatives free. Plant essential oils were found to be a promising antimicrobial and also antioxidant agent. In this study, *Cymbopogon citratus* (lemongrass), *Laurus nobilis* (bay leaf) and *Backhousia citriodora* (lemon myrtle) essential oils were selected and evaluated for their antimicrobial properties. It was found that *Laurus nobilis* exhibited strong antimicrobial activity against the selected bacteria *Streptococcus saprophyticus* (ATCC 49619), *Streptococcus aureus* (ATCC 22923), *Streptococcus pyogenes* (ATCC 29436), *Pseudomonas aeruginosa* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 22922) with MIC ranging between 7.8 ug/mL to 250 µg/mL. The antioxidant activity of selected essential oils was determined by antioxidant assays which were 1,1-Diphenyl-2-picrylhydrazyl assay (DPPH), determination of ferric reducing antioxidant power assay (FRAP) and β-Carotene/linoleic acid bleaching assay. *Backhousia citriodora* and *Laurus nobilis* showed the highest antioxidant activity. n-Octanal and β-Selinene were identified to be the major components with peak area of 26.37 % and 13.92 % respectively in secondary metabolites analysis by Gas Chromatography-Mass Spectrometry (GC-MS).

Keywords: *Essential oil; Antimicrobial; Antioxidant; DPPH; FRAP; β-Carotene*

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INTRODUCTION

Essential oils (EOs) are typically liquid and the present compounds are volatile constituents that are soluble in lipids and have a low density [1]. Essential oils also portray a very good antimicrobial, antifungal, antiviral and antioxidant properties can be used as food preservation, pharmaceuticals, alternative medicines and natural therapies. Various studies also found that essential oils have antioxidant activity against free radicals which can cause several disorders of immune system and gene expression [2, 3].

Preservatives such as methyl parabens are added during the manufacturing of cosmetics to prevent the growth of harmful bacteria and mold. However, some studies suggested that this component of cosmetic formula could give some risks to human health such as allergies, cell damage or even worse skin cancer [4]. Therefore, there is a growing demand for cosmetics that are preservatives free. Plants like *Cymbopogon citrates* (lemongrass), *Laurus nobilis* (bay leaf) and *Backhousia citriodora* (lemon myrtle) are suggested to possess both high antioxidant and microbial activities. The essential oils of these plants can be used as natural preservatives in cosmetics and might as well serve as natural fragrance for the cosmetics. The identification of the essential oil components was carried out by gas chromatography-mass spectrometry (GC-MS) in which showed efficient separation of different components [5,6].

EXPERIMENTAL

Essential Oils

Essential oils of *Cymbopogon citratus* (lemongrass), *Laurus nobilis* (bay leaf) and *Backhousia citriodora* (lemon myrtle) were obtained from Forest Research Institute of Malaysia (FRIM).

Bacterial Strains

The antibacterial activity was evaluated against the following bacteria: *Streptococcus saprophyticus* (ATCC 49619), *Streptococcus aureus* (ATCC 22923), *Streptococcus pyogenes* (ATCC 29436), *Pseudomonas aeruginosa* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 22922). The selected bacteria were subcultured overnight on Tryptone Soy broth at 37 ºC prior to growing on Mueller-Hinton agar. All overnight cultures were standardized to 0.5 McFarland turbidity standard using sterile saline water.

Agar Well Diffusion Method

Overnight bacterial cultures were prepared in Tryptone Soy broth and diluted to 10^7 CFU ml^{-1} which corresponds to 0.5 Mc Farland standard. The wells made on the Muller-Hinton (MHA) agar were impregnated with 10 µL of 500 µg/mL of the selected essential oils. Ampicillin (10 µg/mL) was used as the positive control while the sterile distilled water was used as the negative control. The zones of inhibition expressed by a clear zone surrounding the wells were measured to the nearest millimeter (mm) and the mean diameter was recorded. The plates were prepared in triplicates.

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Minimum Inhibitory Concentration (MIC) Values and Minimum Bactericidal Concentration (MBC)

This assay was performed using sterile 96-well microplates via broth dilution method [3].

Total Phenolic Content

The total phenolic content was determined colorimetrically using the Folin-Ciocalteau method with some modifications [3].

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

A volume of 200 µL of selected essential oil was added in 50 µL of 1 mM DPPH solution. Then, the mixture was incubated in dark room temperature for 30 minutes. The decolourisation of DPPH was observed using spectrometer at 517 nm. The blank was prepared using a DPPH solution without essential oil. The percentage of scavenging effect was calculated with the equation:

$$I\ \% = [1 - \frac{(A^1 - A^2)}{A^0}] \times 100\% \quad \text{(Equation 1)}$$

Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

Determination of ferric reducing/antioxidant power FRAP is a simple direct test for measuring antioxidant capacity [3].

β-Carotene/Linoleic Acid Bleaching Assay

The β-carotene bleaching assay was performed with ascorbic acid as standard [7].

Chemical Analysis

The analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (FID) using fused silica capillary column BP-5 (25 m X 0.25 mm; 0.25 µm film thickness) [5].

RESULTS AND DISCUSSION

Antibacterial Activity, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The *in vitro* antibacterial activities of *Cymbopogon citratus, Laurus nobilis* and *Backhousia citriodora* essential oils against selected bacteria were quantitatively assessed for the presence or absence of inhibition zones (Table 1). The inhibition zone which was higher than 15 mm is considered as high

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activity, 10-15 mm as moderate activity, 8-9 mm as low activity and below than 8 mm is classified as no activity [8]. The agar-well diffusion method indicated that essential oil extracted from *Laurus nobilis* showed high potential as antibacterial towards the selected bacteria.

Table 1: Zones of growth inhibition (mm) showing antibacterial activity of selected essential oils (400 µg/mL) against selected bacterial strains; well diameter (3 mm).

Essential Oils	Average Zone of Inhibition (mm)					
	***Streptococcus saprophyticus* (ATCC 49619)**	***Streptococcus aureus* (ATCC 22923)**	***Streptococcus pyogenes* (ATCC 29436)**	***Pseudomonas aeruginosa* (ATCC 13048)**	***Klebsiella pneumoniae* (ATCC 700603)**	***Escherichia coli* (ATCC 22922)**
CC	18.70 ± 1.15	9.00 ± 0.00	16.67 ± 1.50	11.70 ± 3.90	6.30 ± 1.50	6.70 ± 2.10
LN	11.30 ± 1.50	12.70 ± 2.50	22.00 ± 2.60	11.20 ± 3.80	8.00 ± 1.00	7.30 ± 4.20
BC	7.70 ± 1.50	8.70 ± 1.50	13.00 ± 2.90	7.30 ± 1.50	6.70 ± 0.60	7.70 ± 0.60
AMP	20.30 ± 0.60	26.30 ± 0.60	15.50 ± 0.50	10.30 ± 0.60	14.30 ± 1.50	15.70 ± 2.10

CC: *Cymbopogon citratus*; LN: *Laurus nobilis*; BC: *Backhousia citriodora*; Positive control: AMP: Amplicilin, 10 ug/mL; bacterial strain source: ATCC, American Type Culture Collection; negative control: Sterile distilled water. Values for the zone of growth inhibition are presented as mean ± SD.

The MIC values of *Laurus nobilis* range from 7.80 µg/mL to 62.50 µg/mL with no MBC values (Table 2). The antimicrobial activity of EOs might be due to the large number of different groups of chemical compounds present in the essential oils, it is most likely that their antibacterial activities are targeting several rather than only one target location in the cells [9]. The essential oils can coagulate the cytoplasm and cause damage to the lipids and proteins. Their mechanisms of action would be similar to those of other phenolics which caused the disturbance towards the proton motive force (PMF), electron flow, active transport, and coagulate the cell's content [10].

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Laurus nobilis.*

Culture	MIC / MBC (µg/mL) *Laurus nobilis*
Streptococcus saprophyticus (ATCC 49619)	7.80 / -
Streptococcus aureus (ATCC 22923)	7.80 / -
Streptococcus pyogenes (ATCC 29436)	7.80 / -
Pseudomonas aeruginosa (ATCC 13048)	62.50 / -
Klebsiella pneumoniae (ATCC 700603)	15.63/-
Escherichia coli (ATCC 22922)	15.63/-

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Total Phenolic Content (TPC), Ferric Reducing Antioxidant Power Activity (FRAP) and Radical Scavenging Activity (DPPH)

Polyphenols are found in many natural products as the major occurring antioxidant components with radical scavenging abilities. The presence of hydroxyl groups directly contributes to the antioxidative action of polyphenols. The total phenolic content (TPC) of the 3 selected essential oils were calculated based on the standard curve prepared using gallic acid. *Laurus nobilis* showed 0.73 mg GAE/g of TPC (Table 3). While in the iron-reducing capacity (FRAP assay), the oil showed a moderate activity. In DPPH assay, *Laurus nobilis* essential oil was capable of scavenging DPPH free radicals but served as a moderate antioxidant activity with IC_{50} of 39.52 ± 1.65 µg/mL. For β-carotene assay, *Laurus nobilis* showed the highest percentage of inhibition with 99.81 ± 0.05 %. Thus, the oil demonstrated antioxidant capacities compared to ascorbic acid.

Table 3: Total phenolic content (TPC), ferric reducing antioxidant activity (FRAP), scavenging activity EC_{50} (DPPH) and percentage of inhibition (I %) of β-carotene bleaching assay of selected essential oils.

Essential oils	TPC (mg GAE/mg)	FRAP activity (µM TE/g)	DPPH EC_{50} (µg/mL)	β-carotene bleaching (I %)
Cymbopogon citratus	0.67 ± 0.07	0.52 ± 0.07	21.90 ± 7.18	99.79 ± 0.08
Laurus nobilis	0.73 ± 0.01	0.25 ± 0.15	39.52 ± 1.65	99.81 ± 0.00
Backhousia citriodora	0.88 ± 0.08	0.72 ± 0.25	66.20 ± 8.61	99.70 ± 0.05
Ascorbic acid	-	-	35.71 ± 5.97	96.00 ± 0.00

Essential oils analysis by Gas Chromatography - Mass Spectrometry (GC-MS) analysis

Secondary metabolites analysis using Gas Chromatography Mass Spectrometry (GCMS), of *Laurus nobilis* essential oils shows six major volatile compounds were detected representing 88.99 % of the total oil. The essential oils contain a high content of n-Octanal and β-Selinene with peak area of 26.37 % and 13.92 % respectively and these compounds play a significant role in inhibiting microbial growth and also give antioxidant properties [11]. The other main constituents are α-Selinene, α-Copaene, E-Nerolidol, n-Decanal, 4-Dodecen-1-al, Humulene Epoxide II and γ-Cadinene with area percentages ranging from 10.45 to 1.30 %. Similar results were also observed from the previous studies with slightly different percentages [11].

CONCLUSIONS

Essential oil of *Laurus nobilis* (bay leaf) was shown to produce bacteriostatic properties towards selected bacteria species which are *Streptococcus saprophyticus* (ATCC 49619), *Streptococcus aureus* (ATCC 22923), *Streptococcus pyogenes* (ATCC 29436), *Pseudomonas aeruginosa* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 22922) with the minimum inhibition concentration (MIC) values of these essential oils range from 7.80 µg/mL to 62.50 µg/mL. The oil also

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demonstrated antioxidant capacities compared to ascorbic acid. Secondary metabolites analysis of *Laurus nobilis* essential oil shows that a high content of n-Octanal and β-Selinene Therefore, further researches should be carried out to deepen our knowledge on these plants because this study has shown that *Laurus nobilis* proved to have dual bioactivities; antimicrobial and antioxidant activity.

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