Isolation and identification of curcumin and bisacurone from rhizome extract of temu glenyeh (Curcuma soloensis. Val)

Rista A Vitasari, Fajar R Wibowo, Soerya D Marliyana and M Widyo Wartono *

Medicinal Chemistry Research Group, Chemistry Department, Faculty of Mathematic and Science, Sebelas Maret University, Jl. Ir. Sutami 36 A, Surakarta, Central Java, Indonesia 57126

*E-mail: widyo@staff.uns.ac.id

Abstract. Temu glenyeh (Curcuma soloensis. Val) is one of the medicinal plants that grow in Surakarta. This plant is similar with C. longa and C. Xanthoriza. Chemical constituents from an extract of the plant have never been studied. In this paper, we report the isolation of a terpenoid and curcumin from the rhizome of C. soloensis. The isolation was employed by soxhlet apparatus using acetone as solvent. The fractionation and purification of the compound from the acetone extracts were undertaken by vacuum liquid chromatography and flash chromatography. Identification of compounds used spectroscopy methods, such as FTIR, NMR (1H NMR, 13C NMR, COSY, HSQC and HMBC) and GC-MS. Isolated compounds were identified as curcumin (1) and bisacurone (2).

1. Introduction
Indonesian society has used various types of plants as medicine, and prevention of various diseases. Medicinal plants are generally based on inheritance or experience without knowing the exact chemical content. Many plants used as drugs in Indonesia are the member of Zingiberaceae family, such as ginger, turmeric, kencur, white ginger, temu ireng, temu glenyeh, temu lawak, lempuyang, and bengle. Plants of this family empirically use to treat stomach pains, carminative, diarrhea, rheumatism, asthma, inflammation, intestinal worms, scabies, and ulcers [1].

One of the medicinal plants, temu glenyeh or temu tis (Curcuma soloensis syn. C. purpurea scenas) has the potential for drug plants. Chemical components of this plant have been identified but limited, only from essential oils of rhizome that contains terpenoids [2-5]. Essential oils of rhizome temu glenyeh have inhibitory against three strains of bacteria such as Staphylococcus aureus, S. epidermis, and Streptococcus haemolyticus [6]. While research on the n-hexane extract, extract of methylene chloride and ethyl acetate extracts of rhizome temu glenyeh, showed that extracts have antifungal activity against Candida albicans. Tested showed phytochemical compounds of the extract contain terpenoids. The highest antifungal activity possessed by the ethyl acetate extract, which also contain phenols compounds [7]. Temu glenyeh research largely focused on essential oils, while the isolation of compounds in the extract of temu glenyeh has not been studied. Therefore, in this study, we report the isolation and identification of curcumin (1) and bisacurone (2) from acetone extract of temu glenyeh rhizome.

2. Experimental detail
The rhizome of Temu glenyeh (C. soloensis) was collected from Boyolali district, Central Java and identified by staff Department of Biology, Universitas Sebelas Maret, Surakarta. Rhizome was
crushed and dried to be a powder. Extraction of material used Soxhlet extractor apparatus and acetone as solvent. Isolation of compound from acetone extract used chromatography methods such as vacuum liquid chromatography (VLC) and flash chromatography. Stationary phase used silica gel G (VLC) and silica gel 60 (0.04 to 0.063 mm) 230-400 mesh ASTM (flash chromatography), all process were guided by thin layer chromatography (Si-gel f$^{254}$) and detected by UV 254 nm and cerium (IV) sulfate 2% as spray reagent. Spectrophotometer Fourier Transform Infra Red (FTIR) used Shimadzu Prestige-21, NMR spectroscopy by Agilent VNMR 400 MHz (CDCl$_3$ and TMS as standard), mass spectra by GC-MS QP-2010S SHIMADZU with AGILENT DB-1 column.

Dry powder rhizome of *Curcuma soloensis* (1.16 kg) was extracted using acetone (2.2 L) by Soxhlet apparatus. The filtrate then evaporated to resulting acetone extract (220 g) and fractionated using VLC and eluted with \( n \)-hexane : ethyl acetate ratio (9:1); (8:2) (2x); (7:3) (4x); (6:4) (4x); (5:5) (2x) and (4:6) (2x), (@150 mL) to obtain 17 fractions (figure 1), namely fraction A (0.533 g), B (0.976 g ), C (0.501 g), D (0.522 g), E (0.822 g), F (0.276 g), G (0.212 g), H (0.183 g), I (0.131 g), J (0.202 g), K (0. 385 g), L (0.403 g), M (0.85 g), N (0.937 g), O (0.695 g), P (0.397 g), and Q (0.752 g). Fraction M is bright orange powder, and analysis of the compound gives curcumin (1).

Fraction P and Q then purified using flash chromatography using \( n \)-heksane: ethyl acetate ratio (7:3) (50 mL); (6:4) (150 mL); and (1:1) (100 mL), and gives 37 fraction and from fraction 29 – 37 gives compound 2 (108 mg). Compound 2 (bisacurone): viscouse oil, IR (KBr) \( v_{\text{max}} \) cm$^{-1}$: 3385, 2960, 2930, 1678, 1600; EI -MS (70 ev): 216 (M$^+$-2H$_2$O), 201, 173, 159, 145, 132, 119, 105, 91, 83(100), 65, 55, 39; \(^1\)H and \(^{13}\)C NMR see table 2.

3. Results and Discussion

Fraction M is bright orange powder, insoluble in water but soluble in acetone and ethanol. \(^1\)H NMR spectra indicated similarities with previous research that identified as curcumin [8, 9]. Comparison of \(^1\)H NMR spectra of isolated compound and curcumin is shown in table 1[8].

| No | \( \delta \) H (ppm) (\( \Sigma \)H, m, J)                  | Isolated compound (1)                  |
|----|----------------------------------------------------------|---------------------------------------|
| 4, 4' | 7.59 (2H, d, \( J=15.8 \))                               | 7.60 (2H, \( dd, J=5.88 \); 15.56)   |
| 6, 6' | 7.15 (2H, \( d, J=2 \))                                   | 7.12 (2H, \( dd, J=1.76 \); 8.08)   |
| 10, 10' | 7.08 (2H, \( dd, J=1.8 \); 9)                             | 7.05 (2H, \( d, J=1.6 \))            |
| 9, 9' | 6.93 (2H, \( d, J=8.2 \))                                 | 6.92 (2H, \( d, J=8.2 \))            |
| 3, 3' | 6.48 (2H, \( d, J=15.8 \))                               | 6.48 (2H, \( dd, J=3.56 \); 15.72)  |
| 1   | 5.81 (1H, s)                                              | 5.86 (1H, s)                         |
| 7, 7'-2 OMe | 3.97; 3.93 (6H, s, s)                                 | 3.95 (6H, s)                         |

\(^{13}\)C NMR spectra of isolated 2 showed a peak of 15 carbon atoms that indicated a sesquiterpene. Carbonyl atom showed at the peak of 200.73 ppm. Signals of four carbons alkenes appear on the \( \delta \)C area from 123.94 to 155.78 ppm. The area of \( \delta \)C 48.72 to 16.93 ppm is carbons alkane comprising of two methine (CH), two methylenes (CH$_2$), and four methyls (CH$_3$), while the peak of \( \delta \)C 70.54 and 73.36 ppm indicated as two carbon atoms that binding to Oxygen (C=O).
$^1$H NMR of compound 2 indicated 14 proton signals. Three signals derived from alkene proton (CH) at $\delta_H$ 5.64 (s, 1H), 5.64 (s, 1H) and 6.05 (s, 1H). This shows that there are no aromatic groups. Methine proton usually found on isoprene. It is supported by the presence of four methyls at 0.88 (d, 3H), 1.30 (s, 3H), 1.88 (s, 3H), and 2.12 (s, 3H). Another methine protons were shown at 2.19 (m, 1H), 2.29 (m, 1H) and 3.79 (dd, 1H, $J = 3.32; 7.24$ Hz). Proton signal methyl indicated at $\delta_H$ 0.88 (d, 3H, 6.6 Hz). For methylene protons themselves are shown in $\delta_H$ (ppm) (m, 1H), 2.25 (m, 1H), 2.45 (dd, 1H, $J = 4.96; 14.92$ Hz).

The relationship between carbon with a proton in the bond, it can be seen from the HSQC spectra. There is three carbon signal that has no correlation with proton signals; it is possible a quaternary carbon. COSY spectra can be used to determine the correlation between proton-proton neighbors. The relationship between proton and carbon within 2-3 ties can be seen from the data HMBC. Correlation HSQC, HMBC, dan COSY are shown in table 2.

Table 2. $^1$H NMR (400 MHz), $^{13}$C NMR (100 MHz, CDCl$_3$) and HSQC, HMBC, dan COSY Correlation of compound 2.

| No. | $\delta_C$ (ppm) | $\delta_H$ (ppm)( m, J Hz) (HSQC corr) | HMBC corr. | COSY |
|-----|-----------------|----------------------------------------|------------|------|
| 1   | 36.51           | 2.29 (1H, m)                           | C7, C14    | H7, H2 |
| 2   | 132.00          | 5.64 (1H, s)                           | C1, C4     | H1   |
| 3   | 132.30          | 5.64 (1H, s)                           | C1, C4     | -    |
| 4   | 70.54           | -                                       | -          | -    |
| 5   | 73.36           | 3.79 (1H, dd, $J=7.24; 3.32$)          | -          | -    |
| 6a  |                | 1.72 (1H, m)                           | C7, C1, C4, C5, C2, C3 | H1, H5 |
| 6b  | 27.86           | 1.82 (1H, ddd, $J=13.8; 6.4; 3.36$)    | -          | H1, H5 |
| 7   | 33.12           | 2.19 (1H, m)                           | C1, C8     | H14, H8b |
| 8a  |                | 2.25 (1H, m)                           | C14, C7    | -    |
| 8b  | 48.72           | 2.45 (1H, ddd, $J=4.96; 14.92$)        | C14, C7, C9 | H7 |
| 9   | 200.73          | -                                      | -          | -    |
| 10  | 123.94          | 6.05 (1H, s)                           | C12, C13, C9 | H13, H12 |
| 11  | 155.78          | -                                      | -          | -    |
| 12  | 20.78           | 2.12 (3H, s)                           | C13, C10, C11 | H10 |
| 13  | 27.70           | 1.88 (3H, s)                           | C12, C10, C11 | H10 |
| 14  | 16.93           | 0.88 (3H, $d, J=6.6$)                  | C7, C1, C8 | -    |
| 15  | 23.82           | 1.30 (3H, s)                           | C4, C5, C3 | -    |

FTIR spectra (figure 1) of isolated compound 2 showed several functional groups, the presence of hydroxyl group (3385 cm$^{-1}$), Carbonyl (1678 cm$^{-1}$) and C-O at 1035 cm$^{-1}$. NMR spectra of isolated 2 suggested the compound have 15 C atoms, 24 H atoms and three O atoms with the molecular formula C$_{15}$H$_{20}$O$_3$. However, mass spectra of compound 2 (figure 1) showed peak maximum at 216 that suggested molecular formula C$_{15}$H$_{20}$O. This fact indicated that compound 2 release two water molecules from chromatography heating process before ionization in the mass spectrometry (figure 2).
Figure 1. Mass spectra and FTIR spectra of compound 2.

Figure 2. The reaction of bisacurone in the GC column under high temperature before ionization in the mass spectroscopy.

4. Conclusion
Based on data analysis FTIR, $^1$H NMR, $^{13}$C NMR, HSQC, HMBC, and COSY, suggested structure compounds 2 are derivatives of bisabolane terpenoids with molecular formula C$_{15}$H$_{24}$O$_3$ (MW = 252), A known compound that is bisacurone. Compound 2 previously has been isolated from the rhizome of the rhizome of Curcuma xanthorhiza [10] and Curcuma longa [11]. Comparison of the spectra suggested that the isolated compound 2 is bisacurone.

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