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Original Article

Phytochemical Constituents and Free Radical Scavenging Activity of Madang Gatal (*Schima wallichii*) Choisy Stem Bark

Galuh Widiyarti[1,*], Winda Fitrianingsih[2]

ABSTRACT

Introduction: Madang gatal *(Schima wallichii)* Choisy is one of typical Indonesian plants that used traditionally as insomnia and hypertension drug. **Background:** The environment is highly polluted by free radicals which may contribute to the aging process of tissue and cause chronic diseases. The aims of the research were to study the phytochemical constituents and free radical scavenging activity of *Schima wallichii* stem bark. **Materials and Methods:** Extraction of *S.wallichii* stem bark was carried out by maceration method using methanol and then the methanol extract was partitioned using *n*-hexane, ethyl acetate and butanol successively. The methanol extract was then screened to determine the presence of different classes of secondary metabolites. The ethyl acetate fraction was subjected to silica column chromatography followed by exclusion chromatography on sephadex LH-20 to yield two partially purified isolates, SA and SE4. FTIR and GC-MS analyses indicated SA asa mixture of *pentadecanoic acid, 14-methyl-, methyl ester* and patchouli alcohol, while SE4 was made up predominantly of *pentadecanoic acid, 14-methyl-, 13-hydroxy methyl ester*. Free radical scavenging activity was performed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical oxidation method. **Results:** The phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, saponins, terpenes and quinones. All tested samples, methanol, *n*-hexane, ethyl acetate and butanol extracts showed strong antioxidant activity with IC_{50} values of 8.58, 17.36, 8.17 and 8.79 µg/mL, respectively. The isolates showed strong antioxidant activity with SE4 exhibiting an IC_{50} value of about 5.5 times stronger than quercetin used as a standard antioxidant.

Key words: 14-methyl-, 13-hydroxy methyl ester, Antioxidant activity, DPPH, Chromatography, *Pentadecanoic acid*, *Schima wallichii* stem bark.

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INTRODUCTION

The environment is highly polluted by free radicals which may contribute to the aging process of tissue and cause chronic diseases such as cancer and cardiovascular problems. Therefore, antioxidants are needed for protection of cells and tissues against free radicals.[1-3] Antioxidants are antiradicals that act by donating hydrogen atoms to radical compounds.[4,5] The function of antioxidants are as free radicals neutralizer, thus the aging process that cause the degenerative diseases can be inhibited.[3,6,7]

DPPH method is one of the most widely used and simplest method for analyzing antioxidant activity. DPPH is a stable free radical compound so that it can react with hydrogen atoms derived from an antioxidant compound to form reduced DPPH. When DPPH accepts electrons or hydrogen radicals, a stable diamagnetic molecule is formed. The interaction between antioxidant and DPPH either transfer of electrons or hydrogen radicals will neutralize free radical character of DPPH.[8,9] If all the electrons in free radicals are paired, the colour of DPPH solution will change from purple to yellow light. The colour change can be measured stoichiometrically based on the number of electrons or hydrogen atoms that are captured by DPPH molecules due to the presence of antioxidants.[10,11]

Schima wallichii Choisy, locally known as madang gatal or puspa is used traditionally in Indonesia as insomnia and hypertension drug. *S. wallichii* produces high quality carpentry wood. The plant belongs to the genus *Schima* and tea family (Theaceae).[12,13] Previous studies reported that *S. wallichii* extract have anti-inflammatory, antipyretic and analgesic,[14] antimicrobial[15] and antimutagenic[16] activities. Some bioactive compounds isolated from *S. wallichii* such as kaemferol-3-O-ramnosida have bioactivity as antimalarial,[17] anticancer,[18,19] antiplasmodial,[20] rotenone as antimicrobial and antifungi[14] and theanine has psychoactive properties so that it is useful for overcoming anxiety, depression, stress, insomnia, sleep disorders, hypertension and some symptoms of schizophrenia.[21] In this research the phytochemical constituents and free radical scavenging activity of *S. wallichii* stem bark were studied.

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MATERIALS AND METHODS

Materials

S. wallichii stem bark was collected from PUSPIPTEK provincial garden, Banten, Indonesia. This plant was identified by a botanist of the Biology Laboratory, Biology Research Center, Cibinong West Java, Indonesia and voucher specimens have been stored at the Biology Laboratory (herbarium). All technical solvents such as methanol, *n*-hexane, ethyl acetate, butanol and chloroform were distilled according to standard procedure. All chemicals were obtained from *E. Merck* and used without further purification. Analytical chemicals were used for phytochemical tests including Bouchardat reagent, Mayer reagent, Dragendorf reagent, ethanol, magnesium powder, ether, anhydride acetic acid, sulfuric acid, $FeCl_3$ 1%, sodium acetate, NaOH 1N and DPPH reagent for antioxidant test. Silica gel plates (Kiesel gel $60F_{254}$ 0.25 mm) for Thin Layer Chromatography (TLC) analysis, silica gel 60 (230-400 mesh) and Sephadex LH-20 for coloumn chromatography to isolate bioactive compound of the *S. wallichii* stem bark extract.

Equipment used in this study were macerator, column chromatography and rotary evaporator for extraction and isolation of bioactive compound from *S. wallichii* stem bark, UV lamp 254 nm and 365 nm Camag for detecting the spots on the TLC, FTIR Shimadzu prestige 21 using KBr pellets and GCMS Agilent Tecnologies 7890C for analysis of bioactive compound obtained, UV-Vis spectrophotometer Hitachi U2001 for analysis of antioxidant activity.

Method

A amount of 539.91 g of *S. wallichii* stem barks in macerator macerated and extracted using methanol. Maceration was done for 1 x 24 h. This maceration was repeated 3 times. The percolate/liquid extract was then evaporated using a rotary evaporator until a crude methanol extract obtained. The crude methanol extract was then dissolved in aquades and partitioned using *n*-hexane (1:1) for 3 times. The *n*-hexane phase was evaporated by using rotary evaporator to obtain a *n*-hexane extract. Partition followed by ethyl acetate (1:1) for 3 times. The ethyl acetate phase was then evaporated to obtain an ethyl acetate extract. The partition is continued with butanol (1:1) for 3 times. Furthermore, butanol and aquadest phases are dried by using forced fan oven to evaporate the solvent to obtain butanol and aqueous extract.[22] Crude methanol extract was then phytochemically tested including alkaloids,[23,24] flavonoids,[23,25] terpenes,[23] tannins,[23] saponins[23,26] and quinones test[23] according to standard procedure.

Isolation of the bioactive compounds were carried out on extract that had the highest antioxidant activity by chromatography column using silica gel as the stationary phase and a mixture solvent of *n*-hexane and a gradient of EtOAc to 100 % as the mobile phase and then followed by exclusion chromatography on sephadex LH-20 as stationary phase with methanol-chloroform (1:1) as a system solvent for purification of the isolates. The pure isolates that have been obtained were then identified. IR spectrum was analyzed on a FT-IR Shimadzu prestige 21 using KBr pellets, while mass spectrum (MS) was analyzed by GC-MS Agilent Tecnologies 7890C.

Free radical scavenging activity test

Free radical scavenging (antioxidant) activity test was performed using DPPH method.[17,18,27] First, a 1000 µg/mL solution of samples were prepared. Futhermore, series solutions of 10, 50 and 100 µg/mL were prepared squeeze successively from the mother liquor. Subsequently the series solution was diluted with methanol so the volume of the solution became 2000 µL at each concentration. The each samples test solution were added 500 µL of DPPH solution and incubated for 30 min in the dark. After that, absorbance measurements were conducted using UV-Vis spectrophotometer at 516 nm. The absorbance value obtained is used for calculating the value of % inhibition.

RESULTS AND DISCUSSION

The percentage yields of dried extracts of *S. walliichii* stem bark were calculated based on the percentage weight of extracted material divided by the weight of crude sample. The highest % yield was the methanol extract (22.18%). The % yields of butanol (20%) and aqueous (19.67%) extracts were also high, while the % yields for ethyl acetate and *n*-hexane extracts were only 2.92 and 0.06%, respectively. Therefore it can be concluded that the constituents of *S. walliichii* stem bark were mainly polar compounds. The % yields of *S. walliichii* stem bark extracts are shown in **Table 1.**

Phytochemical test is the examination or screening of the chemical contents qualitatively to know the class of constituents contained in a plant.[22-26] The extract for phytochemical test was methanolic extract before to being partitioned. These phytochemical tests including the test of alkaloids, flavonoids, terpenes, tannins, saponins, as well as quinones constituents. The phytochemical test result indicated the presence of alkaloid, flavonoid, tannin, saponin, terpene and quinone constituents in the extract.

The antioxidant activity of extracts were assessed using spectrophtometry method. The antioxidant assay was performed by using DPPH as sources of free radical, which captured hydrogen from plant extract containing antioxidant. The DPPH solution turned from purple to yellow, indicating the conversion of 2,2-diphenyl-1-picrylhydrazyl into 1,1-difenil-2-pikrilhidrazin. The test was carried out in various concentration of solution prepared from dried extracts: 10, 50, 100 and 200 µg/mL in order to find the IC_{50} of the tested solutions. An extract was chategorized as active if its IC_{50} was less than 200 µg/mL.[8,9,25] Measurement of sample absorbance was performed using UV-Vis spectrophotometer at wavelength 516 nm. Furthermore, absorbance measurements were calculated to find out the most active extracts based on their IC_{50} values. This DPPH method used quercetin as standard antioxidant. The using of standard material was to know if the tested solutions had the same, less or higher activity compared to the standard material. The concentrations of quercetin solutions were 10, 15, 20 and 25 µg/mL.

The antioxidant activity test result showed that all of *S. wallichii* stem bark extracts were active as antioxidant indicated by the DPPH solution colour change, from purple to yellow colour. Based on the IC_{50} value as shown in **Figure 1**, it showed that all extracts have IC_{50} value less than 50 µg/mL. It indicates all of *S. wallichii* stem bark extracts very active as antioxidant. The sample is very active/strong as antioxidant if its IC_{50} value 50-100 µg/mL, while as active/strong antioxidant if its IC_{50} value 100-150 µg/mL and weak antioxidant if its IC_{50} value is 151-200 µg/mL.[8,25-26]

Based on the IC_{50} value, the aqueous extract has smallest IC_{50} value. It indicates the aqueous extract is the highest activity as antioxidant. The IC_{50} value of compound smaller indicates the minimum concentration of compound required to absorb free radicals is smaller. It means this compound more active than the others. However, aqueous extracts are

Table 1: Rendement of *S. wallichii* stem bark extract.

Name of extract	Percentage yield (%)
Methanol (MeOH)	22.18
n-Hexane [*n*H]	0.01
Ethyl acetate (EtOAc)	2.92
Butanol (BuOH)	20.00
Aqueous	19.67

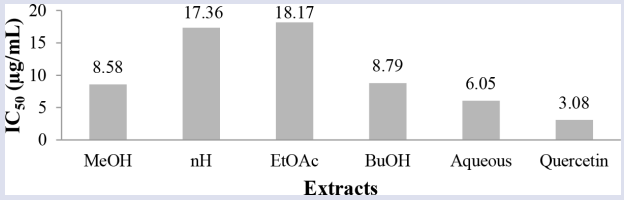

Figure 1: Antioxidant activity of *S. wallichii* stem bark extracts.

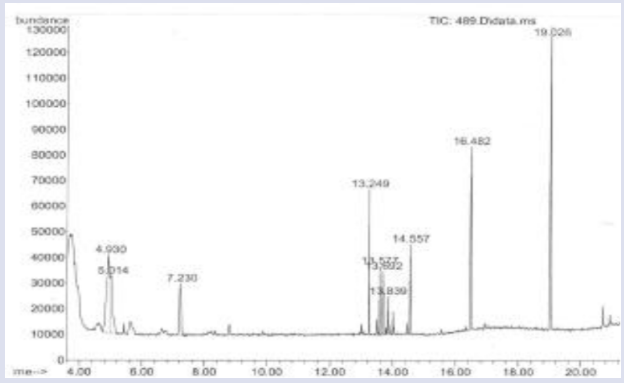

Figure 2: GCMS chromatogram of isolate SA.

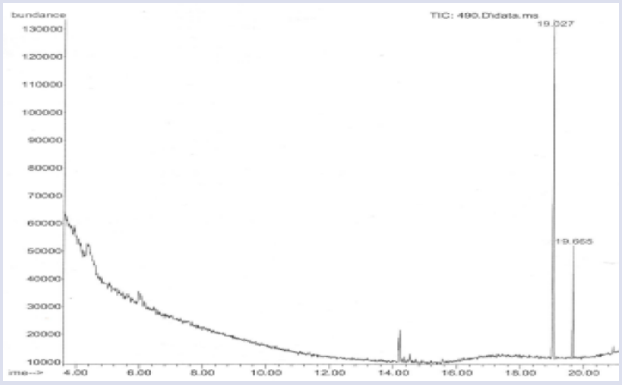

Figure 3: GCMS chromatogram of isolate SE4.

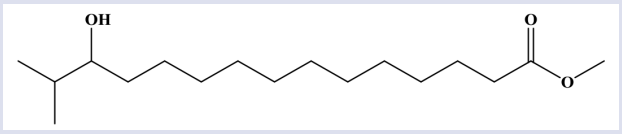

Figure 4: Molecular structure of *methyl 13-hydroxy 14-methyl pentadecanoate* according to literature.

rarely used for antioxidants because they are difficult to evaporate and easily moldy, so that ethyl acetate extract that has the larger antioxidant activity than others extract was further isolated.

Isolation of bioactive compounds from ethyl acetate extract was carried out using chromatography column techniques on silica gel 60 GF_{254} with a gradient solvent system of *n*-hexane:EtOAc as mobile phase to obtain 30 fractions (F1- F30) and then purification was continued by exclusion chromatography using sephadex LH-20 as stationary phase with methanol-chloroform (1:1) as a system solvent.[28,29] Purification of fraction 23 and 25 (F23 and F25) by column chromatography on sephadex LH-20 resulted 2 isolates, named SA (3 mg) and SE4 (15.8 mg). The isolates were then spectroscopy analyzed by FTIR to determine the functional groups of the isolates and GC-MS to determine the alleged constituents of isolates.[30,31]

The IR spectrum of SA showed an absorption at 3423.65 cm^{-1} indicating the presence of hydroxyl (OH) groups. At 2927.94 cm^{-1} and 2856 cm^{-1} indicating the presence of alkyl (C-H) groups. Furthermore, at 1629.85 cm^{-1} indicating the presence of carbonyl (C=O) groups, at 1402.25 cm^{-1} indicating the presence of bending vibration of methyl (CH_3) and at 1109.07cm^{-1} indicating the presence of C-O-group.

The GC-MS chromatogram of SA (**Figure 2**) showed that isolate SA is still an impure compound or mixture of some compounds. There is a dominant peak at retention time of 19.026 minindicating a dominant compound that has highest abundance. According to the GC-MS database, this compound has a similarity 96% to *Pentadecanoic acid*, 14-methyl-, methyl ester or methyl 14-methyl pentadecanoate compound ($C_{17}H_{34}O_2$, MS 270.459 g/mol). The GC-MS chromatogram at **Figure 2** also showed a peak with high enough abundance at retention time of 16.482 min indicating a compound with similarity 99% to patchouli alcohol ($C_{15}H_{26}O$, MS 222 g/mol).

An absorption at 3219.19 cm^{-1}on IR spectrum of SE4 indicated the presence of hydroxyl (OH) groups, while an absorption at 2973.59 cm^{-1} indicated the presence of streching vibration of C-H group. Furthermore, an absorption at 1610.56 cm^{-1} indicated the presence of carbonyl (C=O) groups, at 1300-1100 cm^{-1} indicated the presence of C-O group and at 985.62 cm^{-1} and 827.46 cm^{-1} indicated absorption of streching vibration of C-H group.

GC-MS chromatogram of SE4 showed that SE4 was a mixture of 2 compounds. The compound that has highest abundance detected at retention time of 19.027 min and has similarity 98% to *Pentadecanoic acid*, 14-methyl-, methyl ester. The second compound that detected at retention time of 19.665 min and has abundance lower than first compound was predicted as a contaminant/impurity. The GCMS chromatogram of SE4 was given in **Figure 3**.

From IR spectrum and GCMS chromatogram, SE4 was predicted as *methyl 13-hydroxy-14 methyl pentadecanoate*, a *pentadecanoic acid, 14-methyl-, methyl ester* compound substituted hydroxyl (OH) group, $C_{17}H_{34}O_3$, MS 286.457 g/mol. According to sigma aldrich literature, moleculars tructure of the SE4 ester compoundas shown in **Figure 4**.

Antioxidant activity test result showed that 2 isolates were active as antioxidant as shown in **Figure 5**.

The IC_{50} value of antioxidant activity of isolates were less than 100 µg/mL, so categorized very active as antioxidant.[8] The IC_{50} value of SE4 lower than IC_{50} value of quercetin as standard material indicated that antioxidant activity of SE4 higher than quercetin. Antioxidant activity of SE4 about 5.5 times stronger than quercetin. This maybe caused by molecular structure of SE4 (methyl 13-hydroxy 14-methyl pentadecanoate) whereas the compound was *Pentadecanoic acid*, 14-methyl-methyl ester substituted hydroxyl (OH) group. *Pentadecanoic acid*, 14-methyl-methyl ester has been reported has bioactivy as antioxidant.[29,30] The hydroxyl (OH) group is a good free radical scavenger that can scaveng of free radical

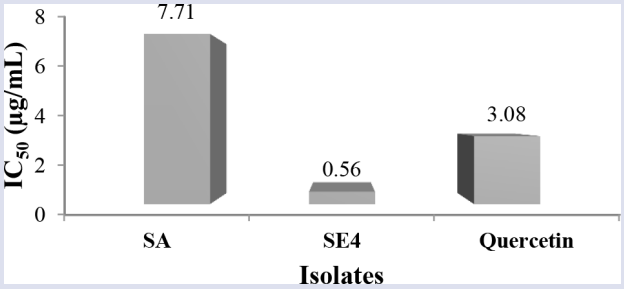

Figure 5: Antioxidant activity of isolate SA and SE4.

so that hydroxyl (OH) group substituted on the ester compound (SE4) improve the antioxidant activity of the ester compound (SE4).

Otherwise, the IC_{50} value of SA that also contents *pentadecanoic acid, 14-methyl-methyl ester* compound is still high compare than quercetin as standard material, although the isolate contents patchouli alcohol that also contents of hydroxyl (OH) group. This occurs due to the impurity of the isolate is still many that are maybe antagonistic to the antioxidant activity of major compound so that decrease the antioxidant activity of SA.

CONCLUSION

Phytochemical screening indicated that *S. wallichii* stem bark contents alkaloid, flavonoid, tannin, saponin, terpene and quinon compounds. Ethyl acetate extract was very active as antioxidant with IC_{50} value 8.167 µg/mL. Isolation of the extract produced 2 isolate, named SA predicted as mixture of *pentadecanoic acid, 14-methyl-, methyl ester* and patchouli alcohol, while SE4 predicted as *methyl 13-hydroxy 14-methyl pentadecanoate* that was *pentadecanoic acid, 14-methyl-, methyl ester* substituted hydroxyl (OH) group. The isolates have strong antioxidant activity with IC_{50} value of SA and SE4 were 7.71 and 0.56 µg/mL, where antioxidant activity of SE4 about 5.5 times stronger than quercetin.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

ABBREVIATIONS

MeOH: Methanol; ***n*H:** *n*-hexane; **EtOAc:** Ethyl acetate; **BuOH:** Butanol; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IC_{50}:** 50% of inhibitory concentration; **µg/mL:** Microgram per mililiter; **min:** Minutes; **h:** Hours.

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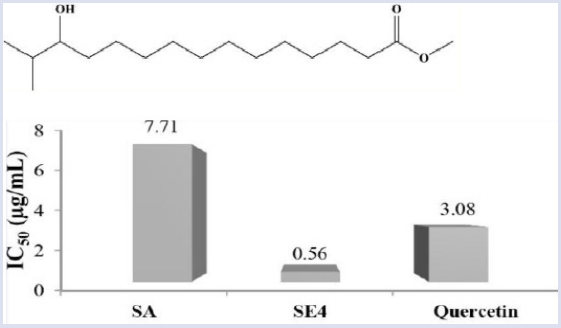
GRAPHICAL ABSTRACT



SUMMARY

- The environment is highly polluted by free radicals which may contribute to the aging process of tissue and cause chronic diseases. Madang gatal (*Schima wallichii*) Choisy is one of typical Indonesian plants that used traditionally as insomnia, hypertension drug and potentially as an antioxidant. The aims of the research were to study the phytochemical constituents and free radical scavenging activity of *S. wallichii* stem bark. The extracts of *S. wallichii* stem bark were very active as free radical scavenger where the isolate of the ethyl acetate extract named SE4 made up predominantly of pentadecanoic acid, 14-methyl-, 13-hydroxy methyl ester had antioxidant activity about 5.5 stronger than quercetin used as standard antioxidant.

ABOUT AUTHORS

Galuh Widiyarti obtained her Ph.D. degree in 2017, from Department of Chemistry Universitas Indonesia, Depok, Indonesia, on the Synthesis of Citronellol and Geraniol Esters and *in silico* Cytotoxic Activity Test Against Murine Leukemia and *in vitro* Against Murine Leukemia P388 and breast cancer MCF7 Cells, after an M.SC. degree in natural product chemistry from Department of Chemistry Universitas Indonesia and bachelor degree in chemical engineering from Universitas Diponegoro, Semarang Indonesia. Currently, she is is a researcher at Research Center for Chemistry (RCChem) – Indonesian Institute of Sciences (LIPI) where she has broadened her areas of expertise to develop a research in the field of natural product and its derivatives for antioxidant and anticancer agent candidates. She is also the author or coauthor of some publications in national and international recognized journals and some presentations in international conferences.

Winda Fitrianingsih obtained her bachelor degree at Departement of Chemistry, Universitas Jendral Soedirman with research entitled "Isolation, identification and antioxidant activity test of secondary metabolite of *S.wallichii* stem bark" under supervision of Dr. Galuh Widiyarti, Dr. Suwandri and M. Chasani, M.SC.