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Chemical Components of the Rhizome Oil of *Curcuma Heyneana* Val.

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ABSTRACT The chemical composition of the essential oil of *Curcuma heyneana* isolated by hydrodistillation was analyzed by capillary GC and GC/MS. The main constituents found in the oil were sesquiterpenoids, curcumanolide (19.6%), dehydrocurdione (17.2%), isocurcumenol (16.5%), curcumenol (13.7%), curcumenone (6.4%), and germacrone (5.0%). A diterpenoid identified as labda-8(17), 12-diene-15, 16-dial (4.8%) was also found as one of the components in the oil. These constituents were also isolated and identified from the chloroform extract of the dried rhizomes.

ABSTRAK Komposisi kimia minyak pati *Curcuma heyneana* yang diperoleh secara penyulingan hidro dianalisis dengan KG rerambut dan KG/SJ. Komponen utamanya terdiri daripada seskuiterpena, kurkumanolida (19.6%), dehidrokurdion (17.2%), isokurkumenol (13.7%), kurkumenon (6.4%), dan germakron (5.0%). Sebatian diterpenoid, labda-8(17),12-diena-15,16-dial (4.8%) juga dikenalpasti sebagai satu daripada komponen dalam minyak pati ini. Komponen kimia ini juga telah diasingkan dan dikenalpasti daripada ekstrak kloroform rizom kering.

(**Keywords:** *Curcuma heyneana*, essential oil composition, sesquiterpenoids, diterpene, cytotoxicity.)

INTRODUCTION

Curcuma heyneana Val. & V. Zijp (Zingiberaceae) or locally known as temu giring is one of the medicinal plants indigenous to Indonesia. The rhizomes are aromatic, pale yellow, and do not contain curcumin, and are useful for the treatment of skin diseases. Isolation of the essential oil extracted from the dried rhizomes has been reported by Firman *et al.* [1]. The oil consists mainly sesquiterpenoids, identified as germacrone, dehydrocurdione, isocurcumenol, curcumenol, curcumanolide A and B, zerumbone and oxycurcumenol. However, they did not study chemical compositions of the essential oil in detail. 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 [2]. Our study showed a different result, therefore we reinvestigate the rhizomes oil again, and here we would like to report the chemical compositions of the fresh rhizomes and the isolation-identification of phytochemicals obtained from *C. heyneana* collected from Padang, Sumatera, Indonesia.

EXPERIMENTAL

Plant material

Fresh rhizomes of *C. heyneana* were collected from Bukit Tinggi, Padang, Sumatera, Indonesia. A voucher specimen had been deposited at the Herbarium of the Department of Botany, University of Andalas, Padang, Sumatera, Indonesia.

Isolation Procedure

Fresh rhizomes (500 g) were hydrodistilled in an all-glass apparatus for 8h. Extraction of the distillate with diethyl ether, followed by drying over anhydrous magnesium sulphate and removal of the solvent yielded 0.43% (w/w) of oil. The oil constituents were determined by GC and GC/MS.

GC

A Hewlett-Packard HP 5880A system equipped with an FID and an Ultra 1 gas chromatograph capillary column (cross-linked methyl silicone gum), (25m x 0.32 mm; 0.20 μm film thickness) was employed. Operating conditions: initial oven temperatural 50°C for 5 min, 50° - 300°C at 6°C/min and then 300°C for 30 min; injector temperature, 250°C, carrier gas 1.0 mL/min He, sample size 0.4 μL. The percentage composition of the oil was computed electronically from the GC peak areas without the use of an internal standard or detector response factors.

GC/MS
A Hewlett-Packard 5989A GC/MS system equipped with Wiley Library software was used. Capillary GC conditions as above were employed. Significant operating parameters; ionization voltage, 70 eV; ion source temprature, 200°C; scan mass range, 40 - 350μ.

Component identification
Sample components were identified by matching their mass spectra with those recorded in the MS Wiley Library and further supported by comparison of their GC retention times, TLC and MS with those of reference compounds when the latter were available.

Isolation of Phytochemicals
The essential oil (0.75 g) was purified by gravity column chromatography using silica gel (70 g) in a column (diameter 3 cm) with a mixture of PE:Et_2O (99:1, 9:1 and 4:1) as eluents to give 207 fractions.

Germacrone (1)
Fractions (24-42) were combined and concentrated to give germacrone **(1)** (21.6 mg, 2.88 %) as a colourless crystal, mp. 52-54°C (lit. [1] 55-56°C, lit. [3] 53-54°C and lit. [4] 53-55°C). IR ($CHCl_3$) ν_{max} cm^{-1}: 2923, 1679, 1439 and 1386; ^{1}H NMR ($CDCl_3$): δ 1.40 (3H, s, H-14), 1.59 (3H, s, H-15), 1.69 (3H, s, H-13), 1.74 (3H, s, H-12), 2.0-2.40 (4H, m, H-2 and H-3), 2.80-3.00 (3H, m, H-6 and H-9a), 3.38 (1H, d, *J* 10.4 Hz, H-9b), 4.68 (1H, br.d, *J* 10.4 Hz, H-5) and 4.95 (1H, br.d, *J* 11.6 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 15.6 (C-14), 16.7 (C-15), 19.9 (C-12), 22.3 (C-13), 24.1 (C-2), 29.2 (C-6), 38.1 (C-3), 55.9 (C-9), 125.4 (C-5), 126.7 (C-4), 129.5 (C-7), 132.7 (C-1), 135.0 (C-10), 137.2 (C-11) and 207.9 (C-8); MS: *m/z* (%) 218 (19) [M^+, $C_{15}H_{22}O$], 136 (81), 135 (73), 107 (100) and 67 (63).

Curcumanolide A (2) and Curcumanolide B (3)
Fractions (64-82) were combined and the solvent was evaporated *in vacuo* using rotary evaporator to yield a mixture of curcumanolide A (106) and curcumanolide B (107) (76.6 mg, 10.21 %) as a yellow liquid. IR ν_{max} cm^{-1}: 2959, 1744, 1666, 1449, 1376 and 1269; ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, d, *J* 6.8 Hz, H-$14_{(2)}$), 0.94 (3H, d, *J* 6.0 Hz, H-$14_{(3)}$), 1.55 (3H, s, H-$15_{(3)}$), 1.70 (3H, s, H-$15_{(2)}$), 1.81 (6H, s, H-$13_{(2),\ (3)}$), 2.21 (6H, t, *J* 2.0 Hz, H-$12_{(2),\ (3)}$), 2.43 (3H, s, H-$6_{(2)}$ and H-$6a_{(3)}$), 1.5-2.35 (10H, m, H-$2_{(2),\ (2)}$, H-$3_{(2),\ (2)}$ dan H-$4_{(2),\ (3)}$), 2.8-2.90 (3H, m, H-$1_{(2),\ (3)}$ dan H-$6b_{(3)}$), 4.73 (2H, s, H-$9a_{(2),\ (3)}$), 4.85 (1H, s, H-$9b_{(3)}$) and 4.92 (1H, s, H-$9b_{(2)}$); ^{13}C NMR ($CDCl_3$): δ 13.0 (C-$14_{(3)}$), 13.1 (C-$14_{(2)}$), 19.8 (C-$15_{(3)}$), 19.9 (C-$15_{(2)}$), 21.9 (C-$13_{(3)}$), 23.1 (C-$3_{(2)}$), 23.9 (C-$13_{(3)}$), 24.4 (C-$12_{(2),\ (3)}$), 26.3 (C-$2_{(2)}$), 27.4 (C-$6_{(3)}$), 27.5 (C-$6_{(2)}$), 30.8 (C-$3_{(3)}$), 33.8 (C-$2_{(3)}$), 42.7 (C-$4_{(2)}$), 45.2 (C-$4_{(3)}$), 52.1 (C-$1_{(2)}$), 56.0 (C-$1_{(3)}$), 89.6 (C-$5_{(2)}$), 91.6 (C-$5_{(3)}$), 112.7 (C-$9_{(2)}$), 114.0 (C-$9_{(3)}$), 120.7 (C-$7_{(2),\ (3)}$), 143.7 (C-$11_{(2)}$), 145.3 (C-$11_{(3)}$), 149.3 (C-$10_{(2),\ (3)}$) dan 169.8 (C-$8_{(2),\ (3)}$); MS: *m/z* (%) 234 (33) [M^+, $C_{15}H_{22}O_2$], 178 (50), 165 (80), 164 (100) dan 152 (85).

Isocurcumenol (4)
Fractions (105-118) were combined and concentrated, followed by recrystallization using petroleum ether to give isocurcumenol **(4)** (54.8 mg, 7.31 %) as colourless crystals, mp. 145-147°C (lit. [1] 144-146°C). IR ($CHCl_3$) ν_{max} cm^{-1}: 3405, 2928, 1644, 1453, 1373 and 1216; ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, d, *J* 6.4 Hz, H-14), 1.54 (3H, s, H-13), 1.77 (3H, s, H-12), 1.6-2.00 (6H, m, H-2, H-3 and H-6), 2.19 (1H, t, *J* 14.0 Hz, H-4), 2.4-2.60 (2H, m, H-9), 2.65 (1H, d, *J* 14.0 Hz, H-1), 2.78 (1H, s, OH), 4.71 (1H, t, *J* 2.0 Hz, H-15a) and 4.75 (1H, t, *J* 2.0 Hz, H-15b); ^{13}C NMR($CDCl_3$): δ 12.4 (C-14), 18.9 (C-13), 22.5 (C-12), 28.3 (C-2), 30.8 (C-3), 36.2 (C-9), 38.9 (C-6), 41.6 (C-4), 52.8 (C-1), 87.1 (C-5), 104.0 (C-8), 112.2 (C-15), 126.9 (C-11), 133.9 (C-7) and 145.1 (C-10); MS: *m/z* (%) 234 (3) [M^+, $C_{15}H_{22}O_2$], 191 (35), 121 (48), 105 (100), 67 (55) and 41 (87).

Curcumenol (5)
Fractions (127-159) were combined, followed by washing with petroleum ether repeatedly to give curcumenol **(5)** (45.6 mg, 6.08 %) as colourless needles, mp 113-115°C (lit. [1] 114-116°C). IR ($CHCl_3$) ν_{max} cm^{-1}: 3378, 2957, 2873 and 1653; ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, d, *J* 6.4 Hz, H-14), 1.55 (3H, s, H-15), 1.61 (3H, s, H-13), 1.77 (3H, s, H-12), 1.8-2.00 (6H, m, H-2, H-3 and H-6), 2.07 (1H, d, *J* 15.6 Hz, H-1), 2.61 (1H, d, *J* 15.6 Hz, H-4), 3.05 (1H, s, OH) and 5.71 (1H, s, H-9); ^{13}C NMR ($CDCl_3$): δ 11.8 (C-14), 20.9 (C-15), 18.9 (C-13), 22.3 (C-12), 27.5 (C-2), 31.2 (C-3), 37.1 (C-6), 40.3 (C-4), 51.2 (C-1), 85.7 (C-5), 101.5 (C-8), 122.1 (C-11), 125.8 (C-9), 137.2 (C-10) and 139.0 (C-7); MS: *m/z* (%) 234 (15) [M^+, $C_{15}H_{22}O_2$], 189 (26), 147 (30), 133 (38), 105 (100) and 91 (38).

Curcumenone (6)
Fractions (182-201) were combined and the solvent was evaporated to yield curcumenone **(6)** (23.6 mg, 3.15 %) as a viscous oil [5]. IR ν_{max} cm^{-1}: 2926, 1716, 1678, 1600, 1439 and 1369; ^{1}H NMR ($CDCl_3$): δ 0.41 (1H, dt, *J* 4.4 and 7.2 Hz, H-1), 0.64 (1H, q, *J* 4.4 Hz, H-5), 1.09 (3H, s, H-15), 1.57 (2H, q, *J* 7.2 Hz, H-2), 1.76 (3H, s, H-13), 2.06 (3H, s, H-12), 2.10

(3H, s, H-14), 2.44 (2H, t, *J* 7.2 Hz, H-3), 2.49 (1H, s, H-9a), 2.51 (1H, s, H-9b) and 2.78 (2H, br.s, H-6); ^{13}C NMR ($CDCl_3$): δ 19.0 (C-15), 20.1 (C-10), 23.3 (C-14), 23.4 (C-12), 23.4 (C-13), 24.1 (C-5), 24.2 (C-2), 28.0 (C-6), 30.0 (C-1), 43.9 (C-3), 48.9 (C-9), 128.0 (C-7), 147.3 (C-11), 201.6 (C-8) and 208.7 (C-4); MS: *m/z* (%) 234 (14) [M^+, $C_{15}H_{22}O_2$], 176 (98), 161 (58), 133 (40), 107 (44), 91 (45) and 68 (100).

Soxhlet Extraction of *C. Heyneana*

The dried rhizomes (59.4 g) was extracted using chloroform in a Soxhlet apparatus for 20 hr. Evaporation of the chloroform gave the crude extract (14.11 g, 23.75%) as brown oil.

Isolation and Identification of Constituents from the Soxhlet Extraction

The crude extract (8 g) was fractionated using vacuum liquid chromatography with petroleum ether and ether as eluents produced 25 fractions. The fractions were then combined based on the TLC pattern to 3 fractions, fraction 1 (0.75 g), fraction 2 (4.18 g) and fraction 3 (2.09 g). Fraction 1 was purified using column chromatography to give germacrone **(1)** (0.23 g, 2.88%) as colourless needles, mp. 52-54°C (lit. [1] 55-56°C, lit. [3] 53-54°C and lit. [4] 53-55°C); Fraction 2 was purified by column chromatography to give 172 fractions. Fractions were combined based on the TLC profile to yield a diterpene, labd-8(17), 12-dien-15, 16-dial **(7)** (76.5 mg, 0.96 %) as yellowish oil [6]; dehydrocurdione **(8)** (0.45 g) as a colourless oil [7]; isocurcumenol **(4)** (0.37 g, 4.63 %) as colourless crystals, mp. 145-147°C (lit. [1] 144-146°C); and curcumenol **(5)** (0.35 g, 4.38 %) as colourless crystals, mp. 113-115°C (lit. [1] 114-116°C). Fraction 3 (2.09 g) was chromatographed to give curcumenone **(6)** (0.11 g, 1.38 %) as a yellow oil.

Labda-8(17), 12-dien-15, 16-dial (7)

IR ν_{max} cm^{-1}: 2930, 2844, 2717, 1727, 1685, 1642 and 889; ^{1}H NMR ($CDCl_3$): δ 0.70 (3H, s, H-18), 0.79 (3H, s, H-19), 0.86 (3H, s, H-20), 1.0-2.10 (12H, m, H-1, H-2, H-3, H-5, H-6, H-7 and H-9), 2.3-2.50 (2H, m, H-11), 3.38 (1H, d, *J* 16.8 Hz, H-14a), 3.47 (1H, d, *J* 16.8 Hz, H-14b), 4.34 (1H, s, H-17a), 4.83 (1H, s, H-17b), 6.74 (1H, t, *J* 6.6 Hz, H-12), 9.38 (1H, s, H-16) and 9.61 (1H, s, H-15); ^{13}C NMR ($CDCl_3$): δ 14.3 (C-20), 19.2 (C-2), 21.7 (C-19), 24.1 (C-6), 24.6 (C-11), 33.5 (C-4), 33.5 (C-18), 37.8 (C-14), 39.2 (C-1), 39.3 (C-7), 39.5 (C-10), 41.9 (C-3), 55.3 (C-5), 56.4 (C-9), 107.8 (C-17), 134.8 (C-13), 147.9 (C-8), 160.0 (C-12), 193.6 (C-16) and 197.1(C-15); MS: *m/z* (%) 302 (9) [M^+, $C_{20}H_{30}O_2$], 137 (100), 123 (50), 95 (84) and 81 (94).

Dehydrocurdione (8)

^{1}H NMR $CDCl_3$): δ 1.00 (3H, d, *J* 7.2 Hz, H-14), 1.63 (3H, s, H-15), 1.73 (3H, s, H-13), 1.76 (3H, s, H-12), 1.0-2.10 (12H, m, H-1, H-2, H-3, H-5, H-6, H-7 and H-9), 2.0-2.20 (4H, m, H-2 and H-3), 2.3-2.50 (3H, m, H-4), 3.0-3.15 (4H, m, H-6 and H-9), and5.13 (1H, br.s, H-1). Germacrone (**1**), isocurcumenol **(4)**, curcumenol **(5),** curcumenone **(6),** and dehydrocurdione **(8)** isolated from the crude extract were identical to those obtained from the essential oil of the fresh rhizomes.

Toxicity screening

Bioassay screening of the rhizome oil against brine shrimps, *Artemia salina* was carried out using standard methods [10–11].

Antimicrobial screening

Screening on the rhizome oil and isolated compounds was carried out using disc diffusion assay [12–13] against three gram positive (*Staphylococcus aureus, Bacillus subtilis, B. cereus*, and five gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Salmonella typhii* and *Streptococcus faecalis)* as well as four fungi with streptomycin sulfate and nystatin hydrate as positive controls respectively.

RESULTS AND DISCUSSIONS

The rhizomes of *C. heyneana* yielded ~ 0.43% oil. Twenty four compounds representing more than 95% of the oil were identified. They are listed in Table I. Among these compounds were: 14 sesquiterpenes (87.3%), one diterpene (4.8%), and 8 monoterpenes (3.0%). The major sesquiterpene were curcumanolides A and B (19.6%), dehydrocurdione (17.1%) and isocurcumenol (16.5%). The rhizome oil of *C. heyneana* was found to be deficient in monoterpenoids. Most of the compounds found in the rhizomes oil reported earlier [2] contained different constituents; the only similar component present was 2-undecanone with only 0.3%. The essential oil reported earlier did not contain high amounts of sesquiterpenoids and also did not contain any diterpenoid constituent. In this study, only eight compounds of monoterpenes were detected in the oil, with linalool (1.2%) as the major monoterpenoid. The differences between the results of the present analysis and the published data suggest that there may be two chemotypes of *C. heyneana*, but without further study this is merely a point of interest.

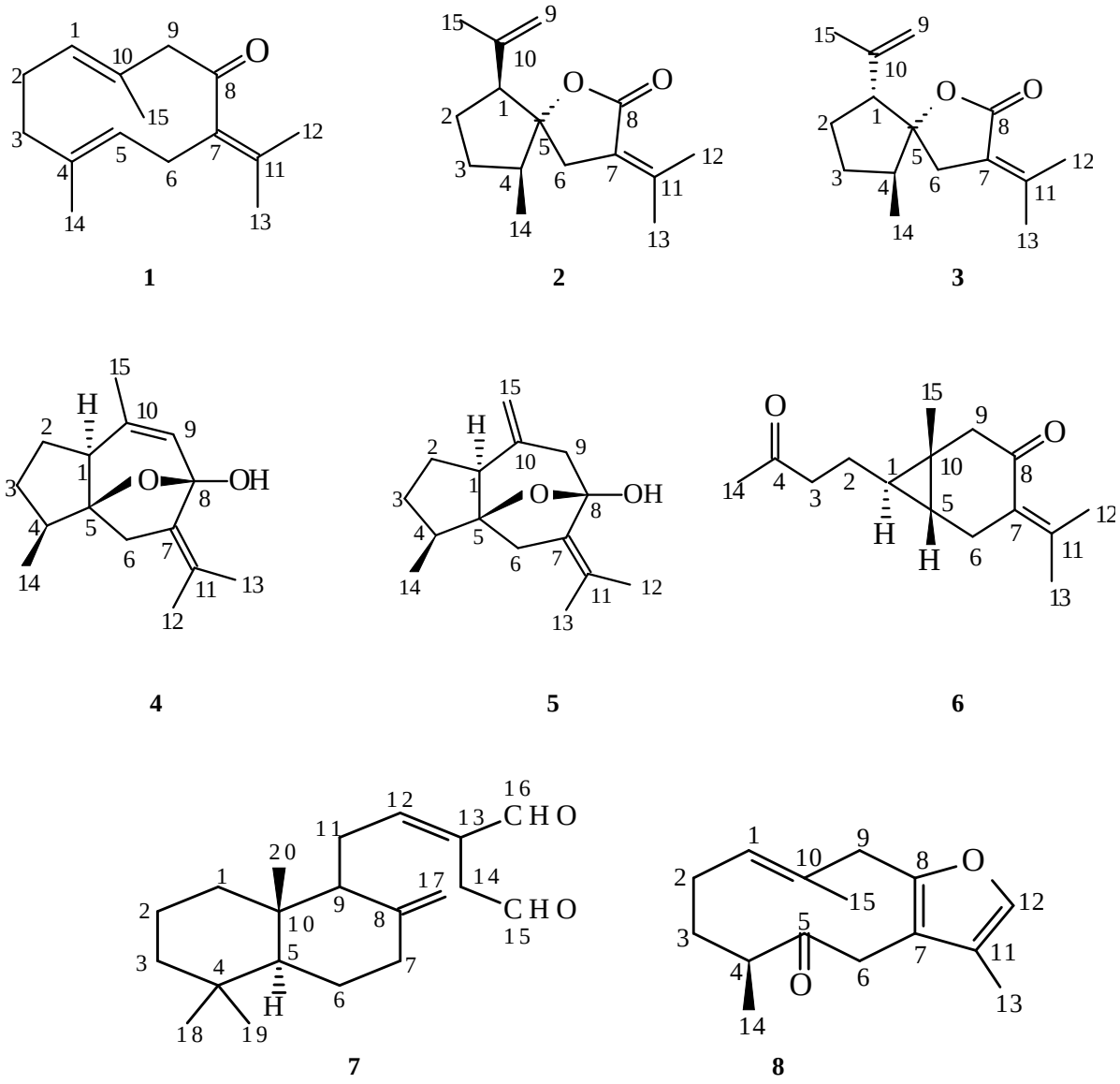
Table 1. Chemical Composition of the rhizome oil of *Curcuma heyneana*

Constituent	**Area %**	**Method of identification**
1, 8-Cineole	0.2	GC/MS
Linalool	1.2	GC/MS
Camphene hydrate	0.1	GC/MS
Endoborneol	0.3	GC/MS
Borneol	0.3	GC/MS
p-Cymene-8-ol	0.1	GC/MS
α-Terpineol	0.3	GC/MS
2-Undecanone	0.3	GC/MS
Isocaryophyllene	0.6	GC/MS
γ-Elemene	0.1	GC/MS
β-Caryophyllene	0.2	GC/MS
α-Humulene	0.4	GC/MS
ar-Curcumene	0.5	GC/MS/CoC
Furanodiene	0.3	GC/MS
Curzerenone	1.7	GC/MS
Caryophyllene oxide	5.1	GC/MS
Isocurcumenol	16.5	GC/MS/CoC
Germacrone	5.0	GC/MS/CoC
Curcumenol	13.7	GC/MS/CoC
Dehydrocurdione	17.2	GC/MS/CoC
Curcumanolide	19.6	GC/MS/CoC
Curcumenone	6.4	GC/MS/CoC
Labda-8(17), 12-diene-15, 16-dial	4.8	GC/MS/CoC
Total	**95%**	

The major constituents in the rhizomes oil, germacrone **(1),** (curcumanolide A **(2)** & B **(3),** isocurcumenol **(4),** curcumenol **(5),** curcumenone **(6),** labda-8(17), 12-diene-15,16-dial **(7)**, and dehydrocurdione **(8)** as shown in Figure 1, have been isolated *via* gravity column chromatography on silica gel, and they were structurally elucidated spectroscopically by IR, ^{1}H & ^{13}C NMR and MS a These data were consistent with those reported earlier in the literatures [1, 3-9].

Preliminary screening on the toxicity of the oil of *C. heyneana* against brine shrimps, (*Artemia salina*) [10–11], showed that the oil was quite toxic with ED_{50} 46.61 ppm. Antibacterial screening revealed that the oil only gave moderate activity against gram negative bacteria, *Pseudomonas aeruginosa*. The result also showed that a sesquiterpene, curcumenone (**6**) was active against *S. aureus, B. subtilis, P. aeruginosa, B. cereus* and *S. faecalis*, whereas a diterpene, labda-8(17),12-dien-15,16-dial (**7**) was active against *S. aureus, B. subtilis, P. aeruginosa*, and *S. typhii*, with the MIC value of 0.05 – 0.025. Labda 8(17),12-dien-15,16-dial (**7**) was the only phytochemical found to be active against fungal

screening, *Cladosporium cladosporoides* and *Trichoderma sp*. with the MIC value of 0.0125 µg/mL. This was consistent with literatures that this labdane diterpene isolated from turmeric leaves [14], and *Alpinia galanga* seeds [15] was found to show antifungal activity against *Candida*.



1 **2** **3**

4 **5** **6**

7 **8**

Figure 1. Chemical components isolated from *Curcuma heyneana*

CONCLUSIONS

The rhizome oil of *Curcuma heyneana* was found to be rich in sesquiterpenes (87.3%) with curcumanolide and dehydrocurdione as the major constituents (19.6%) and (17.2%) respectively. The rhizome oil of *C. heyneana* was also found to be quite toxic against *A. salina* with ED_{50} 46.61 ppm. Isolation and purification of the rhizomes oil yielded pure dehydrocurdione, curcumenol, isocurcumenol, curcumenone, germacrone and labda-8(17),12-diene-15,16-dial, as well as curcumanolides A and B. Zerumbone was not detected in this oil, as it had been reported in the previous literature [1], and this was probably due to the presence of zerumbone in a very minute quantity. Diterpene labda-8(17)12-dien-15, 16-dial (**7**) was reported for the first time in *Curcuma heyneana*. Curcumenone (**6**), and labda-8(17)12-dien-15, 16-dial (**7**) were two pure components that showed moderate antibacterial activity, whereas

labda-8(17)12-dien-15, 16-dial (**7**) was the only constituent that showed antifungal activity.

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