Phytochemicals Screening, GC/MS Characterization, and Antioxidant Activity of Falcataria moluccana Miq. Barneby and J. W. Grimes Methanolic Extract

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ABSTRACT

Introduction: In this study, Falcataria moluccana as a plant of West Java community forest was evaluated for its phytochemical content, characterization of secondary metabolites through GC/MS analysis, and antioxidant activity with the DPPH method. Methods: The extraction of F. moluccana twig used maceration with methanol solvent. Phytochemical compounds in F. moluccana methanolic extract were identified using Gas Chromatography-Mass Spectrometry (GC/MS). The antioxidant activity was tested against 2,2-diphenyl-1-picyrylhydrazyl (DPPH). Results: The phytochemical screening of F. moluccana methanolic extract showed the presence of phenolics, flavonoids, steroids, terpenoids, saponins, and tannins. The results of GC/MS analysis showed that the highest abundance was α-terpinenol from the terpenoid group with a retention time of 6.776 minutes and a percentage area of 25.86%. Total phenolic content in methanolic extract of F. moluccana was 145.21 mg GAE/g, total flavonoid was 95.39 mg QE/g, while antioxidant activity (IC50) was 12.60 ppm. Conclusion: F. moluccana has potential as natural antioxidant and its active compounds can be developed as pharmaceutical raw materials.

Key words: Falcataria moluccana, Phytochemical, GC/MS, Antioxidant, Methanolic extract.

INTRODUCTION

Plants are very rich in secondary metabolite components with specific bioactivity for human needs in the health sector. The factor determining the pharmacological activity of plants is the content of secondary metabolites. Isolated secondary metabolites from plants have interesting bioactivity. Several studies found that secondary plant metabolites have bioactivity as antidiabetic, anti-inflammatory, antihelmintic, and antioxidants. Antioxidants can be produced both synthetically and naturally, but synthetic antioxidants have a toxic effect compared to natural antioxidants. Natural antioxidants can be sourced from plants containing bioactive compounds in the form of secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, and others. Currently, a lot of biological and pharmaceutical exploration is carried out on plants. This is based on the fact that plants contain antioxidants and other bioactive components. Therefore, studies to replace synthetic antioxidants with natural antioxidants are necessary.

One of the potential plants as a source of natural antioxidant compounds is F. moluccana which is a multipurpose species in Southeast Asian plantations, especially Indonesia and Malaysia. The purpose of this study was to find out phytochemical content, characterization of secondary metabolites by GC/MS analysis, and antioxidant activity of F. moluccana methanolic extract.

MATERIAL AND METHODS

Materials

F. moluccana twig were obtained from sengon plantation in Cisempur Village, Jatinangor Sub-District, Sumedang Regency, West Java, Indonesia. All chemical reagents for phytochemical screening i.e. methanol, folin-ciocalteau, phenol, ammonia, toluene, chloroform, hydrochloric acid, dragendorff reagent, mayer reagent were purchased from Merck. Gallic acid, quercetin, 2,2-diphenyl-1-picyrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (MO, USA).

Extraction

The extraction of F. moluccana twig was carried out with a maceration technique using a methanol solvent. Dried branches were crushed to a powder. 50 g of simplicia was macerated using 200 mL of methanol for 72 hours while shaking it in a shaker. Then, the solution was filtered using Whatman No. 42 filter paper. Then, the filtrate was evaporated using a rotary evaporator until obtaining a concentrated extract.

Phytochemical screening

The phytochemical test aims to find out the content of secondary metabolites in a plant-based on reactions that produce color or deposits. In this study, the test for alkaloids, steroids, terpenoids, phenolics, flavonoids, saponins, and tannins was carried out.
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Alkaloids

50 mg was added to 5 mL of chloroform and 3 drops of ammonia. The chloroform fraction was separated, then 45 drops of 2 M H2SO4 were added. The acid fraction (above) was taken and Dragendorf, Mayer, and Wagner reagents were added. The positive result was characterized by the formation of red deposit on the addition of Dragendorf reagent, white deposit in Mayer reagent, and brown deposit in Wagner reagent.

Steroid and terpenoid

5 mg of extract was added to 5 mL of hot ethanol. The solution was filtered and evaporated until dry. Then, 1 mL of diethyl ether was added and homogenized. A drop of concentrated H2SO4 and anhydrous acetic acid were added to the solution. Green or blue color indicated steroid content, while red or purple color indicated terpenoid content.

Phenolic

5 mg of sample was dissolved in 2 mL of methanol. The solution was filtered then the filtrate was mixed with 10% NaOH then heated. The red color indicated phenolic compounds.

Flavonoid

5 mg of the extract was mixed into 5 mL of distilled water, then heated at 80 °C for 5 minutes. The solution was filtered and added with magnesium powder, HCL: ethanol (1:1) solution, and amyl alcohol. The red or orange color of the amyl alcohol layer indicated flavonoids.

Saponins

5 mg of saponin extract was added to 5 mL of distilled water, then heated at 80 °C for 5 minutes. The solution was filtered and added with 3 drops of 10% FeCl3. Dark blue or greenish-black color indicated tannins.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The *F. moluccana* methanolic extract was analyzed using GC/MS ShimadzuQP 2010 (Shimadzu Corp., Kyoto, Japan), RTx5MS (Restek Corp.) columns of 30 m length, 250 °C injectors and detectors, and 50-300 °C operating temperature. The temperature rise at 50-120 °C was regulated with a temperature rise rate of 40 °C/minute and held for 1 minute then a temperature increase of 120-300 °C was adjusted with a temperature rise rate of 60 °C/minute and held for 5 minutes in total retention time (Rt) of 60 minutes. The carrier gas was helium with a molecular weight range of 50-500. The compound was identified by the Wiley/NIST Library software.11

Total phenolic content

The total phenolic content was calculated to determine the amount of phenol in the sample. The extract solution was produced at a concentration of 100 mg/L in the form of the extract dissolved in methanol, then 5 mL extract was added with 0.3 mL 5% NaNO2. Then, it was added with 0.3 mL 10% AlCl3, dissolved with methanol, and rested at room temperature for 5 minutes. Then, it was added with 2 mL 1 M NaOH and the solution volume was added up to 10 mL with distilled water. The absorbance was measured with an UV-Visible spectrophotometer at a wavelength of 510 nm. The standard used was quercetin with a concentration of 10, 20, 30, 40, and 50 ppm. The flavonoid content was expressed by Quercetin Equivalent (QE) mg per gram of dry extract (Quercetin Equivalent (QE) mg/g extract).13

Antioxidant activity

1 mL of *F. moluccana* methanolic extract with 100, 200, 300, 400, and 500 ppm concentrations were mixed with 2 mL of 0.002% DPPH solution and rested for 30 minutes. The absorbance was measured at a wavelength of 517 nm. The positive control used ascorbic acid (5, 10, 15, 20, and 25 ppm concentrations). The inhibitory power was calculated using the following formula:

\[
\text{Inhibitory power (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%
\]

1IC50 (inhibitory concentration) was determined by calculating the concentration with 50% inhibition from linear regression equation.

RESULTS AND DISCUSSION

Phytochemical Screening of *F. moluccana* Methanolic Extract

Phytochemical screening was carried out to test the alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and phenolics. The phytochemical components of *F. moluccana* methanolic extract can be seen in Table 1. The results of phytochemical testing showed that the *F. moluccana* methanolic extract had flavonoids, steroids, terpenoids, phenolics, saponins, and tannins, except alkaloid.

Phenolic and flavonoid compounds showed high antioxidant activity. Flavonoids have the ability to be antiviral, antibacterial, anti-tumor, anti-allergic, anti-inflammatory, and anti-carcinogen.15 Several studies found that secondary metabolites from plants that have many roles as antioxidants and antibacterials are phenolic compounds in the form of flavonoids.16

*F. moluccana* methanolic extract contains active compounds in the form of steroids and terpenoids. Steroid and terpenoid compounds have a carbon structure derived from six isoprene units and are biosynthetically derived from acyclic C30 hydrocarbons such as squalene. Squalene is a natural antioxidant, classified as triterpene compounds, and intermediates in plant and animal sterol biosynthesis. Steroids are natural antioxidant as an anti-radical and antioxidant.17

| No. | Chemical Component | Reagent | Results |
|-----|-------------------|---------|---------|
| 1.  | Alkaloids         | Drangedorf | -       |
|     |                   | Wagner   | -       |
| 2.  | Flavonoids        | HCl, Mg powder, amyl alcohol | +       |
| 3.  | Terpenoids/Steroids | Liebermann-Burchard | +       |
| 4.  | Phenolic          | Methanol and 10% NaOH | +       |
| 5.  | Saponins          | Hot water and shaken | +       |
| 6.  | Tannins           | 10% FeCl3 | +       |

Note: + = positive compound, - = negative compound

Table 1: Phytochemical Screening of *F. moluccana* Methanolic Extract.
**F. moluccana** methanolic extract contains saponin and tannin compounds. Saponins are known as foam substances and are generally used as antioxidants, anticancer, and anti-inflammatory. Tannins are phenolic compounds used therapeutically as antimicrobial, anti-inflammatory, anti-diarrheal, hemostatic, and anti-hemorrhoidal. Secondary metabolites are a very important component in biological activity related to pharmacology.

**GC/MS analysis**

The results of GC/MS analysis showed that 20 compounds were identified in **F. moluccana** methanolic extract. This analysis showed that the highest abundance was α-terpinolene from the terpenoid group with a retention time of 6.776 minutes and a percentage area of 25.85%. α-terpinolene is a terpenoid group (monoterpenes) with a molecular formula of C_{10}H_{16} and a molecular weight of 136.23 g/mol which functions as a food additive or flavoring agent. The **F. moluccana** methanolic extract contains different types of triterpene such as α-pinen, α-terpinolene, α-terpinene, and dl-limonene.

Several compounds have antioxidant and antimicrobial activities such as α-pinen, α-terpinolene, α-terpineol, and dl-limonene. Several phenolic compounds were analyzed in this study.

**Total phenolic content of F. moluccana methanolic extract**

The total phenolic content was calculated with the Folin-Ciocalteau reagent to form a complex solution with phenolic compounds. The total phenolic content was expressed by gallic acid equivalent milligrams per gram of extract. This study used gallic acid as a standard. This study used the complex formation between AlCl₃ and the keto group. The total phenolic content of **F. moluccana** methanolic extract was 145.21 mg GAE/g. The total phenolic content of **F. moluccana** methanolic extract was higher than the total phenolic content of **Averrhoa bilimbi** (79 mg GAE/g) methanolic extract and **Moringa oleifera** (44.03 mg GAE/g) methanolic extract.

Phenolic compounds are known to have beneficial antioxidant activity for the body as an antidote to free radicals. Phenolic compounds reduce free radicals by binding to metal ions and inhibiting enzymatic systems that play a role in the formation of free radicals such as cyclo-oxygenase, mono-oxygenase, or xanthine oxidase. The antioxidant effect on phenolic compounds is due to the ability to reduce and allow phenolic compounds to have free radical scavenging activity mechanisms, metal chelating intermediate activity, and singlet oxygen-reducing activity. In addition, phenolic compounds play an important role in stabilizing lipid peroxidases and inhibiting the oxidation of various enzymes.

**Table 2: GC/MS Analysis of F. moluccana Methanolic Extract.**

| Retention Time (min) | Molecular Formula | Molecular Weight (g/mol) | Peak Area (%) |
|----------------------|-------------------|--------------------------|--------------|
| 1. α-pinene          | C_{10}H_{16}       | 136.23                   | 8.91         |
| 2. Camphene          | C_{10}H_{16}       | 136.23                   | 8.42         |
| 3. l-Phellandrene    | C_{10}H_{16}       | 136.23                   | 0.63         |
| 4. Delta 3-Carene    | C_{10}H_{16}       | 136.23                   | 10.66        |
| 5. Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl- | C_{10}H_{16} | 176.3                   | 12.00        |
| 6. Benzene, methyl(1-methylethyl)- (CAS) Cymol | C_{10}H_{16} | 134.22                   | 2.47         |
| 7. dl-Limonene       | C_{10}H_{16}       | 136.23                   | 12.13        |
| 8. Eucalyptol        | C_{10}H_{16}       | 154.25                   | 2.98         |
| 9. γ-Terpinepine     | C_{10}H_{16}       | 136.23                   | 7.38         |
| 10. α-Terpineolene   | C_{10}H_{16}       | 136.23                   | 25.85        |
| 11. Azulene (CAS) Cyclopentacycloheptene | C_{10}H_{16} | 128.17                   | 1.61         |
| 12. α-Terpineol      | C_{10}H_{16}       | 154.25                   | 2.06         |
| 13. 1-Dodecanol (CAS) n-Dodecanol | C_{10}H_{16} | 186.34                   | 1.89         |
| 14. Butyl Hydroxy Toluene | C_{10}H_{16} | 220.35                   | 1.25         |
| 15. Octadecane (CAS) n-Octadecane | C_{10}H_{16} | 254.5                   | 1.32         |
| 16. Dodecyl trifluoroacetate | C_{10}H_{16} | 282.34                   | 0.67         |
| 17. Oxalic acid, cyclohexylmethyl tridecyl ester | C_{10}H_{16} | 368.5                   | 0.86         |
| 18. Tetradecanoic acid, methyl ester (CAS) Methyl myristate | C_{10}H_{16} | 242.4                   | 1.24         |
| 19. 9-Heptadecanone | C_{10}H_{16}     | 254.5                   | 0.59         |
| 20. Hexadecanoic acid (CAS) Palmitic acid | C_{10}H_{16} | 256.42                   | 0.67         |
IC\textsubscript{50} value of stable ascorbyl free radicals. The lower when compared with ascorbic acid as positive control. Ascorbic acid as positive control can be seen in Table 3. IC\textsubscript{50} of \textit{F. moluccana} is the standard measure of antioxidant activity. The IC\textsubscript{50} value was obtained through a linear regression equation of inhibition percentage and sample concentration. The lower the IC\textsubscript{50} value, the higher the inhibition of the extract against free radicals. The classification of antioxidant activity is based on the IC\textsubscript{50} value, namely very strong antioxidants (IC\textsubscript{50} < 50 ppm), strong antioxidants (IC\textsubscript{50} = 50-100 ppm), moderate antioxidants (IC\textsubscript{50} = 101-150 ppm), and weak antioxidants (IC\textsubscript{50} > 150 ppm).\textsuperscript{31} This study used ascorbic acid as a standard measure of antioxidant activity.

The antioxidant activity of \textit{F. moluccana} methanolic extract and ascorbic acid as positive control can be seen in Table 3. IC\textsubscript{50} of \textit{F. moluccana} methanolic extract was 12.6 ppm (below 50 ppm) so that the secondary metabolite of \textit{F. moluccana} methanolic extract can be expressed as a very strong antioxidant.\textsuperscript{31} IC\textsubscript{50} of ascorbic acid was 2.17 ppm. The antioxidant activity of \textit{F. moluccana} methanolic extract was lower when compared with ascorbic acid as positive control. Ascorbic acid is easily oxidized by donating hydrogen atoms and forms relatively stable ascorbyl free radicals.

IC\textsubscript{50} value of \textit{F. moluccana} methanolic extract was lower than IC\textsubscript{50} of \textit{Averrhoa bilimbi} (4.27 ppm) methanol extract.\textsuperscript{27} The high antioxidant activity of \textit{F. moluccana} methanolic extract is related to the content of compounds in the extract that play an important role in antioxidant activity. The results of phytochemical screening showed that \textit{F. moluccana} methanolic extract has phenols, steroids, terpenoids, flavonoids, saponins, and tannins. The results of GC/MS analysis showed the methanolic extract of \textit{F. moluccana} branches which affect radical inhibiting properties such as phenols and triterpenes.

## CONCLUSION

The phytochemical screening of \textit{F. moluccana} methanolic extract showed the presence of phenolics, flavonoids, steroids, terpenoids, saponins, and tannins. The results of GC/MS analysis showed the presence of compounds with antioxidant activity. \textit{F. moluccana} methanolic extract had a very strong antioxidant activity with an IC\textsubscript{50} value was 12.6 ppm. \textit{F. moluccana} methanolic extract has the potential as a source of bioactive antioxidant compounds.

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Summary
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