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Phytochemicals and Biological Activity Studies of *Curcuma aeruginosa Roxb.*

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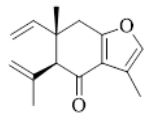
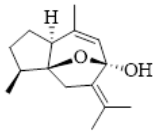
GRAPHICAL ABSTRACT



(1)



(2)

Structure of curzerenone **(1)** and curcumenol **(2)**

ABSTRACT

Curcuma aeruginosa Roxb., also known as temu hitam is one of the species from the Zingiberaceae family. The phytochemicals and bioactivities of this plant species have been studied. Cold maceration technique was used to extract the phytochemicals of the dried rhizomes of *C. aeruginosa* and *n*-hexane, dichloromethane, and methanol were used in this technique as solvents. Fractionation and purification on the crude extracts of the species using vacuum liquid chromatography (VLC) and column chromatography (CC) successfully isolated curzerenone **(1)** and curcumenol **(2)**. Their structures were elucidated using spectroscopic methods such as Infrared (IR), ^{1}H Nuclear Magnetic Resonance (NMR), and Gas Chromatography- Mass Spectrometry (GCMS). The crude extracts and the pure compounds were screened for anti-tyrosinase and anti-acetylcholinesterase activities. Modified dopachrome method with L-DOPA as the substrate and kojic acid as positive control was chosen to screen the anti-tyrosinase activity. Among all the tested samples, the *n*-hexane crude extract and curcumenol **(2)** exhibited the highest activities with 88.7% and 87.9% inhibition against the mushroom tyrosinase. For anti-acetylcholinesterase bioassay, a slightly modified Ellman's method was used with galantamine being used as the positive control and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) or Ellman's reagent was added before being scanned with microplate reader to determine the anti-acetylcholinesterase activity in the samples. It was found that curzerenone **(1)** exhibited acetylcholinesterase inhibitory activity with 88.4% inhibition against acetylcholinesterase enzyme when compared to the positive control, which gave 93.1% acetylcholinesterase inhibition. In conclusion, the objectives of this study were successfully achieved and the plant species exhibits anti-tyrosinase and anti-acetylcholinesterase properties.

Keywords: Curcuma aeruginosa, curzerenone, curcumenol, anti-tyrosinase, anti-acetylcholinesterase

1. INTRODUCTION

Malaysia is well-known to the world as one of the many countries that is rich in natural resources due to the presence of tropical rainforest in this land. The appropriate physiography, geology and climate of Malaysia enables the growth of many tropical plants, many of which are yet to be discovered and studied on. Moreover, the recent and unforeseen revitalization of the role and contributions of natural products chemistry in physical and biological sciences further emphasizes the importance of the discovery of new natural herbal drugs [1].

One of the famous herbal plant species that is being widely used in Malaysia and its surrounding countries *Curcuma aeruginosa*, or also known as *temu hitam* or *temu ireng* [2]. *C. aeruginosa* is from the plant family of Zingiberaceae, which its plants are commonly used as sources of food, spices, medicines, dyes, perfumes, and cosmetics. As for *C. aeruginosa*, it's traditionally applied as purgative during childbirth [2]. Phytochemical studies on this species also unveiled the presence of various monoterpenoids and sesquiterpenoids, which are of potential medicinal uses. Studies had found that some of these compounds extracted from *C. aeruginosa* exhibit biological activities such as anti-androgenic, antioxidant, uterine relaxant, antimicrobial, anti-carcinogenic, and so forth [3-6].

In this research, the focus was on the extraction, isolation, and characterization of compounds from the rhizome of *C. aeruginosa*. In addition, this research also would like to explore some possible biological activities which this plant species might exhibit, namely tyrosinase and acetylcholinesterase inhibition.

2. EXPERIMENTAL

The experiment was divided into two main stages. The first stage was focused on the extraction of the pure compounds. Air-dried *C. aeruginosa* rhizome (300 g) was extracted with cold maceration method with *n*-hexane, dichloromethane (CH_2Cl_2), and methanol (MeOH) (1 L) each for three times at room temperature. The solvents were filtered and concentrated with rotary evaporator and yielded sticky brown gum of *n*-hexane (2.54 g), CH_2Cl_2 (12.81 g), and MeOH (7.73 g) crude extracts. The *n*-hexane and CH_2Cl_2 crude extracts were fractionated with vacuum liquid chromatography (VLC) before being purified with column chromatograph (CC) while MeOH crude extract was directly purified with CC. The fractions of *n*hexane and CH_2Cl_2 were analysed with thin layer chromatography (TLC) and fractions with similar TLC profile were combined and gave six and seven major fractions respectively. Fraction CARH 2 and 3 were combined to be purified with CC and CARD 2 underwent the same step.

Fraction CARD 2-12 was obtained from CC to give yellowish brown gelatinous solid of curzerenone **(1)** (44.8 mg), IR (ATR) spectra ν_{max} cm^{-1} : 3084 (CH sp^2), 2927 (CH sp^3), 1737 (C=O), 1642 (C=C alkene), 1211 (C-O); ^{1}H NMR δ ($CDCl_3$, 300 MHz): 1.27 (3H, *s*, H-15), 1.84 (3H, *s*, H-14), 2.20 (3H, *s*, H-13), 2.66 (1H, *s*, H-5), 2.69, 2.72 (2H, *m*, H-9α, H-9β), 4.97 (2H, *m*, H-2), 5.01 (2H, *m*, H-3), 5.75 (1H, *dd*, *J*= 8.1 and 13.2 Hz, H-1). Fraction CARH23-1 was yielded from CC to obtain yellowish brown gelatinous solid of curcumenol **(2)** (74.2 mg), IR (ATR) spectra ν_{max} cm^{-1} : 3412 (OH), 2930 (sp^2 CH), 2870 (sp^3 CH), 1668 (C=C alkene), 1218 (C-O) ; ^{1}H NMR δ ($CDCl_3$, 300 MHz): 1.00 (3H, *d*, *J*= 6.3 Hz, H-14), 1.57 (3H, *s*, H-13), 1.64 (3H, *s*, H-15), 1.79 (3H, *s*, H-12), 1.88 (6H, *m*, H-2, H-3, H-4), 1.92 (1H, *m*, H-1), 2.09, 2.63 (2H, *m*, H-6α, H-6β), 5.73 (1H, *br s*, H-9).

The second stage was focused on the biological activities of the crude extracts and the pure compounds of *C. aeruginosa*. The crude extracts and pure compounds of *C. aeruginosa* was tested using tyrosinase inhibition assay which uses the modified dopachrome method with L-DOPA as substrate and kojic acid as the positive control. For acetylcholinesterase inhibition assay, galantamine was used as the positive control and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) or Ellman's reagent was added before being scanned with microplate reader to determine the anti-acetylcholinesterase activity in the samples.

3. RESULTS AND DISCUSSION

3.1. Characterization of curzerenone (1)

Extraction and separation on the crude extracts of *n*-hexane and CH_2Cl_2 have led to the isolation and characterization of two sesquiterpenoids, which were identified as curzerenone **(1)**, and curcumenol **(2)**. The IR spectrum of compound **(1)** indicated that the presence of carbonyl group with strong absorption at 1737 cm^{-1}. The ^{1}H-NMR spectrum (Figure 1) showed the presence of three methyl groups at chemical shifts of δ 1.27 (3H, *s*, H-15), δ 1.84 (3H, *s*, H-14), and δ 2.20 (3H, *s*, H-13). Besides, two signals that each attributed to one proton were observed at δ 2.66 (1H, *s*, H-5) and δ 5.75 (1H, *dd*, *J*= 8.1,13.2, Hz, H-1). The signal of H-1 was observed as doublet of doublet because of the coupling with unequivalent olefinic protons, H-2. Enantiotopic protons should be observed for H-9α and H-9β but multiplet were seen at δ 2.69 and 2.75 (2H, *m*, H-9α, H-9β). This might be due to the presence of impurities that affected the splitting. Two multiplets at δ 4.97 (2H, *m*, H-2) and δ 5.01 (2H, *m*, H-3) were assigned to terminal methylene groups of H-2 and H-3, respectively. However, the supposedly downfield signal which should appeared as a singlet around δ 7.00 as a proton of furanodiene system, H-12 was not observed.

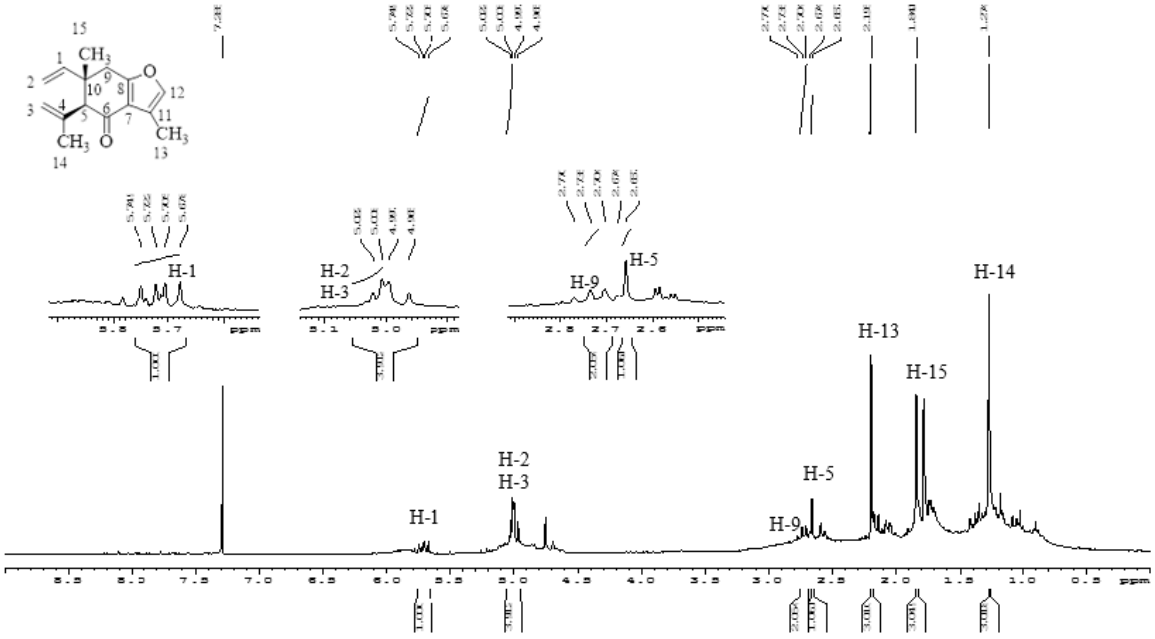


Figure 1. ^{1}H-NMR spectrum of compound **(1)**

3.2. Characterization of curcumenol (2)

Compound **(2)** was also characterised by using IR and ^{1}H–NMR. The IR spectrum of this compound showed the presence of hydroxyl group with a broad absorption band at 3412 cm^{-1}. It also displayed absorption band of sp^3 CH at 2870 cm^{-1}, C=C alkene at 1668 cm^{-1}, and C-O at 1218 cm^{-1}. The ^{1}H NMR spectrum (Figure 2) showed the presence of three methyl groups as three singlets at δ 1.57, 1.64, and 1.79, which attributed to H-13, H-15, and H-12 respectively. A doublet resonated at δ 1.00 (*J*= 6.3 Hz) was assigned the methyl protons of H-14. Two methylene protons, H-2, H-3, and a methine group, H-4 were displayed as multiplet signals at δ 1.88. A multiplet was observed at δ 1.92 instead of a doublet for H-1 as it was overlapped with the multiplet of H-2, H-3, and H-4. A multiplet was also observed for H-6α and H-6β, which supposedly gave a doublet of doublet, at δ 2.09 and 2.63. This might be due to the impurities present that interfered with the proton splittng. A broad singlet at δ 5.73 was attributed to the hydroxyl proton H-9. The MS spectrum (Figure 3) showed the molecular ion peak at *m/z* 234, consistent with molecular formula $C_{15}H_{22}O_2$.The spectrum also showed a base peak at *m/z* 105. Other notable peaks were at *m/z* 189, 147, 133, and 121.

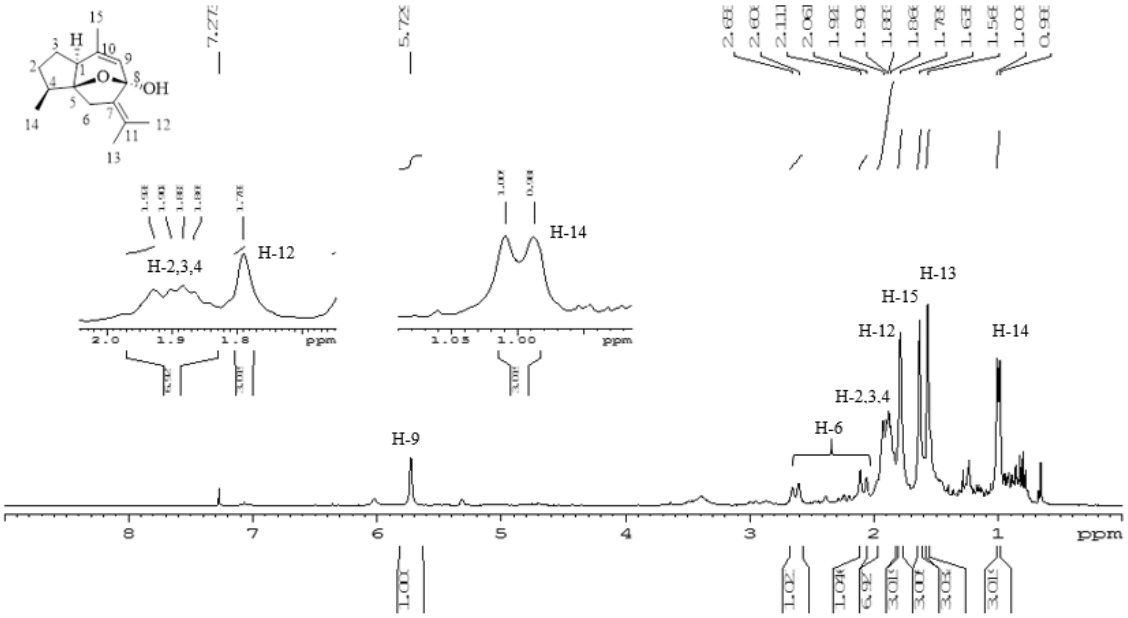


Figure 2. ^{1}H-NMR spectrum for compound **(2)**

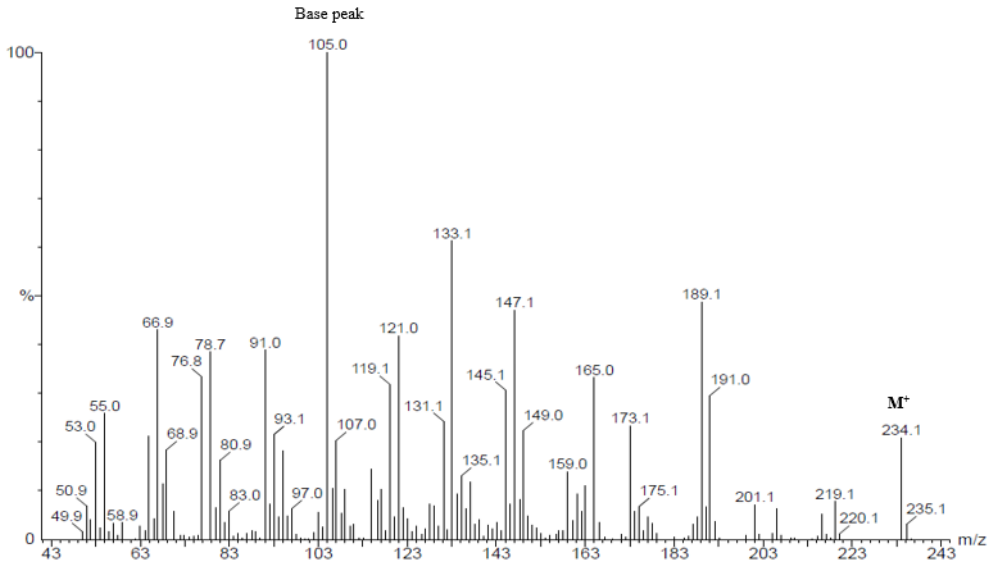


Figure 3. MS spectrum for compound **(2)**

3.3. Anti-tyrosinase activity

The crude extract and pure compounds of *C. aeruginosa* was evaluated for the tyrosinase inhibition activity using modified dopachrome method with L-DOPA as the substrate. Kojic acid was used as the positive control as it exhibits the highest tyrosinase inhibition activity among all other compounds [7, 8]

The results of tyrosinase inhibition potential of the crude extracts and pure compounds are as shown in Table 1. Among the samples, crude extract CARH showed the highest percentage of tyrosinase inhibition (88.7%) and pure compound CARH 23-1 or curcumenol **(2)** which was purified from CARH also displayed a high tyrosinase inhibition (87.9%). The rest of the samples had percentage of tyrosinase inhibition in the range of 11.0-56.0%.

Table 1. Percentage inhibition of tyrosinase inhibitory activity of *C. aeruginosa*

Samples	**Inhibition at 1000 µg/mL (%)**
Crude Extracts	
CARH	88.7
CARD	55.6
CARM	42.9
Isolated Compounds	
Curzerenone (1)	11.1
Curcumenol (2)	87.9
Positive Control	
Kojic Acid	75.0

3.4. Anti-acetylcholinesterase Activity

The crude extract and some pure compounds of *C. aeruginosa* was analyzed for acetylcholinesterase (AChE) inhibition activity using Ellman's method with acetylthiocholine iodide (ATCI) as the substrate. Galantamine was used as the positive control as it is a commercially used AChE inhibitor [9].

The results of AChE inhibition potential of the crude extracts and pure compounds are as shown in Table 2. Among the samples, crude extract CARM showed the highest percentage of AChE inhibition (63.4%) and pure compound CARD 2-12 or curzerenone **(12)** exhibited the highest AChE inhibition (88.4%). The rest of the samples had percentage of tyrosinase inhibition in the range of 13.0-50.0%.

Table 2. Percentage inhibition of AChE Inhibitory Activity of *C. aeruginosa*

Samples	**Inhibition at 1000 µg/mL (%)**
Crude Extracts	
CARH	13.8
CARD	42.5
CARM	63.4
Isolated Compounds	
Curzerenone (1)	88.4
Curcumenol (2)	49.0
Positive Control	
Galantamine	93.1

4. CONCLUSION

The study on the phytochemicals and biological activities of *C. aeruginosa* was successfully carried out. Cold maceration of the rhizomes of *C. aeruginosa* for 72 hours at room temperature using *n*-hexane, CH_2Cl_2, and MeOH followed by the evaporation of each extract using a rotary evaporator had yielded sticky dark green gum of *n*-hexane (2.54 g, 0.9%), CH_2Cl_2 (12.81 g, 4.3%), and MeOH (7.73 g, 2.6%) crude extracts. Fractionation and purification of *n*-hexane and CH_2Cl_2 crude extracts had successfully yielded two sesquiterpenoids, namely curzerenone **(1)** (0.045 g) and curcumenol **(2)** (0.074 g) as yellowish-brown gelatinous solids. The tyrosinase inhibitory activity of *C. aeruginosa* was determined using L-DOPA as substrate and kojic acid as the positive control (75.0%). The crude extract, CARH (88.7%) and pure compound, CARH23-1 or curcumenol **(2)** (87.9%) gave positive results on the inhibition of tyrosinase activity. As for AChE inhibitory activity, ATCI was used as substrate and galantamine as the positive control (93.1%). Pure compound CARD2-12 or curzerenone **(1)** (88.4%) shown positive effect in the inhibition of AChE activity.

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