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ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT FROM INDONESIAN *Curcuma heyneana* RHIZOME

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ABSTRACT

Objectives: Temu giring (*Curcuma heyneana*) is widely used as herbal medicinal plant to treat stomach aches and skin diseases. The objectives of this study are to report the chemical structure and antioxidant activity of the polar fraction of this particular species. **Material and Methods**: The crude methanol extract was fractionated by vacuum liquid chromatography with gradient elution to produce 10 fractions. Fraction F and H were separated by various chromatography technique giving F121 and H13. **Results**: Identification of F121 and H13 with liquid chromatography–mass spectrophotometry (LC-MS) showed 3 compounds in F121 and 2 compounds in H13. The compounds were identified as curcumanolide A, (*R*)-ar-turmerone, (1(10)-*E*,4β,5α,8β)-4,5-epoxy-1(10),11(13)-germacradien-12,8-olide, Dihydrosuberenol, and demethoxycurcumin. Dihydrosuberenol had been reported in this species, while the other compounds had been found in the same genus. The result of the antioxidant activity toward 1,1-diphenyl-2-picrylhydrazil (DPPH) showed moderate activity of H13 and its crude methanol extract with IC_{50} 59.18 and 82.16 µg/mL, respectively, while the F121 showed a low antioxidant activity with IC_{50} value > 100 µg/mL. **Conclusions**: analysis of Indonesian *C. heyneana* rhizomes gave sesquiterpenes of germacrane, cyclic sesquiterpenoid types, and coumarine compounds and have potency as antioxidant activity.

KEYWORD: *Curcuma heyneana*, germacrane, cyclic sesquiterpenoid, coumarine, antioxidant activity, IC_{50}.

INTRODUCTION

Zingiberaceae is one of the most important herbaceous species that can be found in tropical forests. This family comprises of about 52 genera with 1500 species in the world. Plants in the family Zingiberaceae are considered as important sources of food, spices, medicines, dyes, perfumes, and cosmetics. The genus *Curcuma* belongs to the Zingiberaceae family and comprises about 80 species of rhizomatous herbs which mainly occur in the tropical regions of Asia, Australia, and South America. Most of the species can be found in the Malaysian region, which includes Malaysia, Indonesia, the Philippines, and Papua New Guinea. The rhizomes of a few Curcuma species are widely used in indigenous medicine due to their pharmacological properties which have been identified as antimicrobial[1], anticancer[2,3], antidiarrheal[4], anti-inflammatory[5, 6] and anti-oxidant.[6]

Curcuma heyneana is one of the zingiberaceous plants indigenous to Java Island, Indonesia. The rhizome of this plant, which is called 'Temu Giring' in Javanese, is of wide medicinal value in Indonesia, and is considered to be useful for the treatment of skin diseases, abrasions, and injuries. It is not only found commonly as one of the main ingredients in traditional Indonesian mixed herbal medicines ("jamu"), but is also widely used in the form of a juice prepared from fresh rhizome as an anthelminthic against intestinal worms. *C. heyneana* (Zingiberaceae) has also been used as anti-gastropathic, and hepatoprotective agent and as a menstruation promoter.[7]

More recently, analysis of the rhizomes oil of *C. heyneana* gave completely different constituents, mainly sesquiterpenes, i.e. β-pinene, γ-terpinene, guaiazulene, α-copaene, δ-elemene, 2-undecanone, and carvone.[8] Another research, phytochemical investigations on *C. heyneana* have resulted in the isolation of various types of sesquiterpenoids, such as, germacrone, dehydrocurdione, isocurcumenol, curcumenol, curcumanolides A and B, zerumbone, and oxycurcumenol[9], which have been reported to exhibit anti-inflammatory, anti-cancer, and Ca^{2+} channel blocker-like activities.[10-13] Regarding the chemical constituents, sesquiterpenes of germacrane, guaiane, and humulane types as well as labdane-type diterpenes have been reported.[14] However, information on these chemical constituents remains limited, and antioxidant

activity constituents have not been reported.

An amount of evidence showed that free radical-mediated damage plays an important role in several human diseases such as cancer and cardiovascular diseases. Free radical which continuously generated during normal metabolism eventually triggers the onset of degenerative diseases. Exogenous antioxidants derived from dietary components sometimes required preventing this condition. The study about isolation and activity testing of plant-origin antioxidants was significantly increased in recent years. The DPPH (1,1-diphenyl-2-picrylhydrazyl) is general bioassays for pharmacological activities of active phytochemical components of medicinal plants. The DPPH test is a well-established assay for the *In vitro* determination of antioxidant activity in medicinal plant extracts.[15] To the best of our knowledge, there is very little information available regarding to antioxidant activity of *C. heyneana* rhizome. We were thus encouraged to investigate and evaluated for antioxidant activity of the *C. heyneana* rhizome.

MATERIAL AND METHODS

Plant Materials

The rhizome of C. ***heyneana*** was collected from Biopharmaca garden, Bogor, Indonesia in November 2016.

General Experimental Procedures

LC-MS analysis was performed using LC-MS Xevo G2-S QTOF with time of flight (TOF) as mass analyses on low power at 4 V, and high power at 25-70 V in duration time 23 minutes and was used Quardupole-time of flight (Q-TOF) as mass analysis. The LC condition: C18 coloum with size was 2.1 m × 15.0 cm × 1.8 µm with static flow rate, 0.2 mL/minutes, and mobile phase: biner eluent consist of water and methanol with gradient system on 0–2 minutes to 95% air water and 5% methanol, isocratic on 2–3 minutes to 75% air dan 25% methanol, gradient system on 14–15 minutes with 100% water, and isocratic on 19–23 minutes with 95% water and 5% methanol. Chromatogram was analyzed by Masslynx V4.1 software and mass spectrum was analyzed by Chemspider, Massbank, and Human Metabolome Database (HMDB) softwares. Vacuum liquid chromatography was performed by using Si 60 G (Merck) for column packed and Si 60 (0.2-0.5 mm) (Merck) for sample adsorbed. Radial chromatography was carried out by using Si 60 PF_{254} containing gypsum (Merck). Si 60 GF_{254} (Merck) was used for preparative TLC. For TLC analysis, pre-coated silica gel plates (Merck Si 60 GF_{254}, 0.25 mm thickness) and $Ce(SO_4)_2.4H_2O$ 1.5% in H_2SO_4 2N as apparition stain reagent were used, ascorbic acid, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Antioxidant activity in scavenging DPPH was calculated using the following Equation 1. The relation between concentration and activity concentration was determined, and IC_{50} was measured using interpolation. Antioxidant activity appears in the value of IC_{50}.

$$\text{Inhibition } (\%) = \frac{(A-B)}{A} \times 100 \quad (1)$$

A: Blank absorbance (ethanol and DPPH)
B: Sample absorbance (fraction and DPPH)

Extraction, fractionation, and purification

Dried powdered C. *heyneana* rhizomes (750.0 g) were exhaustively extracted three times with methanol at room temperature. After filtering and evaporating the solvent, 121.4 g crude extract was yielded. The crude extract (30 g) was then fractionated using vacuum liquid chromatography with *n*-hexane:ethyl acetate as a solvent to obtain ten major fractions (A-J). Fraction F (268.3 mg) was separated by using radial chromatography with *n*-hexane:ethyl acetate as a solvent to obtain 3 major fraction (F1-F3) and purified of F1 by radial chromatography with *n*-hexane:ethyl acetate as a solvent yielding 3 sub-fractions (F11-F13). Then, F12 was purified by radial chromatography and repeated by TLC preparative with *n*-hexane:ethyl acetate:methanol (8:1:1) as a solvent yielding 1 spots dominant F121 78.0 mg and one spot presumably impurity. Fraction H (1.04 g) was separated by coloumn chormatography with dichloromethane:ethyl acetate as a solvent to obtain 4 major fractions (H1-H4). Then, H1 was purified by gravity coloumn chormatography with dichlorometane:ethyl acetate as a solvent to obtain 4 sub fractions (H11-H14), purified of H13 by TLC preparative with dichloromethane:ethyl acetate (5:5) as a solvent yielding one spot dominant 25.0 mg and one spot presumably impurity. Both F121 and H13 fractions were analyzed by LC-MS.

RESULT AND DISCUSSION

The F121 fraction chromatogram yields several peaks from retention time of 7.24-18.00 minutes (Figure 1). The peak at retention time of 18.07 minute was indicated as solvent peak and only 3 peaks of which provide a high abundance of peaks at retention time of 9.58, 10.68 and 11.05 minutes, respectively which can be analyzed by Masslynx V4.1 software. The peak mass spectrum with an 9.58 minute retention time is possible have a $C_{15}H_{23}O_2$ molecular formula with a 100% resemblance to a base peak of 235.17 identified as $[M + H]^+$ (Figure 2).

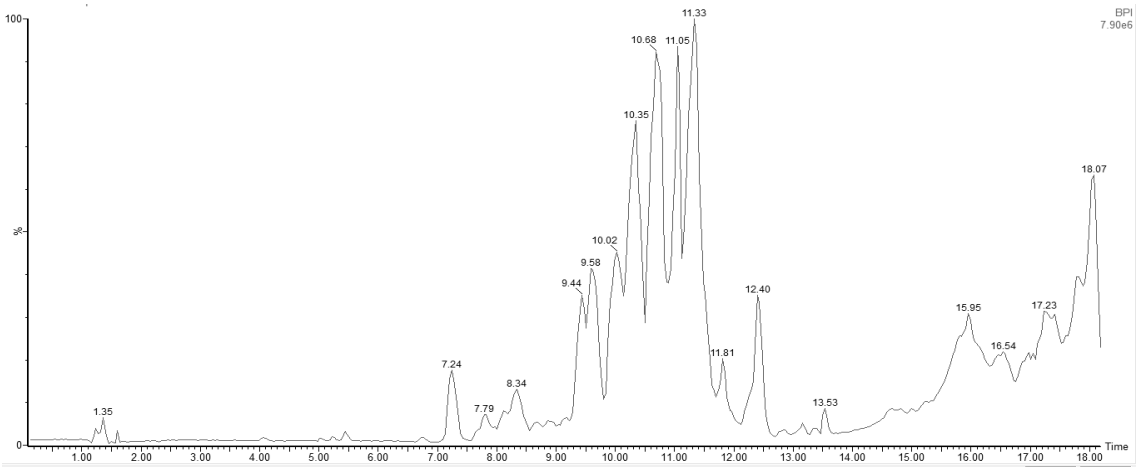


Figure 1: The F121 fraction chromatogram.

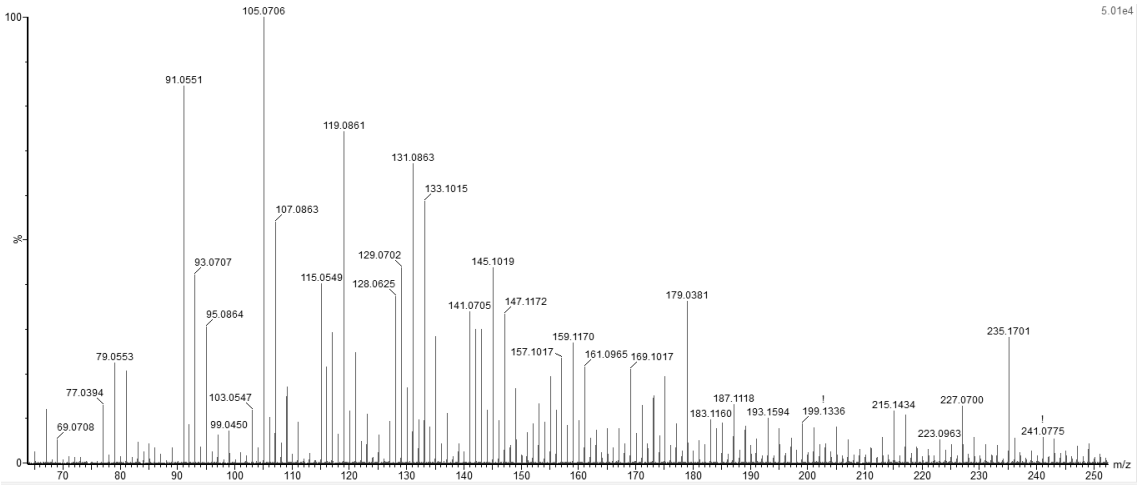


Figure 2: The peak mass spectrum with an 9.58 minute retention time.

Based on search results with Chemspider, Massbank, and Human Metabolome Database (HMDB) softwares, the possibly compound is curcumenol (**1**), curcumenone (**2**), procurcumenol (**3**), zerumbon oxide (**4**), isocurcumenol (**5**), curcumanolide A (**6**), and dehydrocurdion (**7**) (Figure 3). Compounds **1**, **5**, and **6**, had isolated previously on *C. heyneana* from Bandung, West Java[14], compound **3** from C. *heyneana* Jakarta[16], and compound **7** from C. *heyneana* Solo.[17]

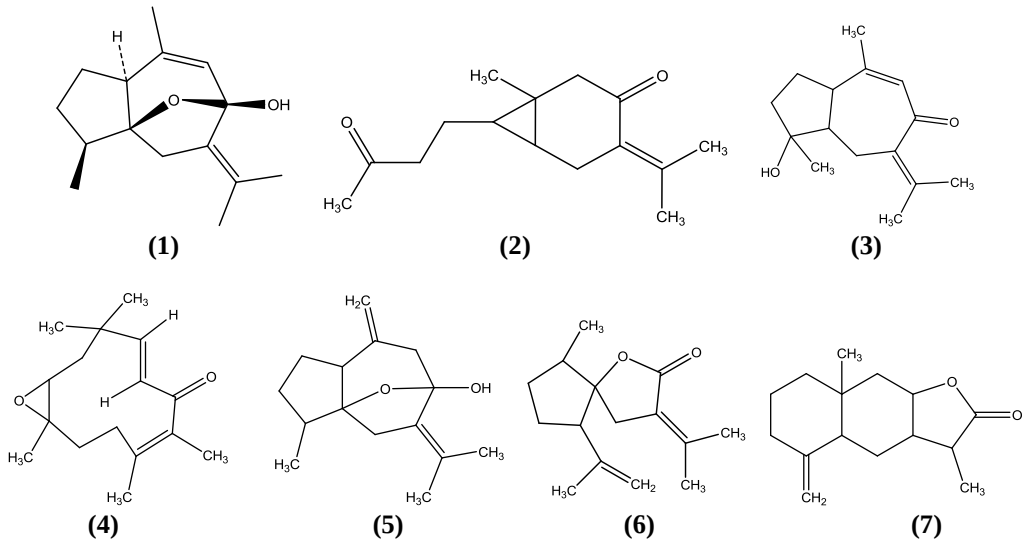


Figure 3: Suitable compounds with an 9.58 minute retention time base on Chemspider, Massbank, and Human Metabolome Database (HMDB) softwares.

The peak mass spectrum with 10.68 minute retention time is possible have a $C_{15}H_{21}O$ molecular formula with a 100% resemblance to a peak base of 217.16 identified as $[M+H]^+$ (Figure 4). Based on search results with Chemspider and PubChem software, the possibly compound is (*R*)-ar-turmerone (**8**) and Isogermafurane (**9**) (Figure 5). The compound **8** belongs to the cyclic sesquiterpenoid group of ketones and is commonly found in C. *Longa*.[18]

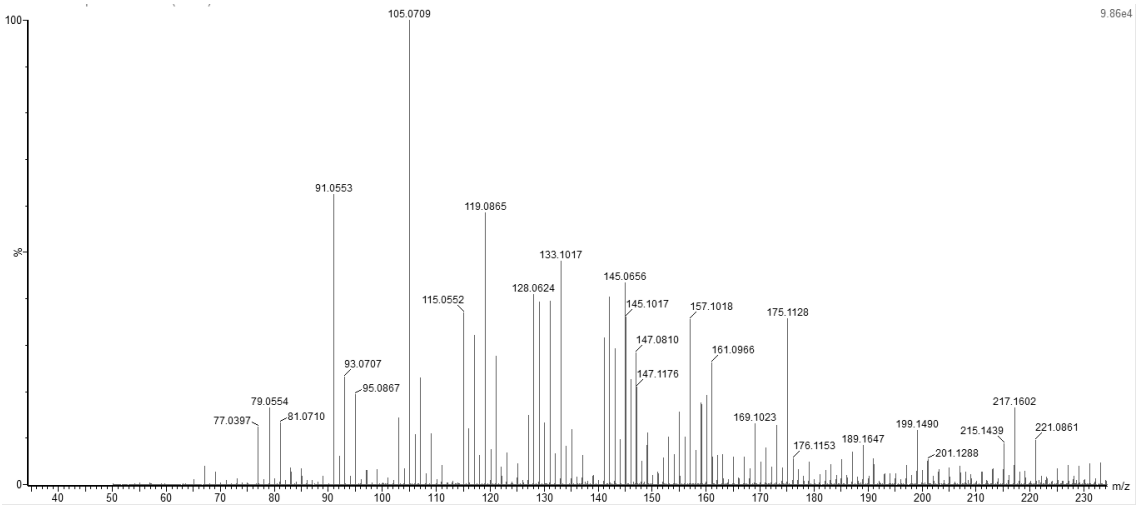
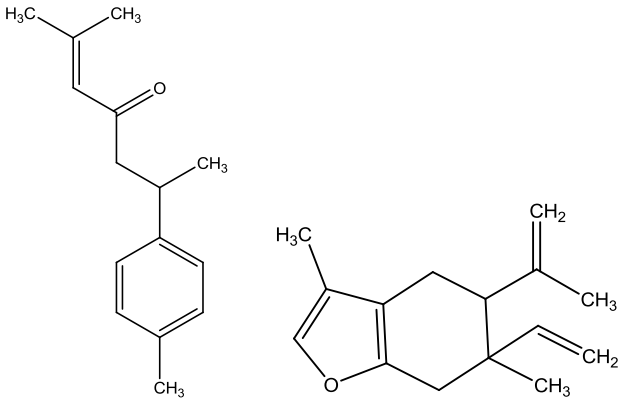


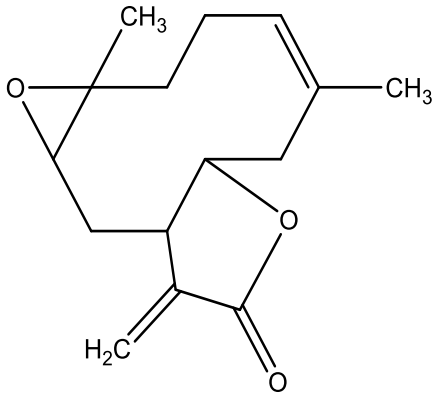
Figure 4. The peak mass spectrum with an 10.68 minute retention time.



(8) **(9)**

Figure 5: Suitable compounds with an 10.68 minute retention time base on Chemspider, Massbank, and Human Metabolome Database (HMDB) softwares.

The peak mass spectrum with an 11.05 minute retention time is possible have a $C_{15}H_{21}O_3$ molecular formula with a 88.55%. resemblance to a base peak of 249.15 identified as $[M+ H]^+$ (Figure 6). Based on search results with Chemspider, Massbank, and Human Metabolome Database (HMDB) softwares, the possibly compound is (1(10)-*E*,4β,5α,8β)-4,5-epoxy-1(10),11(13)-germacradien-12,8-olide (10). Compound 10 belongs to the germacran sesquiterpenes group. This structural approximation is supported by the m/z value 175 in its mass spectrum when it is released radical -$C_3H_5O_2$ (Figure 7). Compounds with germacran skeletons were found in rhizome temu giring grown in Solo, Indonesia[17], and compound 10 has not been reported.



(10)

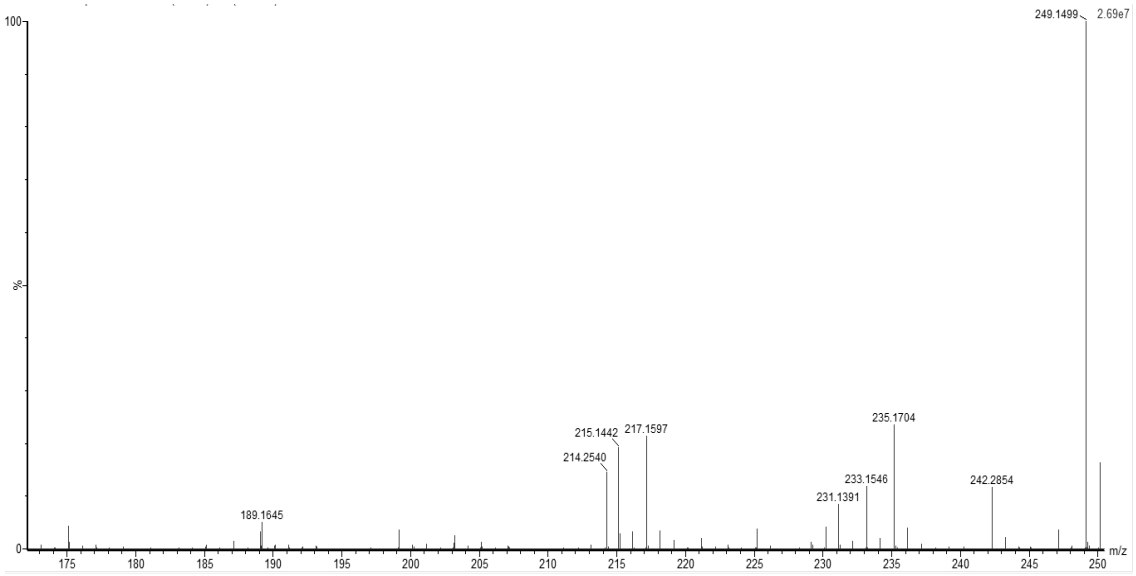


Figure 6: The peak mass spectrum with an 11.05 minute retention time.

The H13 fraction chromatogram yields several peaks from retention time of 1.24-20.00 minutes, 2 peaks of which provide a high abundance of peaks at retention times of 9.50 and 11.15 minutes (Figure 8). These two peaks were determined the possibility of its structure with HMDB and PubChem software. The peak mass spectrum with an 9.50 minute retention time is possible have a $C_{15}H_{19}O_4$ molecular formula with a 95.15% resemblance to a base peak of 263.13 identified as $[M + H]^+$ (Figure 9).

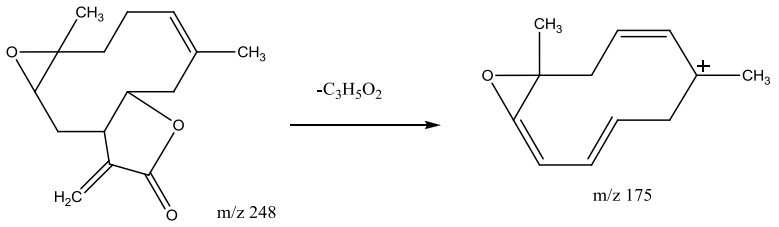


Figure 7: Proposed fragmentation of compound 10.

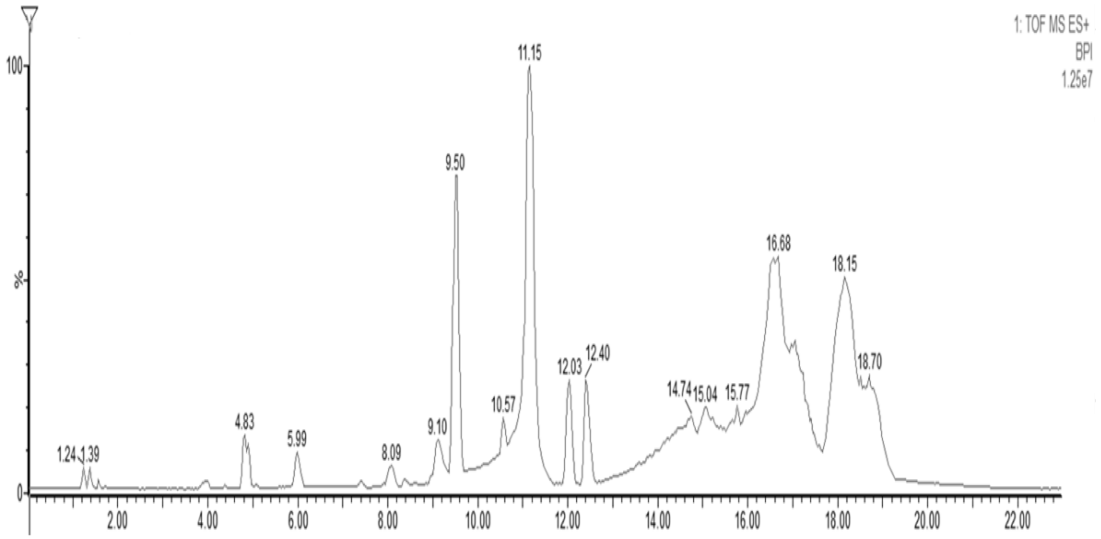


Figure 8: The H13 fraction chromatogram.

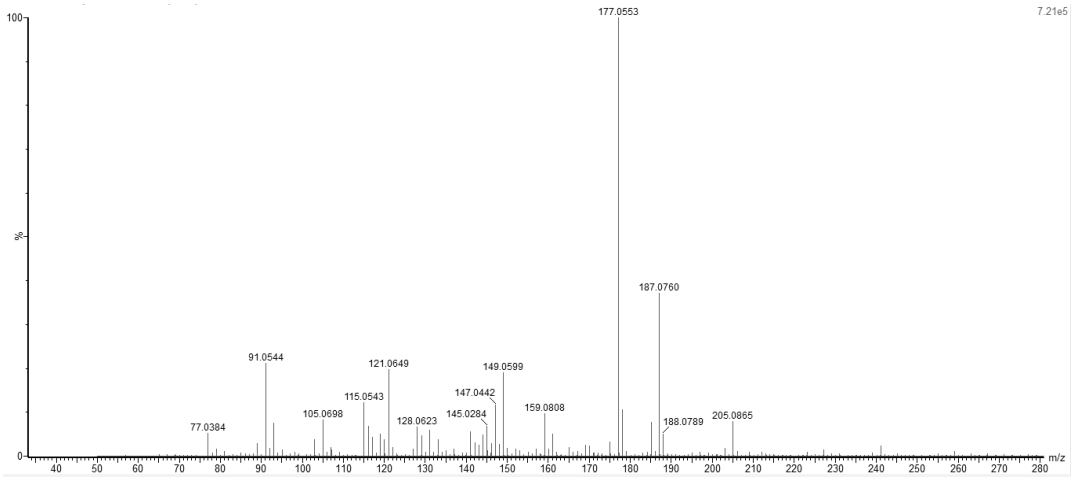
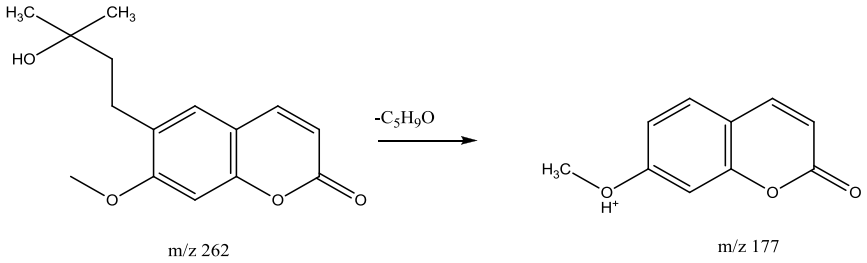


Figure 9: The peak mass spectrum with an 9.50 minute retention time.

Based on search results with HMDB and PubChem software, the possibly compound is the coumarin group compound, dihydrosuberenol (**11**). The presence of the coumarin structure is supported by the appearing of a stable value of m/z 177 in the mass spectrum after the loss of radical ions -C_5H_9O (Figure 10) and coumarin compound has ever been identified in the extract of *C. Xanthorrhiza*.[19]

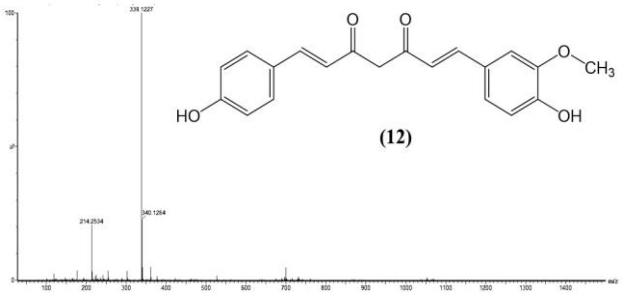


m/z 262 m/z 177

(11)

Figure 10: Proposed fragmentation of compound 11.

The peak mass spectrum with an 11.15 minute retention time is possible have a $C_{20}H_{19}O_5$ molecular formula with a 73.55% resemblance to a base peak of 339.12 identified as $[M+H]^+$ (Figure 11). Based on search results with HMDB, Chemspider, and Massbank softwares, the possibly compound is demethoxycurcumin (**12**) which has been found also in other *Curcuma* genus that is in C. *Longa*.[20]



(12)

Figure 11: The peak mass spectrum with an 11.15 minute retention time.

The result for the DPPH free radical scavenging effect of the crude methanol extract of *C. heyneana*, ascorbic acid, fraction F121, and H13 were shown in Table 1. None of the extracts of *C. heyneana* were found to be more active than the standard (ascorbic acid, IC_{50}, 5.25 ppm) for DPPH scavenging activity. Good antioxidant activity when IC_{50} value is low. The crude methanol extract of *C. heyneana* and H13 fractions have moderate antioxidant activity due to have a value of 100 ppm $<IC_{50}<$150 ppm. Antioxidant activity of crude methanol extract of *C. heyneana* is higher than crude methanol extract of *C. aeruginosa* rhizome (IC_{50} 124,12 ppm) that have ever reported by Sugita *et al.*[21], and slightly higher than for extract of temulawak (*C. xanthorriza*) (IC_{50} 87.27 and 92.28 ppm) that have ever reported by Julita.[22] The difference were presumably due to type and content of its compound in the plant extract which were affected by several factors, such as growing area, environmental stress such as heavy metals or ultraviolet exposure, age of plants, genetic factors, and physical factors such as climate, humidity, temperature, and weather.[23]

Table 1: IC_{50} value of crude methanol extract of *C. heyneana* and its fraction on DPPH scavenging activity

Fraction	IC_{50} (ppm)
F121	478.98
H13	59.18
Methanol extract	82.16
Ascorbic acid	5.25

This result indicated that phenolic and flavonoid in all accessions of *C. heyneana* are responsible for their antioxidant activities or alternatively that radical scavenging activity (antioxidant potential). In other medicinal plants, the results of that study are in agreement with other reports.[24]

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