Potassium Permanganate-Catalyzed Alpha-Pinene Oxidation: Formation of Coordination Compound with Zinc(II) and Copper(II), and Growth Inhibition Activity on *Staphylococcus aureus* and *Escherichia coli*

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Received January 21, 2015; Accepted September 4, 2015

ABSTRACT

*Catalytic oxidation of alpha-pinene was investigated using potassium permanganate as an oxidant. The reaction consumed catalyst following stoichiometric amount instead of the catalytic one. The keto-carboxylate compound **2** was afforded as the oxidation product. Further study of its complex compound with copper(II) and zinc(II) was also reported including their activity for inhibiting the growth of Staphylococcus aureus and Escherichia coli. In overall, the complex compound shows important result by inhibiting the bacterial growth.*

Keywords: *alpha-pinene; catalytic oxidation; antibacterial; natural product*

ABSTRAK

*Telah dilakukan reaksi oksidasi alfa-pinena menggunakan kalium permanganate. Reaksi ini bekerja dengan menggunakan katalis dalam jumlah yang stoikiometris. Produk reaksi yang diperoleh adalah senyawa keto-karboksilat **2**. Studi lebih lanjut terhadap pembentukan senyawa komplek produk **2** dengan tembaga(II) dan seng(II) juga telah dilakukan, termasuk pengujian aktivitas penghambatan pertumbuhan pada bakteri Staphylococcus aureus dan Escherichia coli. Secara umum, senyawa-senyawa kompleks yang dihasilkan menunjukkan aktivitas penghambatan pertumbuhan kedua bakteri.*

Kata Kunci: *alfa-pinena; katalitik oksidasi; antibakteri; bahan alam*

INTRODUCTION

The alpha-pinene is a monoterpenoid compound, a member group of secondary metabolite natural product. It contains C10 with a single C=C double bond and bicyclic [2.2.1]. This compound composes Indonesian turpuntine oil about 60-70% [1-3]. It has also been reported in some literatures contains in essential oil extracted from *Cosmos caudatus*, *Eupatorium odoratum*, *Hyptis species* [4], *Cananga odorata* [5], and *Thymus pectinatus* [6]. Purification under a reduced pressure of fractional distillation of Indonesian turpuntine oils provided almost 99% purity alpha-pinene. It was also reported active in the reducing inflammation in cell level [7], inhibiting of bacterial growth but it was lesser effective than the antibiotic control [2]. Further transformation of its functional group by catalytic hydroxylation and acetyloxylation in acidic condition provided a hydroxylated and acetyloxylated of alpha-pinene derivatives. These new structures slightly increase the solubility in water, and also improve in the activity on *S. aureus* and *E. coli* [8]. However, still come up with an issue related activity below the antibiotic standard, volatility and solubility in water.

The oxidation catalyzed by potassium permanganate, osmium tetroxide and chromic acid of the alpha-pinene double bond can proceed to provide two carbonyl groups or a carbonyl and one carboxyl groups [9] by the cleavage of double bond. Some report has been devoted using different catalyst such as ozone [10-12], complex [Mn(TPP-PEO)Cl] catalyst [13], ruthenium [14-17], rhodium [18], palladium [19], complex of metal-oxalate [20], and silica-supported permanganate [21]. The oxidized of alpha-pinene, then, can coordinate with some metal ions such as Zn, Cu, Pt, and Mo. Moreover, many reports show the important antibacterial activity of diketone complex compound with Cu and Zn [22], aromatic O-N-O ligand with Zn, Cu, Pt, and Co [23], oxime complex compound with Cu [24-25], amino acid complex compound with Co [26], and imidazol-Co [27]. This paper discloses the recent investigation on permanganate catalyzed alpha-pinene oxidation and its complex compounds with zinc(II) and copper(II). The activity in inhibiting bacterial growth is reported as well.

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EXPERIMENTAL SECTION

Materials

Chemicals used for the research included potassium permanganate (Merck), ethyl acetate (Smart Lab), n-hexane (Smart Lab), magnesium sulfate anhydrate (Merck), sodium sulfate anhydrate (Merck), silica gel 60 for column chromatography (Merck), pre-coated TLC Silica F254 (Merck). Alpha-pinene was prepared from turpuntine oil provided by PT. Perhutani Anugerah Kimia, Indonesia and was purified using fractional distillation under reduced pressure following procedure Masruri et al. [2].

Instrumentation

Instrument is applied for analysis included gas chromatography-mass spectrometer (Shimadzu GCMS-QP2010S), infrared spectrophotometer (Shimadzu FTIR-8400S), UV-Vis Spectrophotometer (UV-Vis Shimadzu 1601), refractive index, analytical balance (Mettler Toledo). The chemical drawing was performed using ChemBioDraw Ultra 12.0 while 3D structure with ChemBio3D Ultra 12.0 and was optimized using MM2 method, minimum RMS gradient 0.10, and 10000 iteration.

Procedure

Oxidation of alpha-pinene

Alpha-pinene (10 mL, d 0.864 g/mL, 63.42 mmol), acetone (5.0 mL) and potassium permanganate (10.02 g, 63.42 mmol) were placed in a 50 mL-round-bottom flask and solution sodium hydroxide (10 mL, 0.5 M) was added. This reaction mixture was refluxed until all alpha-pinene was consumed (monitored on TLC). The product was neutralized with solution of hydrochloric acid 0.2 M, filtered, and extracted with ethyl acetate (3 × 10 mL). The ethyl acetate layers was combined, dried over sodium sulfate anhydrate and concentrated under vacuum. The product was purified with flash chromatography using ethyl acetate/n-hexane as solvent and isolated as yellow-brown oil (3.10 g, 26.53% yield). Mass Spectrum (EI): m/z 184 (M^+, 1%), 111 (20), 108 (15), 99 (88), 83 (16), 82 (17), 81 (20), 79 (22), 71 (100), 70 (24), 69 (34), 67 (12), 55 (28), 53 (20), 43 (98), 41 (49), 40 (5). Calculated for $C_{10}H_{16}O_3$ was 184.1099, found 184.11; FTIR (thin film, cm^{-1}) 3419 (O-$H_{stretching}$), 2952-2873 (C-$H_{stretching}$), 1714 (C=$O_{stretching}$).

Synthesis coordination compounds

Oxidation product of alpha-pinene (250 mg, 1.36 mmol) in 5.0 mL ethanol was added zinc chloride (1.0 equiv.). This reaction mixture was refluxed for 2 h to afford the product (540 mg, 92.14%) as pale yellow solid. Further analysis was conducted using FTIR and UV-Vis spectrophotometers. Similar procedure was undertaken using copper chloride.

Evaluation of bacterial growth inhibition

The antibacterial evaluation was conducted using disk-diffusion method. The paper disk was loaded in various concentrations of chemicals tested, and aseptically placed on the bacteria. *Staphylococcus aureus* and *Escherichia coli* was taken as a bacterial tested, and the activity was determined by measuring the inhibition diameter (mm) of the bacterial growth each paper disk. Beside that, tube dilution method was also performed following procedure from Masruri et al. [2].

RESULT AND DISCUSSION

Catalytic Oxidation of Alpha-Pinene

The alpha-pinene as starting material was isolated following procedure Rekfa et al. [1] from turpuntine oils of local producer. Degree of purity was determined from GCMS chromatogram. It was a clear oil with 98% purity. This was further applied for oxidation study. A mixture of alpha-pinene and potassium permanganate was added with solution of sodium hydroxide. Stirring under reflux condition transforms the color of reaction mixture from violet to deep black. A few aliquot of the reaction sample was taken with syringe and subjected for thin layer chromatography to monitor the reaction progress. The disappearing of alpha-pinene spot on the TLC plate as an indication the reaction was accomplished. After completion, reaction mixture was neutralized, and the product was filtered off. Separation and purification of the product was further undertaken under flash chromatography using ethyl acetate-hexane provided pure compound **2**.

Theoretically oxidation of olefins using potassium permanganate in a base condition affords two type products, keto-aldehyde product **3** and keto-carboxylate product **2** (Fig. 1). However, separation using flash chromatography did not observed the presence of product **3**. The infrared spectra revealed the presence of hydroxyl (band at 3419 cm^{-1}), carbonyl group (band at 1714 cm^{-1}), and other supporting band such as methyl and methylene groups between 3000 and 2800 cm^{-1}. No double bond band was observed as indication that all alkene was converted to carboxylate and carbonyl group. And also, no C-H band for aldehyde was recorded as well. These results confirm the existence of the keto-carboxylate product **2** instead of keto-aldehyde product **3** (Fig. 2). Mass spectra from

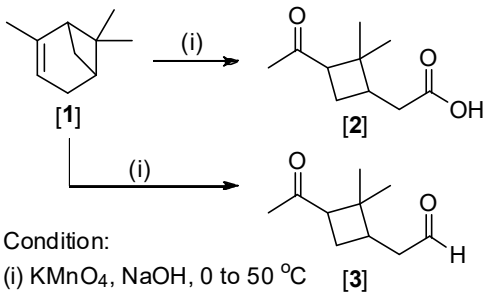

Fig 1. Catalytic oxidation of alpha-pinene with potassium permanganate

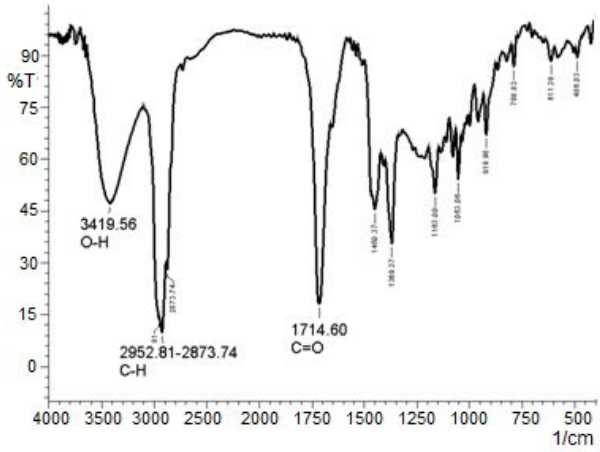

Fig 2. FTIR spectra of the oxidation product from alpha-pinene, **2**

GCMS data also proved product **2**. Radical cation of molecule **2** with m/z 184 was detected beside the base peak at m/z 71.

Complex Compound of 2 with Copper(II) and Zinc(II)

Synthesis of complex compound was undertaken by mixing a mol equivalent of keto-carboxylate product **2** with zinc and copper chloride using ethanol as solvent under reflux. The complex formation was easily observed by changing the reaction color and a solid formation. In Fig. 3, is showed the proposed structure of complex compound of **2** with copper(II) and zinc(II). The complex compounds occur between metal ions with two molecule **2**. Oxygen atom (O13 and O25) of hydroxyl group and oxygen atom (O8 and O21) from carbonyl groups on molecule **2** donates the electron to coordinate with Cu27. Meanwhile a complex compound with zinc(II) was resulted from interaction between Zn27 with oxygen atom (O25 and O13) from hydroxyl group and oxygen atom (O9 and O14) from the carbonyl group. These interactions are significantly supported by the shifting of band absorptions of the main functional groups (hydroxyl and carbonyl groups) the complex compounds in FTIR spectra (Fig. 4).

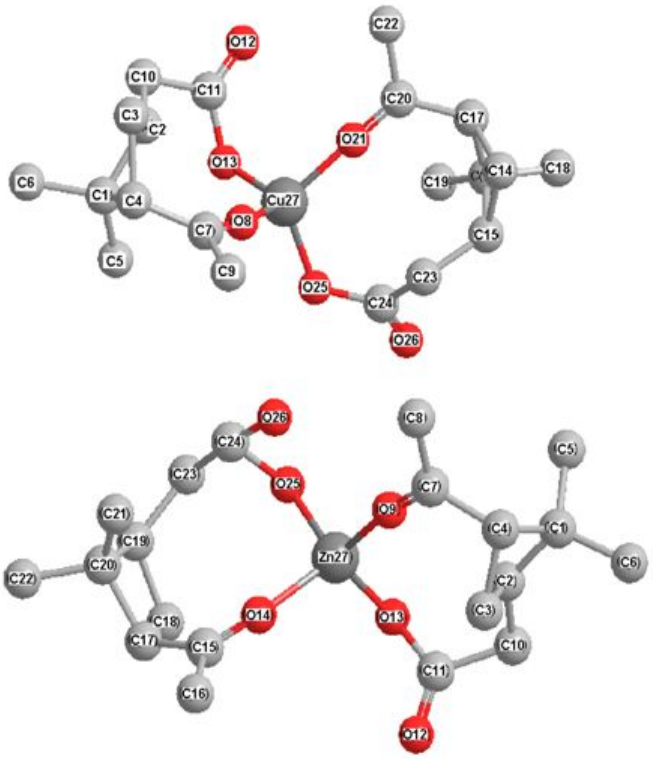

Fig 3. Proposed structure of coordination compound the oxidized product [**2**] with copper(II) and zinc(II). 3D Molecule model was drawn with ChemBio3D Ultra 12.0 from Cambridgesoft and optimized using MM2 method

The complex compound from product **2** with copper(II) shifts the carbonyl group absorption to a lesser value than that in **2** (Fig 4A). It was found in 1650 cm^{-1} while compound **2** was 1714 cm^{-1}. On the other hand, the complex compound with zinc(II) recorded on 1640 cm^{-1}. This absorption is slightly lower than that with copper(II). In addition, the band movement was also observed for hydroxyl group (Fig. 4B). The original hydroxyl absorption was recorded at 3419 cm^{-1}, while in its complex compound with zinc(II), move to a longer wavelength. It was recorded broad in 3600-3200 cm^{-1}. On the other hand, hydroxyl group absorption in complex compound with copper(II) provided two new peaks in 3350 and 3450 cm^{-1}, respectively. Other new vibration pattern was showed in Fig. 4C. These were recorded for Cu-O and Zn-O vibration. It was identified both on 820 and 505 cm^{-1} for Cu-O, and band at 820 and 495 cm^{-1} for Zn-O vibration. These were not sharply observed on FTIR spectra of compound **2**.

Moreover, the ultra violet spectra also provide a clear signal that formation of complex compound between **2** and copper(II) or zinc (II) was occurred (Fig. 5). The right spectra recorded movement of maximum wavelength of **2** to red-shift. The complex compound

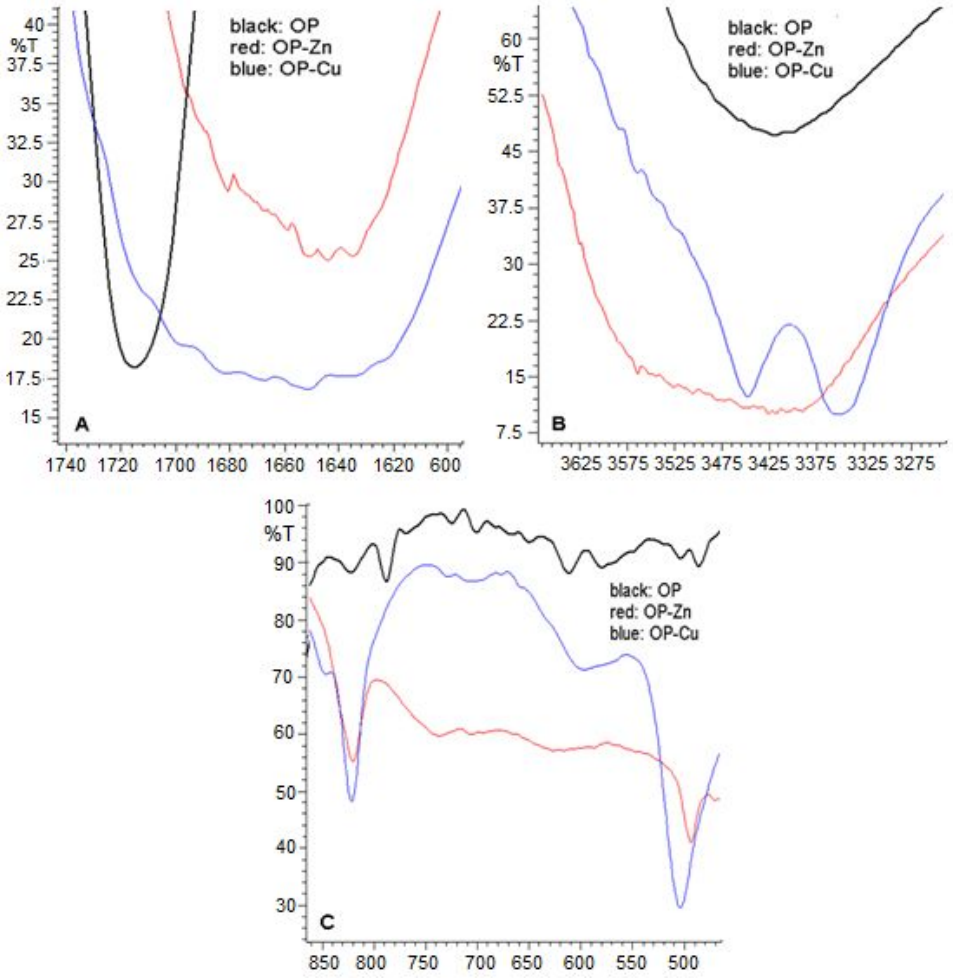

Fig 4. FTIR spectra of complex compound resulted from keto-carboxylate product **2** with copper(II) and zinc(II). **A** is peak expansion for carbonyl group absorption, **B** is a peak expansion for hydroxyl group absorption, and **C** is peaks expansion for Zn-O and Cu-O group vibration. OP is keto-carboxylate product **2,** red line is spectra for complex compound with zinc, and blue line is spectra from complex compound with copper

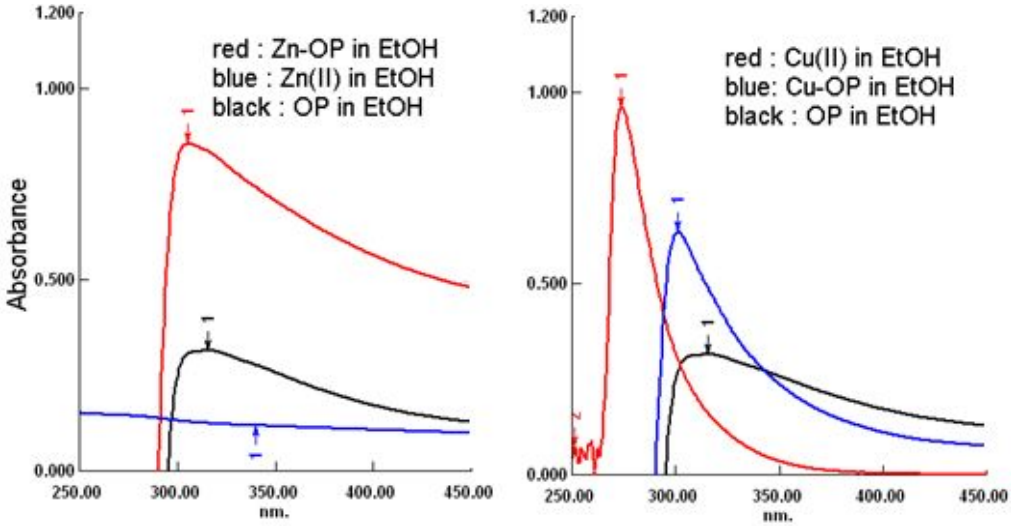

Fig 5. UV-Vis spectra of keto-carboxylate product **2** and its coordination with copper(II) (right) and zinc(II) (left)

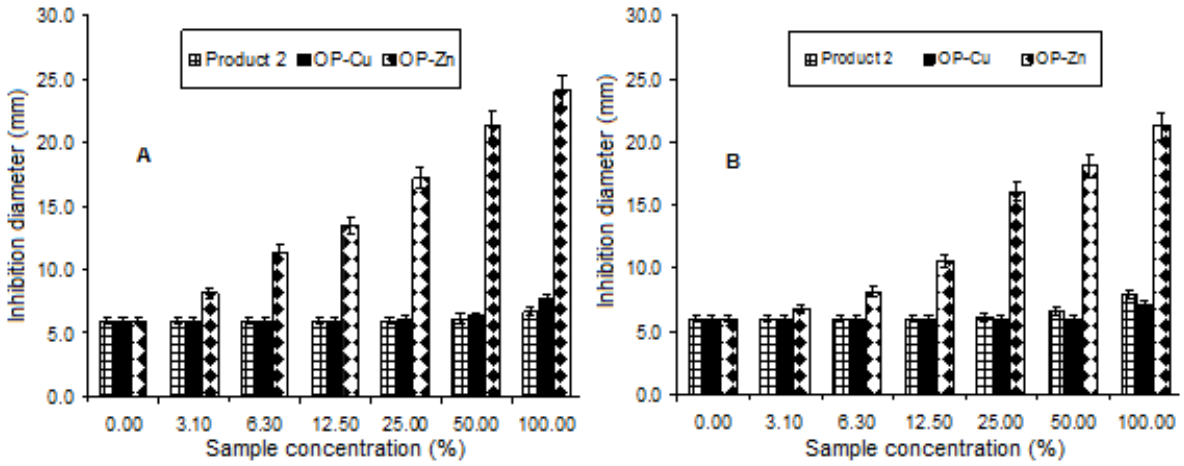

Fig 6. Antibacterial activity of the oxidation product of alpha-pinene, **2** and its coordination compound with copper(II) and zinc(II) using disk diffusion method. Activity of *S. aureus* (**A**) and *E. coli* (**B**). All measurement is conducted using 5% margin error

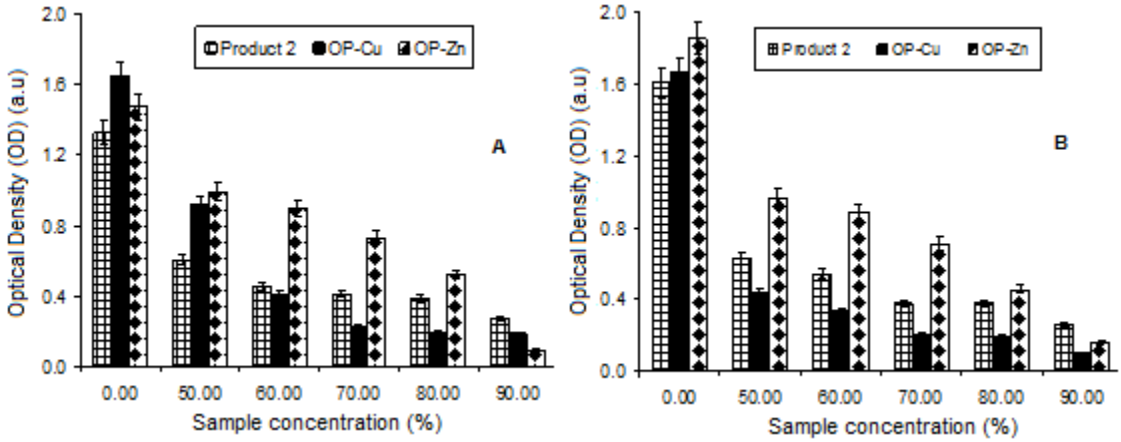

Fig 7. Antibacterial activity of the oxidation product of alpha-pinene, **2** and its coordination compound with copper(II) and zinc(II) evaluated using tube dilution method. Activity on *S. aureus* (**A**) and *E. coli* (**B**). All measurement using margin error 5%

with copper(II) shifts the maximum wavelength from 323 to 305 nm (right), while complex with zinc(II) shifts to 301 nm. Finally, the proposed structure for the complex compound of keto-carboxylate product **2** with metal copper(II) and zinc(II) is concluded as it in Fig. 3.

Antibacterial Evaluation

The antibacterial activity was tested on *S. aureus* and *E. coli* and determined the inhibition of bacterial growth using disk diffusion (Fig. 6) and tube dilution method (Fig. 7). The antibacterial activity for first method was counted based on the diameter inhibition (mm), while the second method using optical density (OD) parameter compared to the control sample. The higher OD values, the higher bacterial concentration growing in the tube, and the lesser its activity.

In a range concentration provided (0 until 100% v/v), the average activity of the oxidation product from alpha-pinene, keto-carboxylate product **2**, gives inhibition diameter less significant in both of bacteria. Similar result was also recorded for sample coordination compound with copper(II). Sharply different, the prospecting result is indicated for sample complex compound with zinc(II). The increasing concentration improves the activity in both bacteria *S. aureus* and *E. coli* (Fig. 6). On the other hand, tube dilution method gives an important result in all range of concentration and in all sample tested for both in *S. aureus* and *E. coli* (Fig. 7). The increasing concentration (from 0 to 90%) improves the bacterial growth inhibition activity. For overall sample tested, the optical density value at 0% recorded between 1.326 and 1.852. This value dropped to between 0.089 and 0.397 when sample tested concentration was 90%.

CONCLUSION

In summary, the catalytic oxidation of alpha-pinene using potassium permanganate as catalyst is working in stoichiometric instead of catalytic amount. The reaction leads to the formation of keto-carboxylate product **2** with low yield. Moreover, the complex compound with copper(II) and zinc(II) was achieved under mild reaction condition in ethanol. The compound resulted has important activity for inhibiting bacterial growth.

ACKNOWLEDGEMENT

This research was supported from DPP/SPP grant from Faculty of Mathematics and Natural Sciences, University of Brawijaya under contract number 11/UN10.9/PG/2013. All of the authors mentioned have an equal contribution to this article.

REFERENCES

1. Amini, R.W., Masruri, and Rahman, M.F., 2014, *Jurnal Ilmu Kimia Universitas Brawijaya*, 1 (1), 147–153.
2. Masruri, Rahman, M.F., and Prasodjo, T.I., 2007, *Jurnal Ilmu-Ilmu Hayati (Life Sciences)*, 19 (1), 32–35.
3. Rodrigues-Corrêa, K.C.S., de Lima, J.C., and Fett-Neto, A.G., 2012, *Food Energy Secur.*, 1 (2), 81–93.
4. Pinheiro, M.A., Magalhães, R.M., Torres, D.M., Cavalcante, R.C., Mota, F.S.X., Coelho, E.M.A.O., Moreira, H.P., Lima, G.C., Araújo, P.C.C., Cardoso, J.H.L., de Souza, A.N.C., and Diniz, L.C.L., 2015, *Pharmacogn. Mag.*, 11 (41), 123–130.
5. Bocci, F., and Frega, N., 1996, *J Am. Oil Chem. Soc.*, 73 (6), 713–716.
6. Vardar-Unlü, G., Candan, F., Sökmen, A., Daferera, D., Polissiou, M., Sökmen, M., Dönmez, E., and Tepe, B., 2003, *J. Agric. Food Chem.*, 51 (1), 63–67.
7. Husnul Khotimah, Diana Lyrawati, and Masruri, 2006, Antiinflammatory effects of alpha-pinene extracted from *Pinus mercusii* on levels of TNFα signalling, iNOS, and apoptosis of neuronal cells, *Proceeding Asian Symposium on Medicinal Plants and Others (ASOMP 2006)*, Padang.
8. Masruri, and Srihardyastuti, A., 2005, *NATURAL J.*, 9 (1), 15–21.
9. Clayden, J., Greeves, N., and Warren, S., 2012, *Organic Chemistry*, 2nd Ed., Oxford University Press, New York.
10. Fisher, G.S., Goldblatt, L.A., and Stinson, J.S., 1951, *Converting alpha-pinene to mixtures of acids*, U.S. Patent 2,750,411
11. Zhang, X., Chen, Z., Wang, H., He, S., and Huang, D., 2009, *Atmos. Environ.*, 43 (29), 4465–4471.
12. Holloway, F., Anderson, H.J., and Rodin, W., 1955, *Ind. Eng. Chem.*, 47 (10), 2111–2113.
13. Liu, S.T., Reddy, K.V., and Lai, R.Y., 2007, *Tetrahedron*, 63 (8), 1821–1825.
14. Carlsen, P.H.J., Katsuki, T., Martin, V.S., and Sharpless, K.B., 1981, *J. Org. Chem.*, 46 (19), 3936–3938.
15. Moglioni, A.G., García-Expósito, E., Aguado, G.P., Parella, T., Branchadell, V., Moltrasio, G.Y., and Ortuño, R.M., 2000, *J. Org. Chem.*, 65 (13), 3934–3940.
16. Ziyat, H., Ali, M.A., Karim, A., Meliet, C., Castanet, Y., and Mortreux, A., 2004, *Acta Chim. Slov.*, 51, 223–230.
17. Padala, K., and Jeganmohan, M., 2012, *Org. Lett.*, 14 (4), 1134–1137.
18. Tan, X., Liu, B., Li, X., Li, B., Xu, S., Song, H., and Wang, B., 2012, *J. Am. Chem. Soc.*, 134 (39), 16163–16166.
19. Liu, Y., Song, R.J., Wu, C.Y., Gong, L.B., Hu, M., Wang, Z.Q., Xie, Y.X., and Li, J.H., 2012, *Adv. Synth. Catal.,* 354 (2-3), 347–353.
20. Rubinstein, A., Jiménez-Lozanao, P., Carbó, J.J., Poblet, J.M., and Neumann, R., 2014, *J. Am. Chem. Soc.*, 136 (31), 10941–10948.
21. Tercio, J., Ferreira, B., Cruz, W.O., Vieira, P.C., and Yonashiro, M., 1987, *J. Org. Chem.*, 52 (16), 3698–3699.
22. Verma, P.N., Sheikh, J.I., and Juneja, H.D., 2011, *World Appl. Sci. J.*, 14 (8), 1154–1157.
23. Aziz, A.A.A., Salem, A.N.M., Sayed, M.A., and Aboaly, M.M., 2012, *J. Mol. Struct.*, 1010, 130–138.
24. Konde, K.J., Patil, V.R., and Malve, S.P., 2004, *Acta Pol. Pharm.*, 61 (2), 123–125.
25. Kuwar, A.S., Shimpi, S.R., Mahulikar, P.P., and Bendre, R.S., 2006, *J. Sci. Ind. Res.*, 65 (8), 665–669.
26. Subhasish, S., Dharumadurai, D., Sarvanan, C., and Annamalai, P., 2009, *FU Phys. Chem. Technol.*, 7 (1), 73–80.
27. Chang, E.L., Simmers, C., and Knight, D.A., 2010, *Pharmaceutical*, 3, 1711–1728.