Profiling polyisoprenoids in kopasanda (*Chromolaena odorata*) stem barks and roots

M Basyuni¹*, M A Fitri² and Sumardi²

¹ Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatera, 20155, Indonesia
² Faculty of Pharmacy, Universitas Tjut Nyak Dien, Medan, Indonesia

*E-mail: m.basyuni@usu.ac.id

Abstract. Kopasanda (*Chromolaena odorata (L.) R.M. Rob, Asteraceae*) distributed in tropical and subtropical countries, including Indonesia. The kopasanda leaves contain several major compounds, such as tannins, phenols, flavonoids, saponins, and steroids. However, polyisoprenoids have no previously reported from this plant. This study aimed to determine the distribution of polyisoprenoid compounds in the bark and roots of the kopasanda plant. Total lipid on the kopasanda bark and roots was 61 and 48 mg/g dry weight. In comparison, the value of polyisoprenoid ranges from 11.8 and 27.3 mg/g dry weight. Results showed that polyisoprenoid compounds found in the roots were polyprenols and dolichols, regarded as type-II of polyisoprenoids. In contrast, in the bark of kopasanda, polyisoprenoid compounds a member of are a type I. Because dolichol dominated on bark, no polyprenols were detected. The present study confirmed the occurrence of polyisoprenoids in kopasanda roots and barks.

1. Introduction
Indonesia is a tropical country that has been known as a producer of various agricultural commodities, including medicinal plants. The people in Indonesia have long done the use of plants as medicinal materials. The use of plants as medicine is also increasingly diverse ethnic [1]. One of the plants commonly used by the community as therapeutic content is the kopasanda plant (*Chromolaena odorata*). The leaves contain several significant compounds, such as tannins, phenols, flavonoids, saponins, and steroids [2-4]. Kopasanda plants that have been tested is the antifungal activity of kopasanda leaf extract on the antifungal test carried out on the fungus *Candida albicans* *Microsporum canis* [5-6].

Moreover, the antibacterial activity test of kopasanda leaf extracts positively against *Staphylococcus aureus* and human pathogens [5-6]. Kopasanda leaf has traditionally been used as a medicine in wound healing, mouthwash to treat sore throat, cough medicine, malaria medicine, antimicrobial, headache, antidiarrheal, antiseptic, antihypertensive, anti-inflammatory and diuretic [7]. However, research on polyisoprenoid content profiles in the bark and roots of kopasanda plants has never been scientifically studied. Scientific information is expected to increase knowledge so that analysis can be utilized further on kopasanda plants.

Polyisoprenoid is divided into polyprenol and dolichol. Polyisoprenoid is composed of straight polymers consisting of several to more than 100 isoprenoid units that have been identified in almost all living things, both animals and plants [8-10]. Polyprenol is found in plant photosynthetic tissues [11], and bacterial cells, whereas dolichol is typical of animal lipids and yeast [12]. They are also found in
plant roots [9-10,13-14]. Polyisoprenoid alcohol is widely distributed in mangrove plants. In thin-layer chromatography analysis of polyisoprenoid compounds, polyprenol and dolichol were found with different lengths and short chains. Two-phase TLC is usually referred to as two-dimensional TLC. It is a method that can allow the use of a broader stationary phase to separate mixtures containing many components. In addition, this method, allowing the separation of mixtures containing components of very different polarity. The present study aimed to determine the distribution of polyisoprenoid compounds in the bark and roots of the kopasanda plant using 2D-TLC.

2. Materials and Method

2.1. Plant materials
Plant material, roots, and barks of kopasanda (Chromolaena odorata (L.) R.M. Rob, Asteraceae) was collected, from Paluh Marbau Tanjung Rejo Percut Sei Tuan, Deli Serdang Regency, North Sumatra (3° 72’ 43.47” E, 98°74’ 10.9” N).

2.2. Isolation of polyisoprenoid
Kopasanda barks and roots were dried at 60°C-75°C for 2-3 days. Then crushed into a fine powder, then weighed with a dry weight of 5 grams and put in a shake bottle, then soaked in solvent chloroform and methanol (2: 1) for 48 hours [8]. After 48 hours filtered with filter paper and funnel into a vial. The filter results are regained before saponification [14]. Lipid extract from Kopasanda, which has been dried, added a concentration of 0.45 g KOH (2 grams), 2 ml of ethanol, and 2 ml of distilled water, then tightly closed with parafilm and duct tape so that water does not enter the vial bottle and damage the sample. After being tightly closed, the saponification sample at 65°C for 24 hours in a water bath. Saponification is carried out until the extract becomes non-lipid saponification) or was soaked. The saponification results are re-oven until completely dry. Kopasanda bark and root, which has been saponified, is then dissolved with n-hexane and ready to be analyzed [14].

2.3. 2D-TLC analyzed samples
The first dimension of TLC was carried out for 60 minutes on silica-gel (20 x 3 cm) with a toluene-ethyl acetate (9: 1) solvent system [13]. Edge longitudinally of the first dimension TLC with a width of 1 cm and a concentration zone of a phase reverse C-18 TLC clamped using two magnets stick to hinder any gel phase. The bound TLC plate is then developed perpendicular to the first dimension to transfer polyprenol and dolichol to the reverse TLC phase concentration zone.

The second dimension of the reverse silica-TLC phase is carried out with acetone solvent for 30 minutes. The polyisoprenoid alcohol position is separated and developed by two-dimensional TLC silica-gel, then identified and visualized with iodine vapor (iodine vapor). Image chromatography obtained is then scanned. The concentrations of polyprenol and dolichol detected in RP-18 HPTLC were measured using Imagel with dolichol and polyprenol standards as a reference [15].

3. Results and Discussion
Table 1 shows the total lipids of polyprenol and dolichol from each of the root tissue and stem bark of C. odorata. Table 1 shows that total lipids in stem bark tissue were 61 mg/g and root tissue 48 mg/g with total lipids. The amount of polyisoprenoid in stem bark tissue was 27.3 mg/g, while the value of polyisoprenoid in root tissue was 11.8 mg/g. The distribution of polyprenols and dolichols in polyisoprenoids can be grouped into three types (I, II, III) [8, 13-14]. In class I, dolichol dominated over polyprenol (more than 90%). In contrast, type II, which traced polyprenol and dolichol compounds can be found with a presence ratio of polyprenol and dolichol. Whereas type III, the dominance of polyprenol compounds over dolichols, which was more than 90%. In the stem bark tissue of Kopasanda, it was found that the bark belongs to type-I, however, in the roots belong to type-II (Table 1).
Table 1. Occurrence and distribution of polyisoprenoids in Kopasanda roots and barks

| Tissue | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in TL | % in PI | Type |
|--------|--------------|--------------|------------|------------|---------|---------|------|
| Roots  | 48.4±4.0     | 11.8±1.5     | 9.0±2.4    | 2.8±1.8    | 24.4    | 18.6    |     |
|         |              |              |            |            |         |         | II   |
| Barks  | 61.6±5.0     | 27.3±2.6     | nd         | 44.3       | nd      | 44.3    |     |
|         |              |              |            |            |         |         | I    |

nd= not detected, TL = Total lipids, PI = Polyisoprenoids, Pol = Polyprenols, Dol = Dolichols. Data are presented as mean of triplicate analyses.

Table 2 shows that dolichol dominates in the bark of kopasanda, no polyrenold found. In contrast to the roots, there was dolichol and the majority of polyprenols. For kopasanda roots, it contained a carbon chain length of polyprenols of C70-C 90 and dolichols of C70-C80. While for bark had a long chain of carbon dolichol C50 - C 75. Analysis of the content of polyisoprenoid compounds on the bark and roots of Kopasanda was similar to the previous reports [16-17]. The study was contrary to other studies that dolichols were predominated in the roots of mangrove plants [13], coastal plants [8], rambutan [14], and soybean [18].

Table 2. Carbon-chain lengths of polyprenol and dolichol of four mangrove associates

| Tissue | Polyprenol | Dolichol |
|--------|------------|----------|
| Root   | 70 75 80 85 90 | 70 75 80 |
| Bark   | nd         | 50 55 60 65 70 75 |

The polyisoprenoid position is separated and developed into small and large spots with acetone solvent on silica gel and visualized using color using iodine vapor. Polyisoprenoids can be useful for characterizing taxonomies in classifying plants (chemotaxonomy), the carbon chains vary depending on the species, often only found in one or a group of different species [8-9, 12-14]. Several studies were analyzing polyisoprenoid compounds, polyprenol, and dolichol in mangroves and plants showing antibacterial [19-20], antiviral [21-22], and anticancer [23-25]. These studies suggested that polyisoprenoid extracts were potentially for the development of antimicrobial, antiviral, and anticancer drugs and agents. Furthermore, polyisoprenoid plants observed in this study prove that the polyisoprenoid found in the bark and roots and needed further investigations the function of polyisoprenoids in kopasanda plant.

4. Conclusions

Polyisoprenoid compounds found in the roots of kopasanda belong to type II polyisoprenoid, both polyprenol and dolichol. Whereas in the stem bark of kopasanda was a member of type I polyisoprenoid, which dolichol dominated over polyprenol.

Acknowledgment

This work was funded by TALENTA grant 2018 from Universitas Sumatera Utara (No. 227/UN5.2.3.1/PPM/KP-TALENTA USU 2018).

References

[1] Hamilton AC 2004 Medicinal plants, conservation, and livelihoods. Biodivers Conserv 13: 1477–1517.
[2] Vijayaraghavan, K., Rajkumar, J., & Seyed, M. A. (2018). Phytochemical screening, free radical scavenging and antimicrobial potential of Chromolaena odorata leaf extracts against pathogenic bacterium in wound infections–a multispectrum perspective. Biocatalysis and agricultural biotechnology, 15, 103-112.
[3] Akinmoladun, A. C., Ibukun, E. O., & Dan-Ologe, I. A. (2007). Phytochemical constituents and antioxidant properties of extracts from the leaves of Chromolaena odorata. Scientific research and essays, 2(6), 191-194.

[4] Suriyavathana, M., Parameswari, G., & Shiyan, S. P. (2012). Biochemical and antimicrobial study of Boerhavia erecta and Chromolaena odorata (L.) King & Robinson. International Journal of Pharmaceutical Sciences and Research, 3(2), 465.

[5] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[6] Stanley, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[7] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[8] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[9] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[10] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[11] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[12] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[13] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[14] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[15] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[16] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[17] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[18] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[19] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.

[20] Vital, P. G., & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. Journal of medicinal plants Research, 3(7), 511-518.
[21] Wang, C. Z., Li, W. J., Tao, R., Ye, J. Z., & Zhang, H. Y. (2015). Antiviral activity of a nanoemulsion of polypropenols from ginkgo leaves against influenza A H3N2 and hepatitis B virus in vitro. Molecules, 20(3), 5137-5151.

[22] Safatov, A. S., Sergeev, A. N., Shishkina, L. N., Pyankov, O. V., Poryvaev, V. D., Bulychev, L. E., ... & Boldyrev, A. N. (2000). Effect of intramuscularly injected polypropenols on influenza virus infection in mice. Antiviral Chemistry and Chemotherapy, 11(3), 239-247.

[23] Sari, D. P., Basyuni, M., Hasibuan, P. A., Sumardi, S., Nuryawan, A., & Wati, R. (2018). Cytotoxic and antiproliferative activity of polyisoprenoids in seventeen mangroves species against WiDr colon cancer cells. Asian Pacific Journal of Cancer Prevention: APJCP, 19(12), 3393.

[24] Illian, D. N., Basyuni, M., Wati, R., & Hasibuan, P. A. Z. (2018). Polyisoprenoids from Avicennia marina and Avicennia lanata inhibit WiDr cells proliferation. Pharmacognosy Magazine, 14(58), 513.

[25] Istiqomah, M. A., Basyuni, M., & Hasibuan, P. A. Z. (2020). Apoptotic with Double-Staining Test, P53, and Cyclooxygenase-2 to Proliferation Colon Cancer Cell (WiDr) of Dolichol in Three Mangrove Leaves. Open Access Macedonian Journal of Medical Sciences, 8(A), 37-42.