

ISOLATION AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF PHENOLIC CONSTITUENT OF THE *Garcinia silyguifolia***ISOLASI DAN EVALUASI AKTIVITAS ANTIOKSIDAN SENYAWA FENOLIK DARI *Garcinia silyguifolia*****Muharni^{1*}, Elfita¹, Didi Pratama¹**¹Department of Chemistry, Faculty of Mathematics and Natural Sciences
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Received March 20, 2017; **Accepted** May 26, 2017; **Available online** May 30, 2017**ABSTRACT**

Garcinia silyguifolia is a native plant to the South Sulawesi region popularly known as sula and has been cultivated in several regions in Indonesia. The plant by local community use as a food and source of wood, but has not found information on chemical content and biological activity. Therefore, this study was carried out to Isolation and evaluation of the antioxidant activity of the phenolic constituent of the *G. silyguifolia*. A 30.0 g portion of ethyl acetate extract of the stem bark *G. silyguifolia* were separated by column chromatography method using silica G 60 F₂₅₄ (230-400 mesh), eluted gradient polarity mixtures of *n*-hexane-ethyl acetate were collected and sorted into fractions. Fraction F 1 (5.2 g) were separated and purified again by chromatography method until pure compound obtained. The structure of the isolated compound was determined using UV, IR, and NMR spectroscopy. The antioxidant activity was tested by DPPH method. The isolated pure compound was a yellow solid with melting point 148-149 °C. Base on spectroscopy data and by comparison with data from the literature, isolated compound is a known compound 2,4-dihydroxyphenylethanone. The compound exhibited antioxidant activity with IC₅₀ 96 µg/mL against DPPH.

Keywords: Antioxidant, dihydroxyphenylethanone, *Garcinia silyguifolia*, phenolic.**ABSTRAK**

Garcinia silyguifolia merupakan tumbuhan yang berasal dari daerah Sulawesi Selatan dikenal dengan nama sula, dan telah dibudidayakan di beberapa daerah diseluruh Indonesia. Tanaman ini oleh masyarakat lokal dimanfaatkan sebagai bahan makanan dan sumber kayu, namun belum ditemukan informasi kandungan kimia dan aktivitas biologisnya. Oleh karena itu, dilakukan penelitian untuk mengisolasi dan menentukan aktivitas antioksidan dari senyawa fenol dari *G. silyguifolia*. Sebanyak 30 g ekstrak etil asetat kulit batang *G. silyguifolia* dipisahkan dengan metode kromatografi kolom menggunakan fasa diam silika G 60 F₂₅₄ (230-400 mesh) dengan eluen kepolaran meningkat campuran *n*-heksana-etil asetat dan diperoleh fraksi-fraksi. Fraksi F1 (5,2 g) kembali dipisahkan dan dimurnikan dengan metode kromatografi sampai diperoleh senyawa murni. Struktur senyawa murni hasil isolasi ditentukan menggunakan data spektroskopi UV, IR, dan NMR. Aktivitas antioksidan ditentukan dengan metode DPPH. Senyawa murni hasil isolasi berupa padatan kuning dengan titik leleh 148-149 °C. Berdasarkan analisa data spektroskopi dan dibandingkan dengan data pada literatur senyawa hasil isolasi adalah senyawa yang sudah dikenal yaitu 2,4-dihidroksifeniletanon. Senyawa ini dengan metode DPPH menunjukkan aktivitas antioksidan dengan IC₅₀ 96 µg/mL

Kata kunci: Antioksidan, dihidroksifeniletanon, *Garcinia silyguifolia*, fenolik.**INTRODUCTION**

The genus *Garcinia* belonging to the family Guttiferae also called Clusiaceae with the most numerous genus and commonly as *Rhedia*. Many drug compounds were found from the genus of *Garcinia* with more than 1000 species widely distributed in tropical Asia, Africa, New Caledonia, Polynesia and Brazil (Piccinelli *et al.*, 2005). In Brazilian Several species of the Guttiferae family are used in traditional medicine to cure

various ailments such as for treating cancer, hypertency and diabetes. In Thailand, Sri Lanka, Malaysia, the Philippines and India, parts used in traditional medicine ripe fruits are used to treat abdominal pain, diarrhea, dysentery, wound infections, suppuration and chronic ulcer (Cui *et al.*, 2010).

Several members of the *Garcinia* genus has been used in Indonesia traditional medicine for indigestion, treat abdominal pain, anti-dandruff, cough, Stomachic,

digestive, and skin diseases. Base on literature study has been reported that genus *Garcinia* rich sources of xanthenes (Wu *et al.*, 2013), flavonoids (A. Shukla, R. Shukla, Vikas, Dilip, & Jain, 2014), benzophenone (Jantan and Saputri, 2012), and phenolic acid (He Zhong *et al.*, 2010). This class of compounds having phenolic functional groups and ring three cyclic linear, so it has the biological and pharmacological activity of diverse. The groups of these compound gives rise to several interesting pharmacological properties were these constituents have been reported to several biological activities such as antibacterial activity (Dharmaratne, Yoshikazu, Piyasena, & Thevanesam, 2013), anti-inflammatory (Castardo *et al.*, 2008; Santa *et al.*, 2011), antioxidant (Gantijo *et al.*, 2011), antitumour (Coelho *et al.*, 2008) therapies, and anticancer activity (Sales *et al.*, 2014).

Garcinia silygifolia known Indonesia name Sula is a small tree with prop roots and that endemic to south Sulawesi. The plant is obtained from the wild for local use as a food and source of wood, In Indonesia cultivated as a plant for vegetable. The root of this has been reported to Preservative, and fruit can to eat as pickle (Heyne, 1987). Base on literature study cannot provide information that chemical compound and biological activity. Preliminary test from ethyl acetate extract stem bark of *Garcinia silygifolia* showed contained phenolic compounds. Phenolic compounds usually showed active as the antioxidant (M. Maria, N. Susana, D. Susana, Ana, & A. Maria, 2014). In previous paper have been reported one compound polyprenylated benzophenone of the stem bark *Garcinia silygifolia* (Muharni, Elfita & Bastian, 2014).

In this paper will be reported the identification and the evaluation of the antioxidant activities of the main phenolic constituent of the stem bark *G. silygifolia*. Isolation begins with maceration extraction method was used to get the corresponding extracts of n-hexane, ethyl acetate, and methanol. Therefore, ethyl acetate fraction was subjected to further separation by column chromatographic methods to obtain the pure compound. The pure compound were analyzed by UV, IR, and ¹H NMR dan ¹³C NMR and 2D NMR method. The antioxidant activities assay of the pure compound using

DPPH method using ascorbic acid as standard.

EXPERIMENTAL SECTION

Materials

G. silygifolia stem bark was collected from the Hutan Raya Bogor, South Java in Mei 2013. The botanical identification of the samples was confirmed by Dr. Laila Hanum. A voucher specimen (number UNB 112) was deposited at the Herbarium Departement Biology Sriwijaya University

Material for isolation: silica gel 60 (70 - 230 mesh.), thin layer chromatography (TLC) using Merck (Art.5554) silica gel 60 F₂₅₄ (230-400), silica gel G 60 (70-230), methanol, n-hexane, ethyl acetate, dichloromethane, acetone. The organic solvents were used from distilled technical grade. Reagent for antioxidant activity: methanol p.a, DMSO (dimethyl sulfoxide), DPPH (1,1-diphenyl-2-picrylhydrazyl), and ascorbic acid.

Procedures

The extracts and fractions were concentrated using a rotary evaporator R-114 Buchi with vacuum system Buchi B-169 under reduced pressure at 45^o C. The ethyl acetate extract was purified by column chromatography (CC), using silica gel G 60 (70 -230 mesh) Merck as the stationary phase, eluted with increasing polarity mixtures of n-hexane-ethyl acetate and ethyl acetate-methanol. The substances were stained with 1% FeCl₃ in ethanol reagent and visualized using ultra-violet CAMAG 254 nm, radiation ($\lambda = 254$ and 366 nm). Melting point is measured by Fisher Jhon melting point apparatus, UV spectrum was obtained with spectrophotometer ultraviolet Beck DU-7500. The IR spectrum was measured by FTIR-Perkin Elmer-Spectrum One) using KBr pellets. The ¹H and ¹³C NMR spectra measured on 500 MHz (¹H) and 125 MHz (¹³C) on JEOL JNM ECA-500 spectrometer. The HMQC and HMBC contour maps were collected on a 500 MHz Varian. The *invitro* antioxidant activity experiments were monitored by UV-visible spectrophotometry using a single beam merk Shimadzu-UV mini 1240 instrument. The radical scavenging experiment was observed at $\lambda = 517$ nm.

Preparation of extracts

The stem bark of *Garcinia silygifolia* was air-dried at 30^oC for 14 days with

continuous moisture monitoring. After the material was completely dry, it was pulverized in a knife grinder, producing 1000 g of ground sample. The dried, ground stem bark was subjected to exhaustive extraction in maceration apparatus using an increasing polarity solvent system, with n-hexane, ethyl acetate, and methanol as solvents for 24 h each. The extracts were then concentrated under reduced pressure, yielding 5.37 g of n-hexane extract, 40.41 g ethyl acetate, and 25.56 g of methanol.

Purification and isolation of chemical constituent

Ethyl acetate extract (30 g) was separated by column chromatography using silica G 60 F₂₅₄ (230-400 mesh, eluted with step gradient polarity mixtures of n-hexane-ethyl acetate (1:0 → 0:1) were collected, analyzed by TLC and combined into five groups [F1 (5.2 g), F2 (1.7 g), F3 (3.9 g), F4 (4.2 g) and F5 (7.24 g)]. The 1st fraction (5,2 g) was further purified by rechromatographed using silica gel 60 (70-230 mesh), eluted with gradient polarity mixtures of n-hexane-ethyl acetate (10:0, 8:2, 6:4, 4:6, 2:8 and 0:10), and methanol. Base on TLC analysis to obtained five fractions F_{1.1} (0.4 g), F_{1.2} (0.8 g), F_{1.3} (1.3 g), F_{1.4} (0.9 g) and F_{1.5} (1.4 g). Furthermore, fraction 3rd (F_{1.3}) was rechromatographed using the same method (silica gel, eluted with gradient polarity mixtures n-hexane-ethyl acetate (5:5 → 3:7) to yield three fraction F_{1.3.1} (200 mg), F_{1.3.2} (80 mg) and F_{1.3.3} (600 mg). Fractions 1.3.2 from group 1 were completely pure, compound (50 mg) obtained yellow solid.

The Purity Test

The purity test of isolated compounds by TLC analysis using different types of eluent and measuring the melting point. Otherwise pure compound when the analysis by TLC with various eluent still give single spot and give the melting point with a small range (≤ 2).

Characterisation

The structure of the isolated compound was elucidated using UV, IR, ¹H and ¹³C NMR, and NMR 2D HMQC, and HMBC spectroscopy and by comparison with data from the literature.

Evaluation of antioxidant activity

DPPH radical-scavenging activity the isolated compound in the evaluation according to the method of Gulcin *et al.*, (2005). A concentration series (7.81;15.62; 31.25; 62.5; 125; 25; and 500 μ g/mL in DMSO) was prepared. A 0.2 mL aliquot the sample solution was mixed with 3.8 mL of DPPH (0.5 mM in methanol) where 1.98 mg DPPH was placed on a 100 mL volumetric flask then add methanol until the volume 100 mL. This mixture was vigorously shaken at room temperature for 30 min. The absorbance of the mixture was then measured by UV-Vis spectrophotometer at λ_{maks} 517 nm (Gulcin *et al.*,2005). The most effective radical scavenging activity was expressed with a low absorbance value. The isolated compound solution was analyzed in triplicate, and the average values were plotted to obtain the IC₅₀ against DPPH by linear regression. The activity antioxidant of ascorbic acid, a recognized was used as a standard over the same range of concentrations. Determination of radical-scavenging activity declared as the percentage of inhibition according to the following equation: % inhibition = [(absorbance of control - absorbance the sample)/absorbance of control]x100.

RESULTS AND DISCUSSION

A compound was isolated from the ethyl acetate extract of stem bark *G. silyguifolia* by chromatography technique. This type of chromatography is employed to separate semi-polar substances, which may be lost or obtained in low yield when separation by other chromatography techniques, due to irreversible adsorption use usual stationary phases such as silica. The isolated compound was as a yellow solid (50 mg). The purity test the isolated compound based on the analysis of TLC with different eluent n-hexane- ethyl acetate 5:5 and 3:7 showed a single spot with R_f values of 0.25 and 0.63 on the UV lamp at λ 366 nm (**Figure 1**).

The purity test of isolated compounds were also conducted with measurement of the melting point. The measurement result obtained melting point isolated compound is 148-149 °C, the narrow melting point range (≤ 2 °C) indicated the compound was pure.

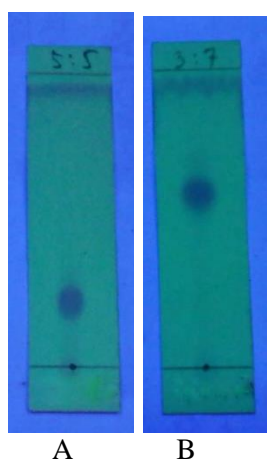


Figure 1. Performance TLC isolated compound, eluent: n-hexane-ethyl acetate 5:5 (A) and 3:7 (B)

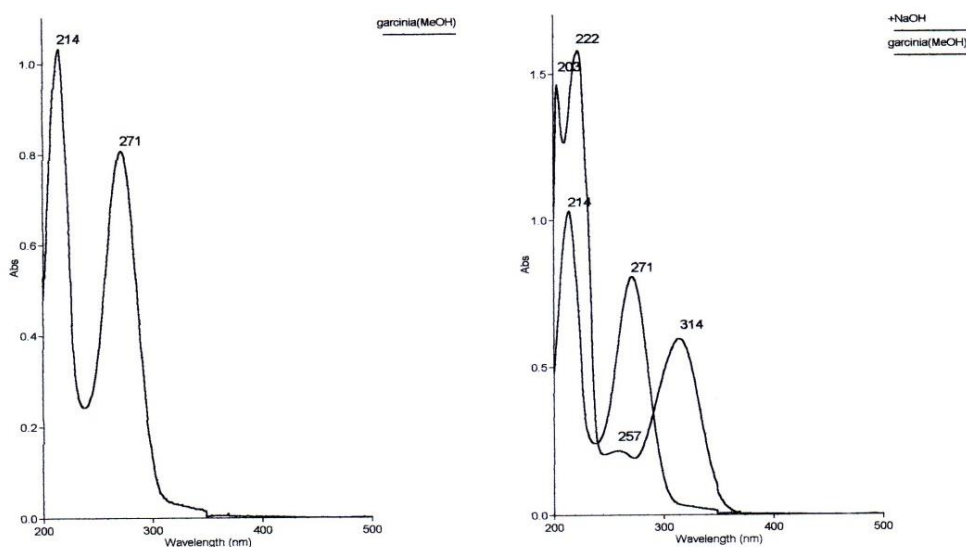


Figure 2. UV spectrum of 2,5-dihydroxyphenylethanone

The compound was identified as 2,4-dihydroxyphenylethanone based on spectroscopic data UV, IR, ^1H and ^{13}C NMR, and NMR 2D HMQC, and HMBC spectroscopy and by comparison with data from the literature. The UV spectrum in MeOH (**Figure 2**) displayed absorptions at λ_{max} at 214 nm and 271 nm for the pure compound. Adding NaOH produced absorptions at 222 nm and 314 nm. The bathochromic shift in also of NaOH showed wavelength shift there λ_{max} 214 nm to 222 nm and 271 nm to 314 nm. The shifts in the UV spectra produced by adding NaOH indicated the presence of phenol hydroxyl groups.

The IR spectrum (**Figure 3**) displayed absorption bands, λ_{max} at 3405 (OH phenol), 3082 (C-H aromatic), 2927-2860 cm^{-1} (C-H aliphatic), 1618, 1562, and 1456 (C=C aromatic), 1238 (C-O alcohol), 1651 (C=O carbonyl), and 836 cm^{-1} (C-H). Absorption of carbonyl groups usually occurs in the area

around 1700, but if there is conjugation with a double bond will decrease the character of the double bond so that carbonyl absorption will occur at a lower wave numbers. Base on literature absorption at λ_{max} at 1651 as C=O carbonyl conjugated with C=C (aromatic ring). The molecular structure of isolated compound, was determined by comparative analysis of the ^1H NMR, ^{13}C NMR HMQC and HMBC spectra the compound. The signals at δ_{H} 6.32 ppm (1H; *d*; $J=5\text{Hz}$) and δ_{H} 7.68 ppm (1H; *d*; $J=5\text{Hz}$) (**Table 1**) in the ^1H NMR spectrum (**Figure 4**) base on coupling constants indicated the presence of aromatic hydrogen groups as orto coupling (Creswell, Runquist, & Campbell, 1982). The signal at δ_{H} 7.74 ppm (1H; *s*) that aromatic proton have not splitting proton. ^1H NMR spectral data showed only three signals for the aromatic protons, so that predictable isolated compound is aromatic in the form of three substitutions aromatic.

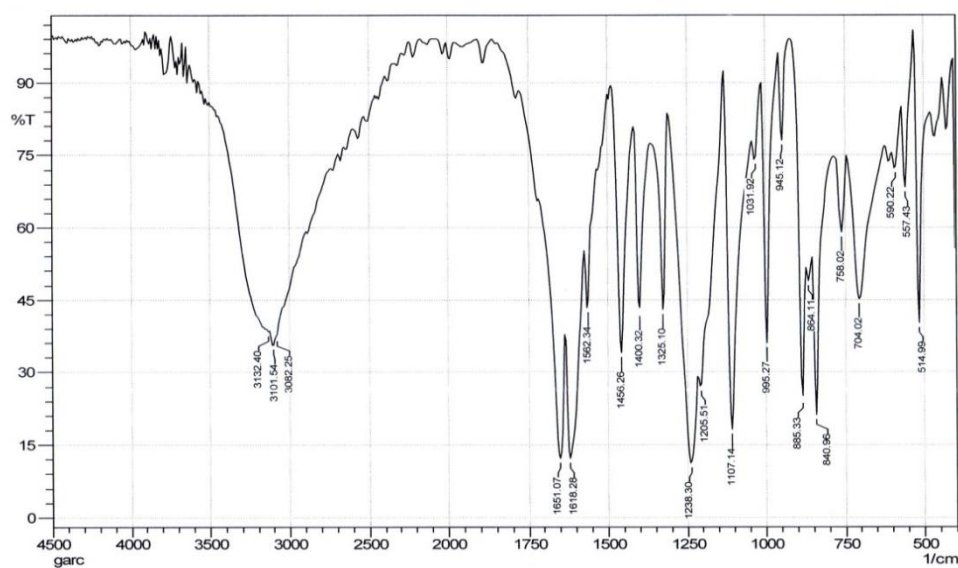


Figure 3. IR spectrum of 2,5-dihydroxyphenylethanone

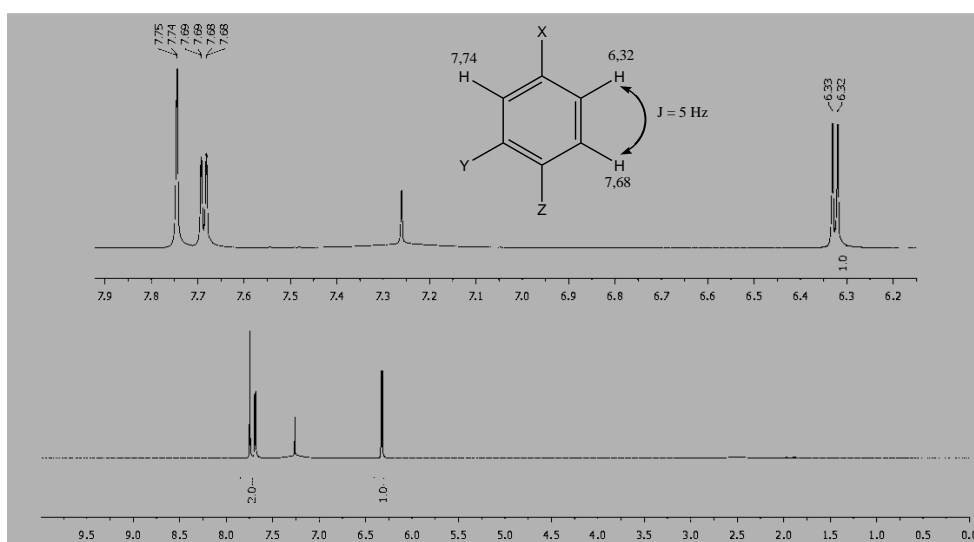


Figure 4. ^1H NMR spectrum of 2,5-dihydroxyphenylethanone

The ^{13}C -NMR spectrum (**Figure 5**) of compound (**Table 1**) showed just eight signal carbon, seven carbon for C sp^2 at δ_{C} 113.6, 138.8, 138.9, 146.6, 155.3, 173.5, and 207.0 ppm and one signal C sp^3 at δ_{C} 30.0 ppm. ^{13}C NMR spectrum showed characteristic signals for a group of aromatic compounds, which are found only one signal on the chemical shift values below 100 ppm. This is also reinforced by the ^1H NMR data. The signal at δ_{C} 207.0 characteristic fo C carbonyl at ketone in the form of cyclo. Base on literature study has been reported that genus *Garcinia* rich sources of xanthenes (Wu *et al.*, 2013), flavonoids (A. Shukla, R, Shukla, Vikas, Dilip, & Jain, 2014), and benzophenone (Jantan and Saputri, 2012).

The group of these compounds has a basic structure with a carbon number of at least 13 signals, while the isolated compound has only eight signals carbon atoms. ^{13}C NMR spectral data indicated isolated compound were not xanthenone, benzophenone or flavonoid groups. NMR 2D analysis for HMQC spectrum (**Figure 6**) showing the proton at δ_{H} 6.32 ppm correlation to carbon at δ_{H} 113.6 ppm and proton at δ_{H} 7.68 ppm correlation to carbon at δ_{C} 155.3 ppm. The proton at δ_{H} 6.32 ppm the appearance of a long-range ($^2J_{\text{CH}}$) heteronuclear correlation with to carbon at δ_{C} 146.6 and 155.3 ppm in the HMBC spectrum (**Figure 7**) confirmed that it was linked to carbon that position of between 146.6 and 155.3 ppm carbon.

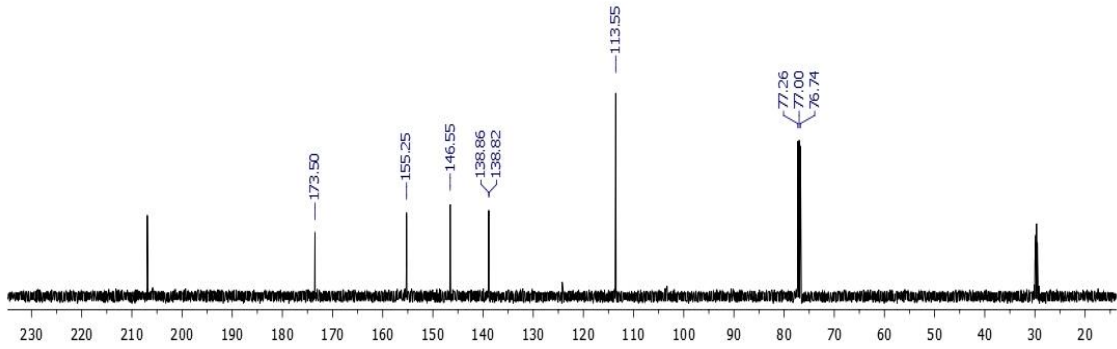


Figure 5. ¹³C NMR spectrum of 2,5-dihydroxyphenylethanone

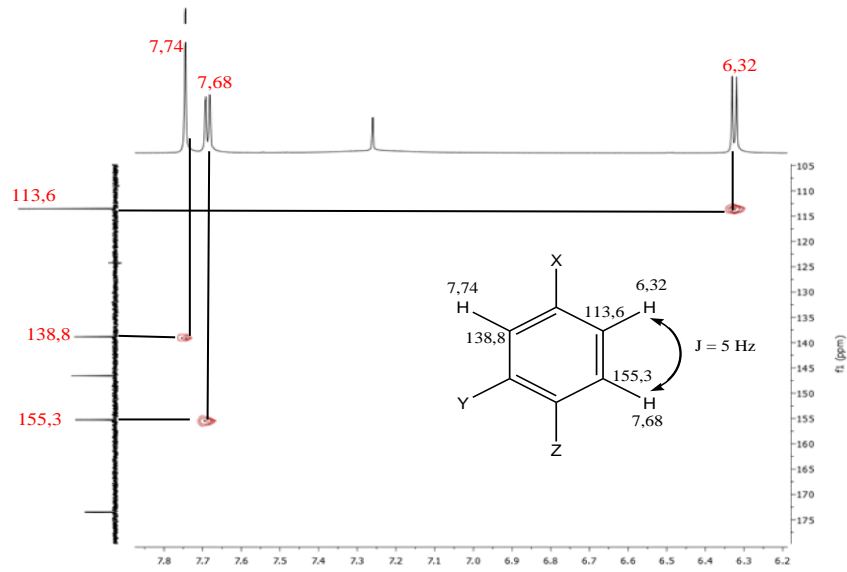


Figure 6. HMQC Spectrum of 2,5-dihydroxyphenylethanone

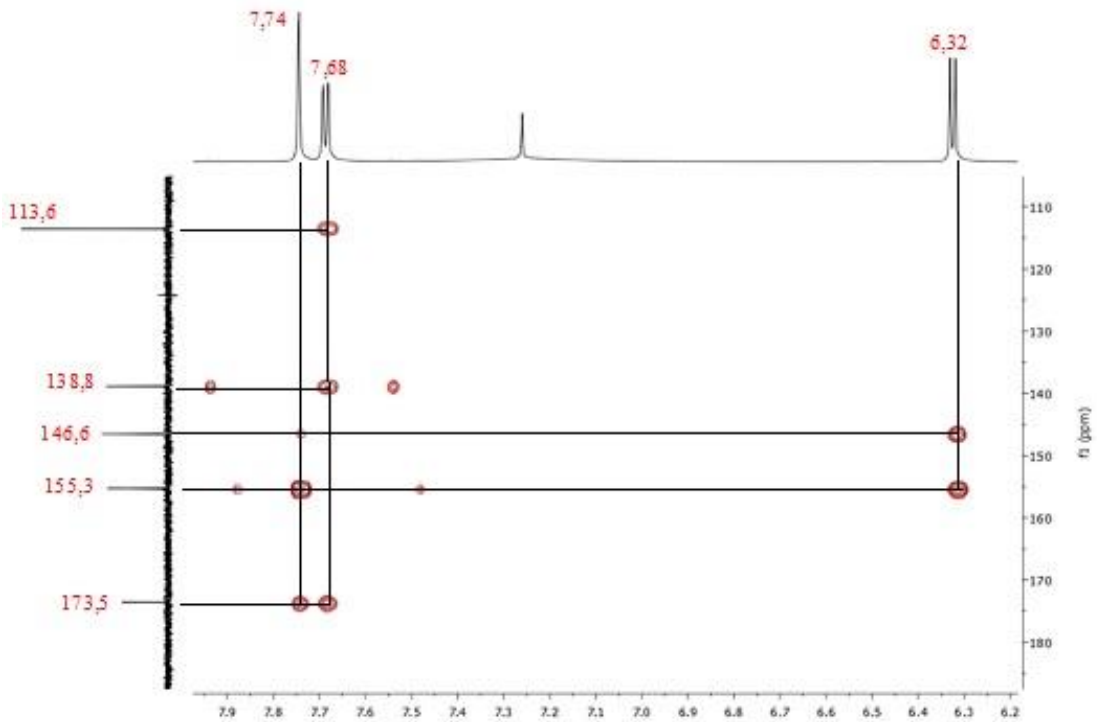


Figure 7. HMBC spectrum of 2,5-dihydroxyphenylethanone

The proton at δ_H 7.68 ppm showed of a long-range ($^2J_{CH}$) heteronuclear correlation with to carbon at δ_C 113.6 ppm and δ_C 178.5 ppm in HMBC spectrum and ($^3J_{CH}$) to carbon δ_C 138.8 ppm. Base on This signal could be assigned to the hydroxyl group at δ_C 178.5 ppm but no correlation was observed between the protons hydroxyl with carbon at δ_C 155.3 or 138.8 ppm in the HMBC spectrum (**Table 1, Figure 7**). The proton at δ_H 7.74 ppm the appearance of correlation heteronuclear correlation with to carbon at δ_C 138.8 in HMQC spectrum and the appearance of a long-range ($^2J_{CH}$) heteronuclear correlation with to carbon at δ_C 138.9 and 173.5 ppm (**Figure 8**). The complete ^{13}C and 1H assignments (**Table 1**), we determined that the structure the isolated compound was phenolic that 2,5-dihydroxyphenylethanone with molecular formula $C_8H_8O_3$ with structure showed in **Figure 8**. This proposal is also strengthened by the similarity of spectroscopic data for 2,5-dihydroxyphenyl-ethanone in the literature (K. Trivedi, Tallapragada, Branton, D. Trivedi, & Nayak, 2015).

2,5-dihydroxyphenylethanone known by other names 2',5'-dihydroxy acetophenone and used cosmetic agents. Isolated compound base on Dictionary Natural Products data base 2,5-dihydroxyphenylethanone was not new compound which has been reported previously from the Chinese medicinal plant *Cynanchum auriculatum* Royle ex Wight (Sun, Liu, Wang, & Zhu, 2009), and from the steamed root of *Rehmannia glutinosa* Libosch and evaluated the effects of 2,5-dihydroxyacetophenone (DHAP) on inflammatory responses in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophages (Han *et al.*, 2012) but it was new for *Garcinia silygifolia*. Exploration of secondary metabolites needs to be done in order to get the profile of organic compounds produced by *Garcinia silygifolia*. Base on literature study 2,5-dihydroxyacetophenone have been applied at sublimation provides efficient ionization of lipid species (Hayasaka, Naoko, Noritaka, Koji, & Mitsutoshi, 2014).

Table 1. 1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz), and HMBC spectral data of of 2,5-dihydroxyphenylethanone recorded in CD_3OD .

No. C	δ_C ppm	δ_H ppm (ΣH ; multiplicity; J (Hz)	HMBC
1	138.9		
2	146.6		
3	113.6	6.32 (1H, <i>d</i> , $J = 5$ Hz)	146.6; 155.3
4	155.3	7.68 (1H, <i>d</i> , $J = 5$ Hz)	113.6; 173.5; 138.8
5	173.5		
6	138.8	7.74 (1H, <i>s</i>)	146.5; 155.3; 173.5
7	207.0		
8	3.,0		

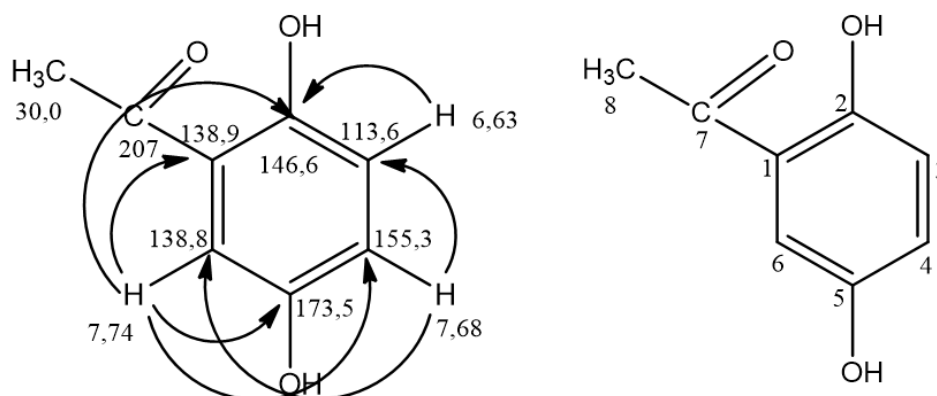


Figure 8. HMBC correlation and structure 2,5-dihydroxy-phenylethanone

Antioxidant activity

The evaluation antioxidant activity of this compound used DPPH method base on absorption DPPH at λ_{maks} 517 nm. Antioxidant reacts with 1,1-diphenyl-2-picrylhydrazyl, which is a stable free radical and converts it to 1,1-diphenyl-2-picrylhydrazine. The degree of discolouration indicates the scavenging potentials of the antioxidant compound (Meryem *et al.*, 2016). Determination of antioxidant activity by DPPH method is a classical and simple method based on the reduction of DPPH (reduction of purple) to hydrazine (yellow) (Gülçin, Alici, & Cesur, 2005). The reaction can be monitored spectrophotometrically by following the decrease in absorbance at 517 nm. The ability of a compound to reducing DPPH radical at a certain concentration, calculated by percent inhibition (% I). Standart antioxidant compound used ascorbic acid.

The activity of the compound is attributed to their hydrogen donating ability (Sravani and Padmaa, 2012). Antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming the stable end product. The effectiveness of DPPH radical reduction determined by calculating the IC_{50} value through linear regression calculation, the concentration of test compound which can reduce 50% of DPPH radical. Based on the regression ascorbic acid were used as standards antioxidant produced IC_{50} values of at 6.0571 $\mu\text{g/mL}$ against DPPH, and isolated compound showed IC_{50} values of at 96 $\mu\text{g/mL}$. According to Selvi, Joseph, and Jayaprakasha (2003) categorized as a pure compound has potent antioxidant activity when it has a value of $IC_{50} < 10 \mu\text{g/mL}$. IC_{50} 10-100 $\mu\text{g/mL}$ categorized as active antioxidants and when have $IC_{50} > 100 \mu\text{g/mL}$ categorized as inactive. Based on this statement, the isolated compound considered active antioxidants but its activity under ascorbic acid

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids (Maria *et al.*, 2014). Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom. Caffeic acid has been found to have high

activity comparable to that of the flavonoid, quercetin. Flavonoid constituents found in plants are commonly are flavonol aglycones such as quercetin, myricetin, kaempferol, and their glycosides (Maria *et al.*, 2014). Generally, flavonoid group that has several hydroxyl groups have higher antioxidant activity compared with the phenolic acids. However, the flavonoid glycosides (rutin, naringin, and hesperidin) usually have low antioxidant value. The antioxidant activities of flavonoids increased with the number of hydroxyl groups substituted on the B-ring, especially at C-3, and a single hydroxy substituent generates little or no additional antioxidant capacity (Rajalakshmi and Narasimham, 1996)

The high antioxidant activity of the phenolic group is often associated with the presence OH groups, which are potent H donors because electron delocalisation across the molecule efficiently stabilizes the resulting phenoxy radicals, which can be observed for the isolated compound. Another important feature the isolated compound is the planarity of the molecule, which permits conjugation and electron delocalisation. These factors are associated with an increase in radical stability. Intramolecular hydrogen bond between neighbouring –OH and C=O groups increase aromatic-ring conjugation and raise the H donation power of the molecule, because the resulting radicals are more delocalised (Jindai and Russell., 2010) The presence of a dihydroxy group ortho to the C=O moiety also increases antioxidant activity. Based on literature study low antioxidant activity of the isolated compound because has no ortho hydroxyl group and have not Intramolecular H-bonds between neighbouring –OH and C=O groups.

CONCLUSION

One compound has been isolated from ethyl acetate extract of the stem bark *G. sizygiifolia* of pure compound in the form of yellow solid (50 mg) with a melting point 148-149°C. Based on the analysis of spectroscopic data suggested a group isolated compounds was 2,5-dihydroxyphenylethanone with molecular formula $C_8H_8O_3$. Pure isolated compound showed antioxidant activity with IC_{50} 96 $\mu\text{g/mL}$. 2,5-dihydroxyphenylethanone was not new

compound, but it was new for *Garcinia silybiifolia*.

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