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# Antibacterial and Ferric Reducing Ability Activities of Selected Herbs Essential Oils

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**Abstract:** In this study, four herbs namely *Baeckea frutescens*, *Cinnamomum zeylanicum*, *Syzygium aromaticum* and *Vitex negundo* were hydrodistilled and the potential antibacterial properties against oral pathogens and ferric reducing antioxidant activities were evaluated. Essential oils extracted from *Cinnamomum zeylanicum* and *Syzygium aromaticum* showed varied effectiveness of antimicrobial properties against oral pathogens. Total phenolic content in the selected essential oil extracts were quantified. Ferric reducing antioxidant activities of the essential oils were analyzed by Ferric Reducing Antioxidant Power (FRAP) assay to determine the free radical scavenging capacity. The total phenolic content of all four essential oils ranged from 0.259-1.575 mg GAE/g and the FRAP values lie between 54.71 and 368.78 μM TE/g. GC-MS analysis showed that eugenol and phenol were the highest compounds present in *Cinnamomum zeylanicum* and *Syzygium aromaticum*. A positive correlation was observed between the antibacterial properties against oral pathogen and the ferric reducing antioxidant activities of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils.

**Key words:** Oral pathogen, herbs essential oil, ferric reducing ability, antimicrobial, antioxidant

## INTRODUCTION

Essential Oils (EOs) are a mixture of aromatic compounds present in medicinal plants which are responsible for many biological activities due to high content in phenolic compounds, terpenes (monoterpenes and sesquiterpenes) antioxidants and antibacterial properties (Botelho *et al*., 2007). At present, there is an increasing interest in the use of naturally occurring substances. Medicinal plants have also been the subject of interest particularly by the pharmaceutical industries in treating dental plaque and to delay the aging process. Dental plaque is a bacteria film that is almost invisible and accumulates on the gums and teeth.

Tooth decay, dental cavities or periodontal disease activities occurred when dental plaque accumulates between teeth and along the gum line (Mazumdar *et al*., 2009). Antioxidants are the bioactive compounds that reduce the activities of Reactive Oxygen Species (ROS) and free radicals hence slowing down the aging process (Wootton-Beard and Ryan, 2011).

To date, no research has been done using the selected essential oils extracted from *Baeckea frutescens* (*B. frutescens*), *Cinnamomum zeylanicum* (*C. zeylanicum*), *Syzygium aromaticum* (*S. aromaticum*) and *Vitex negundo* (*V. negundo*) essential oils on the following oral pathogens *Streptococcus mutans (S. mutans)*, *Streptococcus anginosus (S. anginosus)*, *Streptococcus salivarius* (*S. salivarius*), *Streptococcus mitis* (*S. mitis*), *Streptococcus oralis* (*S. oralis*) and *Streptococcus pneumoniae* (*S. pneumoniae*) strains.

The aims of the present study were: to determine the antibacterial activities of the selected essential oils against oral pathogens: *S. mutans*, *S. anginosus*, *S. salivarius*, *S. mitis*, *S. oralis* and *S. pneumoniae* to determine the total phenolic content and ferric reducing activities of the selected essential oils and to determine the chemical compounds in the selected essential oils by Gas Chromatography-Mass Spectrometry (GC-MS).

## MATERIALS AND METHODS

**Herb materials:** The herb materials were obtained from Forest Research Institute of Malaysia (FRIM). The selected herbs were *B. frutescens*, *C. zeylanicum*, *S. aromaticum* and *V. negundo*.

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**Extraction of the essential oils:** Each of the selected herbs was crushed into small pieces to increase the surface area in order to enhance the extraction of essential oils. The crushed sample was placed in a container and further filled with distilled water up to the sample's surface. The extraction process was performed in a combination of hydrodistillation process with Clevenger apparatus for 8 h.

**Bacterial strains:** The antibacterial activity was evaluated against the following oral pathogens: *S. mutans* ATCC 25175, *S. mitis* ATCC 6249, *S. salivarius* ATCC 13419, *S. anginosus* ATCC 33397, *S. oralis* ATCC 35037 and *S. pneumonia* ATCC 6305. The oral pathogens were obtained from the culture collection of Microbiology Laboratory, Faculty of Applied Sciences, UiTM, Shah Alam. The oral pathogens were subcultured overnight on columbia blood agar at 37°C prior to growing in Mueller-Hinton broth. All overnight cultures were standardized to 0.5 McFarland turbidity standard using sterile saline water.

**Agar well diffusion method:** The agar well diffusion method was carried out on Columbia blood agar plates. Briefly, a bacterial cell suspension (concentration of 10 (Dhanya and Sidhu, 2011) CFU/mL was prepared using an overnight culture growth incubated at 37°C) was spread over the Columbia blood agar plates then 6 μL of 400 μg/mL essential oils was pipetted into the respective wells. The diameter of inhibition zone was measured around each well after 24 h of incubation at 37°C. Ampicillin (40 μg/mL) a known antibiotic drug was used as the positive control. This assay was done in triplicates and the inhibition zones were compared with the positive control.

**Determination of Minimum Inhibitory Concentration (MIC) values:** Minimum Inhibitory Concentration (MIC) was performed on the sterile 96-well microplates using broth dilution method. The 25 μL of essential oil (25-800 μg/mL) was transferred into the 96-well microplate to obtain two-fold serial dilution in DMSO. The inocula (175 μL) containing 10 (Dhanya and Sidhu, 2011) cfu/mL of each bacterial strain were added to each well. Several empty wells were reserved in each plate to test the sterility control of the medium (no inoculum added) and inoculum viability (no compound added). After an incubation period at 37°C for 24 h, the microplates were read using a microplate reader and the appearance of turbidity was taken as an indication of growth. The MIC was recorded as the mean concentration of triplicates.

**Determination of Minimum Bactericidal Concentration (MBC):** The 10 μL from each well with no visible growth were taken and inoculated in muller-hinton agar plates. After 24 h of incubation at 37°C, the bacteria growth was observed. The MBC value was determined as the highest dilution (lowest concentration) at which no growth occurred on plate.

**Estimation of total phenolic content:** Folin-ciocalteu method was used for the determination of Total Phenolic Content (TPC) (Kumar *et al*., 2010). The 100 μL of sample or standard was accurately mixed total with 0.75 mL of folin-ciocalteu reagent. Then, 0.75 mL of 7.5% (w/v) sodium bicarbonate was added to the mixture and mixed thoroughly. The mixture was allowed to stand at room temperature for 90 min. Absorbance was measured against blank reagent at 725 nm using spectrophotometer. Gallic acid was used as the calibration curve with a concentration range of 0.08 and 0.18 mg/mL. The TPC of the sample was expressed as Gallic Acid Equivalent (GAE)/g of essential oil. All experiments were performed in triplicates.

**Analysis of Ferric Reducing Antioxidant Power (FRAP) activity:** FRAP assay was carried out according to the procedures (Gulcin, 2011). The FRAP working solution was prepared fresh. The 25 mL acetate buffer (3.1 g sodium acetate and 16 mL concentrated acetic acid per 1 L of buffer solution) was mixed accurately with 2.5 mL 2, 4, 6-tripyridyl-s-triazine solution (10 mM/L 2, 4, 6-tripyridyl-s-triazine in 40 mM/L hydrochloric acid) and 2.5 mL iron chloride solution (20 mM/L iron chloride hexahydrate in distilled water) as the FRAP working solution. Briefly, 150 μL sample or standard was mixed with 2850 μL FRAP reagent. The mixture was then incubated in a dark at room temperature for 30 min. Absorbance readings were made at 593 nm using a spectrophotometer. The total antioxidant capacity of samples were determined against a standard with known FRAP values and were expressed as μM of Trolox equivalent (μMTE)/g of essential oil. All experiments were carried out in triplicates.

**Gas Chromatography-Mass Spectrometry (GC-MS) analysis conditions:** GC-MS analysis was performed (Kumar *et al*., 2010) agilent 5975C GC/MS equipped with Elite-1 fused silica capillary column (30×0.25 mm 1D×1EM df, composed of 100% dimethylpolysiloxane). Electron ionization system with ionizing energy of 70 eV was used. Carrier gas: helium (99.999%) with a constant flow of 1 L/min. Injection volume of 0.5 El was employed (split ratio of 10:1) injector temperature: 250°C; ion source temperature: 280°C; oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10C/-200°C min then 5°C/-280°C min.

## RESULTS AND DISCUSSION

**Antibacterial activity, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):** The *in vitro* antibacterial activities of *B. frutescens*, *C. zeylanicum*, *S. aromaticum* and *V. negundo* essential oils against the oral pathogens were quantitatively assessed for the presence or absence of inhibition zones (Table 1). The inhibition zone which was >15 mm is considered as high activity, 10-15 mm as moderate activity, 8-9 mm as low activity and than 8 mm is classified as no activity (Prabuseenivasan *et al*., 2006). The agar-well diffusion method indicated that the essential oil extracted from *C. zeylanicum* showed moderate antibacterial activities against *S. mutans*, *S. anginus*, *S. mitis* and *S. pneumonia* with low antibacterial activities against *S. oralis* and *S. salivarius*. The essential oil extracted from *S. aromaticum* also showed moderate potential as antibacterial towards all of the selected oral pathogens except *S. salivarius*. The *B. frutescens* and *V. negundo* essential oils showed low to no antibacterial activities. The MIC and MBC of both essential oils were tabulated in Table 2. Several studies also showed promising inhibitory activities even at the low concentrations (Prabuseenivasan *et al*., 2006). *S. aromaticum* (Dhanya and Sidhu, 2011) and *C. zeylanicum* (Gupta *et al*., 2013) were also reported to have strong biological properties. 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 on several rather than only one target locations in the cells (Burt, 2004). The essential oils can coagulate the cytoplasm and caused 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 (Burt, 2004; Martos *et al*., 2008).

**Total Phenolic Content (TPC) and ferric reducing antioxidant power activity:** Total Polyphenols (TP) are found in many natural products as the major occurring antioxidant components with radical scavenging abilities. The presence of hydroxyl group directly contributes to the antioxidative action of TP. The Total Phenolic Content (TPC) of the 4 selected essential oils were calculated based on the standard curve prepared using gallic acid. The TPC was presented as Gallic Acid Equivalents (GAE)/g of dry extract. The 4 selected essential oils showed TPC ranging from 0.259-1.575 mg GAE/g (Table 2). Among the 4 selected essential oils, *S. aromaticum* showed the highest TPC with 1.58±0.06 mg GAE/g followed by *C. zeylanicum* (1.52±0.02 mg GAE/g). The lowest TPC was found in *B. frutescens* with 0.26±0.03 mg GAE/g. The iron-reducing capacity of the selected essential oils were determined using FRAP method which depends upon the reduction of ferric-2, 4, 6-tri (2-pyridyl)-s-triazine [Fe(III)-TPTZ] complex to ferrous-2,4,6-tri(2-pyridyl)-s-triazine[Fe(II)-TPTZ]complex that has intensive blue color and can be monitored at

Table 1: Average inhibition zone diameter (mm) of selected essential oils BF: *Baeckea frutescens*; CZ: *Cinnamomum zeylanicum*; SA: *Syzygium aromaticum*; VN: *Vitex negundo* and AMP: Ampicillin

| | Average diameter inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| Cultures | BF | CZ | SA | VN | AMP |
| *Streptococcus mutans* ATCC 25175 | - | 10.67±0.58 | 10.33±0.58 | - | 10.33±0.57 |
| *Steptococcus anginosus* ATCC 33397 | - | 10.67±0.58 | 11±1.00 | - | 31±0 |
| *Streptococcus oralis* ATCC 35037 | - | 9.33±0.57 | 10.33±0.57 | - | 40 ± 0 |
| *Streptococcus mitis* ATCC 6249 | 4.67 ±0.57 | 11.67±0.57 | 10.6±0.57 | 4.33±0.57 | 40±0 |
| *Streptococcus pneumoniae* ATCC 6305 | 5±0 | 11.33±0.58 | 10±1.00 | - | 12.6±0.58 |
| *Streptococcus salivarius* ATCC 13419 | - | 7.66±0.57 | 8±0 | - | 11±1.00 |

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Cinnamomum zeylanicum* and *Syzygium aromaticum*

| | MIC/MBC (µg/mL) | |
|---|---|---|
| Cultures | *Cinnamomum zeylanicum* | *Syzygium aromaticum* |
| *Streptococcus mutans* (ATCC 25175) | 100/400 | 100/400 |
| *Steptococcus anginosus* (ATCC 33397) | 100/400 | 100/200 |
| *Streptococcus mitis* (ATCC 35037 ) | 200/400 | 200/400 |
| *Streptococcus oralis* (ATCC 6249) | 200/200 | 200/400 |
| *Streptococcus pneumoniae* (ATCC 6305) | 100/200 | 100/400 |
| *Streptococcus salivarius* (ATCC 13419) | 100/400 | 200/400 |

Table 3: Total Phenolic Content (TPC) and ferric reducing antioxidant activity of essential oils

| Essential oils | Average total phenolic content (GAE/g) | Average ferric reducing antioxidant activity (μM TE/g) |
|---|---|---|
| *Baeckea frutescens* | 0.26±0.03 | 79.13±9.550 |
| *Cinnamomum zeylanicum* | 1.52±0.02 | 364.18±16.39 |
| *Syzygium aromaticum* | 1.58±0.06 | 368.78±17.10 |
| *Vitex negundo* | 0.41±0.03 | 54.71±3.210 |

Table 4: Compounds present in *Cinnamomum zeylanicum* essential oil by GC-MS analysis

| Compounds | Peak area (%) | RT | Percent quality (%) |
|---|---|---|---|
| Eugenol | 43.28 | 8.17 | 97 |
| Cinnamyl alcohol | 9.51 | 9.31 | 93 |
| Phenol | 9.30 | 10.01 | 92 |
| 1,6-octadien-3-ol | 5.07 | 4.25 | 97 |
| Caryophyllene oxide | 3.72 | 10.67 | 91 |
| 4-((1E)-3-hydroxy-1-propenyl) -2-methoyphenol | 0.40 | 11.97 | 95 |
| Benzyl benzoate | 0.34 | 12.31 | 98 |
| Napthalene | 0.20 | 9.63 | 94 |
| 1-docosene | 0.05 | 17.66 | 96 |
| 1-octadecene | 0.04 | 12.36 | 99 |
| Cyclotetracosane | 0.02 | 20.56 | 98 |
| 9-tricosene | 0.02 | 23.43 | 99 |

593 nm. The range of ferric reducing antioxidant power activities of the essential oils lies between 54.71 and 368.78 μM TE/g (Table 3). Previous research works (Cortes-Rojas *et al*., 2014) reported that *S. aromaticum* have the potentials as antioxidant neutraceutical and food preservative that improve shelf life (Dua *et al*., 2015). Among all 4 essential oils extracts, *S. aromaticum* contain high phenolic content and also exhibited high FRAP value with 368.78±17.10 μM TE/g followed by *C. zeylanicum* with 364.18±16.39 μM TE/g. Similar trends have been reported (Wojdylo *et al*., 2007) as high total phenolic content and FRAP value were observed in *Vetiveria zizanioides*. This is due to presence of the polyphenols which reduce the ferric ions in the ferric-TPTZ complex as compared to the ability in scavenging free radicals using DPPH assay. The lowest FRAP value was found in *V. negundo*.

**Chemical composition:** The chemical composition of the EOs extracted from *C. zeylanicum* and *S. aromaticum* were studied. The main constituents of each essential oil, expressed as relative percentage of the total chromatogram area were summarized in Table 4-5. In the EO extract of *C. zeylanicum*, 12 compounds were identified and the major constituents were eugenol (43.28%) cinnamyl alcohol (9.51%) and phenol (9.30%). The 10 compounds were identified in the EO of *S. aromaticum* and the major constituents were eugenol (24.25%) and phenol (18.21%). The chemical analysis of these EOs shown that the principal active compound was eugenol which was proven to have several biological activities (Burt, 2004).

Table 5: Compounds present in *Syzygium aromaticum* essential oil by GC-MS analysis

| Compounds | Peak area (%) | RT | Percent quality (%) |
|---|---|---|---|
| Eugenol | 24.25 | 8.26 | 97 |
| Phenol | 18.21 | 7.98 | 97 |
| Caryophyllene | 9.42 | 8.87 | 99 |
| Caryophyllene oxide | 2.74 | 10.51 | 91 |
| 10,10-dimethyl-2,6 dimethylbicyclo | 0.75 | 10.98 | 93 |
| Á-farnesene | 0.24 | 9.38 | 93 |
| Naphthalene | 0.16 | 9.66 | 98 |
| Methyl salicyclate | 0.11 | 5.29 | 96 |
| Dodecane | 0.05 | 5.21 | 95 |
| Tridecane | 0.05 | 6.38 | 95 |

## CONCLUSION

This study revealed that the bioactive compounds of *Cinnamomum zeylanicum* and *Syzygium aromaticum* were associated with their antibacterial potencies against oral pathogens and ferric reducing power properties. *Baeckea frutescens* and *Vitex negundo* contain no bioactive compounds from the selected assays studied. A positive correlation between phenolic contents and both biological properties suggested that phenol was the major group that contributed to the antibacterial and ferric reducing power properties of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils.

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