Identification and antioxidant activity of phenolic compound from leaves of *Scurrula parasitica* L

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Abstract: *Scurrula parasitica* L is one of the Loranthus plant species which is an herbal plant recorded in ancient documents and widely used as traditional medicine. The purpose of this study was to identify phenolic compounds and to test the antioxidant activity of compounds from *Scurrula parasitica* L. leaf extract. The research method was carried out by extracting dried powder of *S. parasitica* leaves with ethanol. Ethanol extract was partitioned using *n*-hexane, chloroform, and ethyl acetate solvents respectively. The ethyl acetate fraction obtained is then concentrated to form deposits. The precipitate obtained was separated and washed using *n*-hexane and a mixture of ethyl acetate: acetone (1: 1) in sequence. The precipitate obtained is then purified by recrystallization with ethanol as solvent. The crystals obtained were tested for their purity by thin layer chromatography (TLC) and identified using UV-Vis, IR and NMR spectrophotometers. The method for testing antioxidant activity using 2,2- diphenyl-1-picrylhydrazyl (DPPH). The results of spectroscopic identification showed that the isolation compound obtained was quercitrin (quercetin-3-O-rhamnoside). Quercitrin shows high antioxidant activity with IC50 5.00 μg / mL. Thus, *S. parasitica* plant is very potential as a source of natural antioxidants

1. Introduction

Indonesia is a tropical country that has a high level of plant diversity. Some of these plants are widely used as medicinal plants. One of the plants that can be used as medicine is Loranthus or its local name is "Benalu". Loranthus is a parasitic plant that attaches to the stem of host plants, such as *Mangifera indica* (Anacardiaceae), *Melia azedarach* (Meliaceae), and *Psidium guajava* (Myrtaceae) [1; 2]. Several studies have shown that Loranthus plants contain compounds that show cytotoxic properties of cancer cells [3], can inhibit polio virus [4], antimicrobial and anti-inflammatory [5], neuroprotective [6], antioxidant [7], and antidiabetic [8]. One type of Loranthus plant found in Indonesia is *Scurrula parasitica* L. The plant is attached to the stem of the plant *Lagerstroemia speciosa* L. Press or flower plant whose local name is "bungur". Polysaccharide compounds from the leaves of *Scurrula parasitica* are reported to inhibit cancer cells [9]. Several studies have shown the presence of several secondary metabolites from Loranthus plants including quercetin [10] and quersitrine (quercetin-3-O-rhamnosida) [11].

*Lagerstroemia speciosa* is one of the plants as a host for Loranthus. The leaf part of *L. speciosa* contains favonol groups namely 5.3 ', 4'-trihydroxy flavonol, saponins, glycosides, tannins and
terpenoids [12]. L. speciosa are usually used as antidiabetic and anti-obesity [13; 14], anticariogenic [15]. The secondary metabolite content of the plant Loranthus is usually influenced by the type of host plant, so there is a possibility that the secondary metabolites found in plants attached to the stem are similar to those found in L. speciosa. In this article we will report the identification and testing of antioxidant activity of compounds obtained from the leaves of the Scurrula parasitica plant which is hemiparasit on L. speciosa.

2. Experimental Section

2.1 Apparatus and reagent
The equipment used in this study included evaporator Buchi Rotavapor R-114, spectrophotometer, vortex mixers, and glassware. The materials used include organic solvents such as ethanol, methanol, n-hexane, ethyl acetate, and acetone, 2,2-diphenyl-1-picrilhidrazil (DPPH, Aldrich), ascorbic acid (Aldrich), leaves of Scurrula parasitica plant that hemiparasite on L. speciosa, and distilled water.

2.2 Extraction, isolation, and identification of phenolic compounds
Extraction was carried out by maceration method of leaf dry powder Scurrula parasitica for 24 hours and repeated twice. The maceration results were concentrated with the Buchi evaporator, then partitioned with n-hexane, chloroform, ethyl acetate solvents in succession. The results of the partition from ethyl acetate solvents were then concentrated with the Buchi evaporator, to obtain concentrated ethyl acetate fractions of 84.42 g. Solids formed from concentrated fractions of ethyl acetate are separated and purified. Purification was carried out by washing solids from concentrated ethyl acetate fraction using n-hexane and a mixture of ethyl acetate: acetone (1: 1) in sequence. The precipitate obtained is recrystallized by dissolving it in ethanol and left for several days to form crystals. The obtained crystals are filtered and left to dry at room temperature. The crystal obtained is shiny yellow as much as 0.27 g. Furthermore, the purity test was carried out with TLC using ethyl acetate eluent: methanol = 4: 1 and 4.5: 0.5. The TLC chromatogram shows a single compound which means that the compound isolated is a pure compound. Then pure compounds from the isolation were identified using UV-Vis, IR, and NMR spectroscopy.

2.3 Determination antioxidant activity test by DPPH method
The antioxidant activity of the isolated compounds was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [16]. Samples were dissolved in ethanol at various concentrations (50; 25; 12.5; 6.25; 3.125 ug / mL). 5 mL of sample solution in various concentrations were mixed with 5 mL DPPH solution in methanol (0.12 mM) and incubated at room temperature for 30 minutes. The absorbance of each sample was determined using a spectrophotometer at a wavelength of 516 nm. As a positive control in this experiment ascorbic acid was used. Each sample was tested with three replications. Inhibition activity was calculated as a percentage decrease in sample solution compared to DPPH solution without sample. The inhibitory activity obtained from each sample was used to calculate the antioxidant activity expressed as IC\textsubscript{50} (inhibitory activity at a concentration of 50%).

3. Results and discussion
L. speciose or Bungur is a purple flowering plant every September-October. Many of these plants are planted on the roadside or garden. One parasitic plant attached to the plant is S. parasitica. The results of isolation and purification of compounds in the ethyl acetate fraction of S. parasitica leaves were obtained one compound in the form of yellow crystalline solids. The UV-VIS spectrum shows the maximum wavelength (λ\textsubscript{max}) at 351.80; 258.00; 217.60 nm as in Fig.1. The UV-VIS spectrum data shows that the isolated compounds have sinamoil, benzoyl, and conjugated phenol chromophore. The IR spectrum shows absorption at (ν max) 3286.70; 2924.09; 1658.78; 1604.77 and 1505.77; 1357.02 cm\textsuperscript{-1} (Fig. 2.), which indicates the presence of hydroxyl groups, carbonyl groups, and aromatic C = C bonds.
Fig. 1. Spectrum UV-VIS (in MeOH) of isolated compound in the ethyl acetate fraction of *S. parasitica* leaves

Fig. 2. Spectrum IR (KBr pellet) of isolated compound in the ethyl acetate fraction of *S. parasitica* leaves

The $^1$H NMR spectrum data of the isolated compound shows 14 protons. Aromatic proton signals at $\delta$ 6.36 (1H, $d$, $J$=2.3 Hz) and $\delta$ 6.19 (1H, $d$, $J$=2.3 Hz) ppm indicate the presence of benzene rings substituted at positions 1,2,3 and 5. Aromatic proton signals at $\delta$ 7.30 (1H, $dd$, $J$= 8.3 and 2.1 Hz); $\delta$ 6.90 (1H, $d$, $J$=8.3 Hz); and $\delta$ 7.32 (1H, $d$, $J$= 2.1 Hz) ppm indicating the presence of substituted benzene rings at position 1 '; 3 '; 4 '. Aliphatic proton signal at $\delta$ 5.34 (1H, $d$, $J$=1.7 Hz); $\delta$ 4.22 (1H, $d$, $J$=3.3); $\delta$ 3.75 (1H, $dd$, $J$= 3.3 and 3.4 Hz); $\delta$ 3.41 (1H, $m$); $\delta$ 3.34 (1H, $d$, $J$= 6.1 Hz); and $\delta$ 0.94 (3H,
$d, J=6.1$ Hz) ppm showing protons from the rhamnosil group. The presence of the ramnosil group is strengthened by the presence of a methyl group proton at $\delta$ 0.94 (3H, $d, J=6.1$ Hz) The hydroxyl proton signal from the rhamnosil group is indicated by a signal that is high and wide in the area of $\delta$ 4.90 ppm. The $^1$H NMR spectrum data indicates that the isolated compounds are flavonoids with the rhamnosil group substituted. Spectrum $^1$H NMR of this isolated compound can be seen at Fig. 3.

![Spectrum $^1$H NMR (CD$_3$OD) of isolated compound in the ethyl acetate fraction of S. parasitica leaves](image)

The $^{13}$C-NMR spectrum data can be seen in Fig. 4. From the spectrum data, the isolated compound has two benzene rings. Carbon signals at $\delta$ 163.1 (C1), $\delta$ 103.6 (C2), $\delta$ 158.4 (C3), $\delta$ 99, 8 (C4), $\delta$ 165.8 (C5), and $\delta$ 94.7 (C6) ppm which shows the presence of a substituted benzene ring with two hydroxyl groups at positions 3 and 5. Then the second aromatic ring is shown from the carbon signal at $\delta$ 122.9 (C1 '), $\delta$ 116.9 (C2 '), $\delta$ 146, 3 (C3'), $\delta$ 149.7 (C4 '), $\delta$ 116.9 (C5'), and $\delta$ 122.8 (C6) ppm which are substituted benzene rings at position 1 ', 3 ', and 4 '. The carbon signal at $\delta$ 179.6 is a carbonyl group. The carbon signal at $\delta$ 159.2 and $\delta$ 136.2 ppm shows the alkene group. Other carbon signals are at $\delta$ 103.6, $\delta$ 71.8, $\delta$ 72.0, $\delta$ 73.2, $\delta$ 72.1, and $\delta$ 17.6 ppm which indicates signals carbon from the rhamnosil group. Furthermore, to ascertain the structure of the isolated compound used two-dimensional NMR spectrum data including HMQC (Heteronuclear Multiple Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Coherence) and by comparing spectroscopic data with compounds found in the *Dendrophthoe falcata* plant [11]. From these data indicate that the compound isolated is quercitrine which has the structure as in Fig. 5. Quercitrin is the main compound found from *D. falcata* which is an antioxidant [11].
Fig. 4. Spectrum $^{13}\text{C}$ NMR (CD$_3$OD) of isolated compound in the ethyl acetate fraction of $S.$ parasitica leaves

Fig. 5. Structure of isolated compound in the ethyl acetate fraction of $S.$ parasitica leaves
Test of antioxidant activity from isolated compounds and plant extracts can be done by various methods. One of the simplest and easiest methods to do is to use DPPH. The antioxidant activity test using the DPPH method is based on radical capture reactions by antioxidant compounds with the mechanism of transfer of oxygen atoms, which will produce a stable DPPH-H molecule [17]. Reduction of DPPH concentration is indicated by a decrease in the intensity of the sample solution compared to a blank solution (DPPH solution without test sample). The absorbance of each solution was measured using spectrophotometry at a wavelength of 516 nm. Antioxidant capacity is expressed as IC$_{50}$ ($\mu$g/ml) from DPPH capture activity at various concentrations. The absorbance data of each sample is then made into a linear regression equation. From the regression equation used to calculate IC50. The inhibitory activity of each sample and calculation of antioxidant activity data expressed as IC$_{50}$ are shown in table 1.

Table 1. Antioxidant activity (IC$_{50}$) test leaves of isolated compound

| Sample               | Concentration (µg/ml) | Inhibition activity (%) | The regression equation | Antioxidant activity (IC$_{50}$ µg/ml) |
|----------------------|-----------------------|-------------------------|-------------------------|--------------------------------------|
| Isolated compounds   |                       |                         |                         |                                      |
| (Quercitrin)         | 10                    | 87.87                   | y = 8.2225x + 8.8838    | 5.00                                 |
|                      | 5                     | 55.87                   | R² = 0.981              |                                      |
|                      | 2.5                   | 32.82                   |                         |                                      |
|                      | 1.25                  | 16.49                   |                         |                                      |
|                      | 0.625                 | 10.68                   |                         |                                      |
| Ascorbic acid        |                       |                         |                         |                                      |
| (positive control)   | 5.00                  | 95.59                   | y = 13.76x + 32.51      | 1.27                                 |
|                      | 2.50                  | 79.82                   | R² = 0.883              |                                      |
|                      | 1.25                  | 51.45                   |                         |                                      |
|                      | 0.625                 | 32.28                   |                         |                                      |

Quercitrin is a compound that has been found in the flower of *Allamanda cathartica* [18], *Zanthoxylum bungeanum* [19], *Anarcardium occidentale* L [21]. Quercitrin is a major compound in several species of loranthus plants [11]. Quercitrin showed strong antioxidant and moderate antibacterial activities [18]. Ascorbic acid is a widely known antioxidant compound, exhibiting very high antioxidant activity and is commonly used as a positive control [17]. The results showed that IC$_{50}$ quercitrin from compounds isolated from ethyl acetate fraction from *S. parasitica* leaves by 5.00 µg/ml was higher than positive control (ascorbic acid), but still in very active criteria.

4. Conclusion
From this study it can be concluded that the leaves of the plant *S. parasitica* contain quercitrin (quercetin-3-O-rhamnoside). Quercitrin showed high antioxidant activity with IC$_{50}$ of 5.00 µg/mL. Therefore, *S. parasitica* leaves can be developed as natural antioxidants.

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**Acknowledgements**

This research was funded by the Minister of Research and Technology of the Directorate of Higher Education, Indonesia to fundamental research grand 2019. The author would like to express his gratitude for the funding.