Investigation of Total Phenolic and Flavonoid Contents, and Evaluation of Antimicrobial and Antioxidant Activities from Baeckea frutescens Extracts

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Abstract. Baeckea frutescens L. is a medicinal plant endemic to the tropical area and it has been used by locals for topical and oral ailments. This study investigated total phenolic and flavonoid contents and also evaluated in vitro antimicrobial and antioxidant activities of Baeckea frutescens crude extracts. These extracts were assessed for their antibacterial activities against strains of Escherichia coli, Staphylococcus aureus, Salmonella thyphi, and Pseudomonas aeruginosa by the broth micro-dilution methods using a modified tetrazolium-based colorimetric assay (3-(4, 5-dimethylthiazol)-2, 5-diphenyl tetrazolium bromide (MTT) assay). Baeckea frutescens crude extracts were also tested against the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical. The results indicated that Baeckea frutescens water and ethanol extracts possesed remarkable antibacterial activity with the minimum inhibitory concentration less than 100 μg/ml against Escherichia coli and Salmonella thyphi. On the evaluation of the antioxidant activity via DPPH assay, Baeckea frutescens ethanol extracts exhibited a good antioxidant activity with IC₅₀ less than 50 μg/ml and Baeckea frutescens water extracts showed a moderate antioxidant activity with IC₅₀ less than 100 μg/ml.

1. Introduction
Plants have the ability to produce secondary metabolites like proteins, flavonoids, alkaloids, and phenolic substances which responsible to their pharmacological properties [1,2]. Phenolic and flavonoid existed in fruits, seeds and leaves are the most important phytochemical constituents. Phenolic components in natural compounds put a notable source of antioxidant and antifungal metabolites. The source of potent antioxidants is also supplied by flavonoids content from vegetables and fruits which reported could reduce incidence of chronic inflammatory diseases or cancer. The important tool to understand the therapeutic effect of medicinal plants is through investigation of the polyphenols and flavonoids as antioxidant compounds in herbs [3,4].

Baeckea frutescens L., a coastal shrub belongs to the family Myrtaceae. This plant is mostly present throughout Southern China, Australia, and South East Asia including Indonesia, Malaysia, and Vietnam. The leaves have been used to treat fever, headache, snake bites, and rheumatism [5-7]. Previous investigation on its main constituents suggested that the terpenoids, flavonoids, and phloroglucinols possessed the biological effects such as anti-inflammatory, anti-bacterial, and
cytotoxic activities. Prior phytochemical studies of the leaves of this species led to the isolation of flavanone and phloroglucinol derivatives [8,9]. Therefore, the aim of this study was to determine total phenolic and total flavonoid contents in water, ethanol and chloroform extracts of *Baeckea frutescens* and also establish their potential effects on health promotion as antioxidant and antimicrobial agents.

2. Materials and Methods

2.1. Collection of plant materials

The leaves of *Baeckea frutescens* were purchased from Pasar Beringharjo, Yogyakarta, Indonesia.

2.2. Preparation of *Baeckea frutescens* crude extracts

*Baeckea frutescens* leaves were air-dried and powdered. Approximately 20 g of materials were exhaustively macerated with water, ethanol and, chloroform solvents (3 times, with ratio 1:5 w/v) in an ultrasonic bath, for 60 min each at room temperature.

2.3. Determination of the total phenolic contents

The amount of total phenolic content in *Baeckea frutescens* crude extracts was determined according to Folin-Ciocalteu reaction method [10] with slighty modification. Briefly, 0.5 mL of extracts from the stock solution was mixed with 7.5 ml distilled water and 0.5 mL of Folin-Ciocalteu reagent. After 8 min of incubation, 1.5 mL of 20% Na$_2$CO$_3$ solution was added and further incubated for 2 hours. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV Vis Spectrophotometer Hitachi HALO RB-10) against blank sample. The results were expressed as gallic acid equivalents (% GAE) of dry weight and the experiment was conducted in triplicate analysis.

2.4. Determination of the total flavonoid contents

Total flavonoid contents in the *Baeckea frutescens* extracts were measured according to a colorimetric assay (UV Vis Spectrophotometer Hitachi HALO RB-10) [11] by taking 10 μL of each extract and mixed with 60 μL methanol, 10 μL of 10% AlCl$_3$ solution, 10 μL of 1 M KCH$_3$COO, and 120 μL distilled water. After 30 min of incubation, the absorbance was measured at 415 nm. The total flavonoids were determined as quercetin equivalents (% QE) dry weight. The experiment was conducted in triplicate analysis.

2.5. Assay for antimicrobial activity

Antimicrobial activity was evaluated through inhibition of bacterial strains growth by the standard MTT assay according to a previous procedure [12] with slight modifications. A stock solution of samples was prepared at 10 mg/mL in DMSO and varied into different concentrations in 96-well plates that contained microbial strains incubated in Potato Dextro Agar (Merck, Germany) and Nutrient Broth (Merck, Germany) medium. Bacterial strains of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella thyphii*, and *Pseudomonas aeruginosa* were used for this assay. The plate containing bacteria strains and samples was further incubated at 37 °C overnight, and ampicillin (Merck, Germany) was used as a positive control for the bacterial strains. Finally, the 50 mL of the 3- (4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) (Sigma Aldrich, USA) solution (0.5 mg mL$^{-1}$ isopropyl-HCl) was then added to each well and incubated further for 1 h.

2.6. Assay for antioxidant activity

The ability of the plant extracts to scavenge DPPH free radicals was assessed using the microplate assay for radical scavenging activity DPPH standard method [13]. Briefly, in a 96-well plate, successively sample dilutions (standard stocks of different samples 10 mM), in triplicate, received DPPH solution (40 μM in methanol) in a total volume of 0.2 mL. After incubation period of 30 min in darkness at room temperature, the absorbance was recorded with a microplate reader (Thermo-
scientific). Absolute methanol was used as a blank and gallic acid (Sigma Aldrich, USA) as a positive control.

3. Results and Discussion

3.1. Chemical composition of Baeckea frutescens

The ethanol extract of Baeckea frutescens has the highest total phenol content as 5.796 % GAE dw. The total phenol content of ethanol extract was significantly higher compared to water extract, whereas the chloroform extract of Baeckea frutescens showed the absence of phenolic content. The efficiency of the solvents to the phenolic compounds was in the order of ethanol > water > chloroform. Total flavonoid content of the Baeckea frutescens extracts were recorded in least quantities in quercetin equivalents (QE). The ethanol extract of Baeckea frutescens showed the highest total flavonoid content as 0.192 % QE dw, in contrast the phenolic content was not detected in chloroform extract of Baeckea frutescens. The efficiency of the solvents to the flavonoid compounds was in the order of ethanol > water > chloroform.

3.2. Antimicrobial Activity of Baeckea frutescens

The antibacterial activity of different Baeckea frutescens extracts has been demonstrated against E. coli, S. aureus, S. thypi, and P. aeruginosa bacterial strains. The ethanol and water extracts of Baeckea frutescens showed antibacterial activity even against E. coli and S. thypi, but none of the extracts was active against S. aureus and P. aeruginosa. The result showed that the ethanol extract of Baeckea frutescens exhibited strong antibacterial activity against S. thypi, with an MIC value less than 50 μg/mL. Both ethanol and water extracts remarkably inhibited the growth of E. coli and S. thypi, with MIC values less than 50 μg/mL. Each bacterial strain demonstrated a significant degree of sensitivity to the Baeckea frutescens extracts. It has been reported that antibacterial activity of the extracts is due to the flavonoids, aromatic acids, terpenoids, and its esters contents. Flavonoids, one of the major constituents existed in Baeckea frutescens, are found to be very useful as an antimicrobial agent, a mitochondrial adhesion inhibitor, an antiulcer agent, an antiarthritic agent, an antiangiogenic agent, and an anticancer agent [14].

| Extracts   | MIC (μg/mL) | E. coli | S. aureus | S. thypi | P. aeruginosa |
|------------|-------------|---------|-----------|----------|---------------|
| Ethanol    | < 50        | > 200   | < 50      | > 200    |               |
| Chloroform | > 200       | > 200   | > 200     | > 200    |               |
| Water      | < 50        | > 200   | < 50      | > 200    |               |
| Ampicillin | 1.0         | 1.0     | 1.0       | 1.0      |               |

* Ampicillin: Positive control

3.3. Antioxidant Capacity

The antioxidant activity of the extracts was assessed on the basis of the radical scavenging effect using stable 1,1-di-phenyl-2-picrylhydrazyl (DPPH). The antioxidant activity may occur via various mechanisms such as scavenging the radicals and chelating metal ions. The good DPPH scavenging activity was showed by Baeckea frutescens ethanol extract (IC_{50} 41.96±6.74 μg/mL) and moderate DPPH scavenging activity by Baeckea frutescens water extract (IC_{50} 93.3±3.41 μg/mL). In contrast, chloroform extract was not active as compared to gallic acid (IC_{50} 3.81±0.18 μg/mL). Phenolic compounds are secondary metabolites that have ability to donate hydrogen, quench singlet oxygen and act as metal chelators, which can act as antioxidant agents. Flavonoids, as one of major components in Baeckea frutescens consist of a large group of polyphenolic compounds provide benefits in multiple ways to the plant producing them [15]. The phenolic compounds presence in Baeckea frutescens leaves are attributed many of the antioxidant properties.
Table 2
Antioxidant activities of *Baeckea frutescens* L. extracts

| Extracts  | IC<sub>50</sub> (μg/mL) |
|-----------|-------------------------|
| Ethanol   | 41.96 ± 6.74            |
| Chloroform| > 200                   |
| Water     | 93.30 ± 3.41            |
| Gallic acid* | 3.81 ± 0.18         |

*Gallic acid: Positive control

4. Conclusion

The objective of the present work was to find the medicinal value of the plant *Baeckea frutescens*. The phytochemical properties of *Baeckea frutescens* was studied as not much work done earlier. The ethanol extract of *Baeckea frutescens* showed higher antioxidant activity than water extract. In addition, the ethanol and water extracts were also active to inhibit some bacterial strains. The highest antibacterial activity of the *Baeckea frutescens* extracts was observed against bacterial strains of *E. coli* and *S. thypi*, and is thought to be due mainly to the presence the phenolic compounds. The antioxidant and antimicrobial activities of these extracts were positively correlated with their total phenolic and flavonoid contents. It has been shown that *Baeckea frutescens* possesses significant antioxidant and antimicrobial properties as a source of natural antioxidants for use in pharmaceutical applications.

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