Difference Triterpenoid and Phytosterol Profile between *Kandelia candel* and *K. obovata*

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Abstract: *Kandelia*, a genus belonging to Rhizophoraceae has been reported to have two distinct species: *K. candel* and *K. obovata*. Mangrove plants are known to produce secondary metabolite mostly derived from isoprenoid (triterpenoid and phytosterol). Isoprenoid composition of leaves and roots of *K. candel* (L.) Druce and *K. obovata* Sheue, Liu & Yong were investigated and compared. Triterpenoid and phytosterol profile of both species was analyzed using Gas Chromatography with Flame Ionization Detector (GC-FID). Both species displayed difference composition either in the leaves or roots. In the leaves of *K. candel*, eight isoprenoids detected, with dominating of β-amyrin, a member of triterpenoid. The ratio between triterpenoid and phytosterol was 73.2%:26.8%. By contrast, phytosterol dominated the isoprenoid proportional in the roots of *K. candel* (91.7%). Similar results were found in the *K. obovata* leaves and roots, a predominated phytosterol over triterpenoid, 59.6%, and 97.9%, respectively. The present work suggested diversity composition of isoprenoid in both *Kandelia.*

1 INTRODUCTION

Mangroves are known to produce triterpenoids and phytosterols (or called isoprenoids) (Volkman 2005; Basyuni et al., 2007), as well as the cases for other many plant species (Yendo et al., 2010; Thimmappa et al., 2014). These tree species are not only a source of genes encoding enzymes in the triterpenes and phytosterol biosynthetic pathways but also potential plants that may have prospective medicinal and agricultural value (Sari et al., 2018). These mangrove characteristics may expose another prospect of mangrove use.

A number of reports have involved triterpenoids as compatible evidence for the main origin of organic matter from mangrove due to their immovability during sedimentation and diagenesis (Killops and Frewin, 1994; Versteegh et al., 2004; Koch et al., 2011).

Because of their varied array of biological properties, isoprenoids are recognized as necessary as pure prospective sources for medicinal activities. Several biological activities have been described for triterpenes: anti-inflammatory activity for taraxerol, α-amyrin, β-amyrin, lupeol and germanicol (Kim et al. 2005; Melo et al., 2011); anti-carcinogenic activity for taraxerol and germanicol (Jang et al. 2004); insecticidal property for taraxerol (William, 1999), cardioprotective impact in hypercholesterolemic syndrome for lupeol (Sudhahar et al., 2007), hepatoprotective counter to acetaminophen-induced hepatotoxicity for α- and β-amyrin (Olievera et al.,

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2005), Stigmasterol, plentifully available in *Acanthus illicifolius*, has been shown to possess hypercholesterolemic cases (Kokpol et al., 1986), antimicrobial activity for polyisoprenoid (Sumardi et al., 2018), anticancer colon property for polyisoprenoid including dolichol (Illian et al., 2018; Sari et al. 2018a,b).

Triterpenes are mostly accumulated in plants as their glycosides and saponins. In order to get more insight into biological and medicinal functions of triterpenoid and phytosterol in mangrove plants, *Kandelia candel*, and *K. obovata*, it is; therefore, the study is aimed to analyze the composition of isoprenoid. Here we report the different composition of triterpenoid and phytosterol of *Kandelia candel* and *K. obovata* leaves and roots.

2 MATERIALS AND METHOD

Kandelia obovata leaves and roots were collected from Okukubi River, Okinawa, Japan. Leaves and roots of *K. obovata* were obtained from PT. Kandelia Alam, Kubu Raya District, West Kalimantan, Indonesia. These materials were taken directly into dry ice and stored at –20 ºC for further investigation.

The leaves or roots (5 g wet weight, correspondingly) were first crushed in liquid nitrogen and extracted with chloroform-methanol (2:1 by volume) (CM21). The cell wall debris insoluble in CM21 was discarded by filtration through No. 2 filter paper (Advantec, Tokyo, Japan), and the extract was incompletely purified for lipid analysis as reported previously (Basyuni et al., 2007).

The lipid extract consists of 2 mg of total lipid, then saponified at 60 ºC for 24h with 3% KOH in 94% ethanol. The nonsaponifiable lipids (NSL) separated into hexane by robust infuse were determined by gas chromatography (GC 2010). The column used was CBP1-M50-025 (Shimadzu), and the column temperature was set up from 1 min hold at 50 ºC to a final temperature of 300 ºC at a rate of 10 ºC/min as earlier described (Basyuni et al., 2007, 2012).

3 RESULTS

Table 1 shows the triterpenoid and phytosterol composition of *K. candel* leaves and roots. In the leaves, β-amyrin, a member of triterpenoid has the most significant proportion (38.5%), then followed by lupeol and α-amyrin. By contrast, in the roots of *K. candel*, stigmasterol (49.6%), a member of phytosterols predominated over triterpenoids (91.7%: 8.3% in ratio). This finding was supported by previous results on the tree of *K. candel* leaves with major components was β-amyrin (45.2%) and α-amyrin (18.0%) (Basyuni et al. 2007). Similarly, in the roots of *K. candel* tree, the essential compounds were phytosterols (β-sitosterol, stigmasterol, and campesterol) (Basyuni et al., 2007).

Table 1: Triterpenoid and phytosterol composition in *K. candel* leaves and roots.

Tissue	RT (min)	Area	Compound	Proportion (%)
	41.490	2507.3	Campesterol (1)	2.6±0.2
	42.395	24252.7	Stigmasterol (2)	4.9±0.8
	44.285	69363.8	β-sitosterol (3)	14.8±0.6
Leaves	45.072	7228.1	Lanosterol (4)	1.8±0.4
	46.864	180485.3	β-amyrin (5)	38.5±0.8
	46.864	10812.2	Cycloartenol (6)	2.7±0.4
	47.122	100902.4	Lupeol (7)	21.3±0.5
	47.334	60279.2	α-amyrin (8)	13.5±0.2
	41.490	2507.3	Campesterol (1)	18.2±1.1
	42.395	24252.7	Stigmasterol (2)	49.6±0.8
Roots	44.285	69363.8	β-sitosterol (3)	20.3±3.9
	45.072	7228.1	Lanosterol (4)	3.7±1.8
	45.752	180485.3	β-amyrin (5)	2.8±0.9
	46.864	10812.2	Cycloartenol (6)	3.2±0.4
	47.122	100902.4	Lupeol (7)	2.3±0.5

Table 2: Triterpenoid and phytosterol composition in *K. obovata* leaves and roots.

Tissue	RT (min)	Area	Compound	Proportion (%)
	41.750	32959.8	Campesterol (1)	6.3±0.8
	42.661	81775.6	Stigmasterol (2)	18.3±1.7
	44.580	146898.3	β-sitosterol (3)	33.5±3.8
Leaves	45.655	8322.1	Lanosterol (4)	1.4±0.5
	46.031	88652.9	β-amyrin (5)	17.4±1.6
	46.450	9037.7	Lupenone (6)	2.0±0.1
	47.432	108905.9	Lupeol (7)	21.0±2.6
	41.750	2507.3	Campesterol (1)	16.6±0.7
	42.666	24252.7	Stigmasterol (2)	46.8±0.5
Roots	44.565	69363.8	β-sitosterol (3)	34.5±0.2
	45.800	180485.3	β-amyrin (4)	2.1±0.4

Table 2 compiles the isoprenoid profile in the leaves and roots of *K. obovata*. The isoprenoid profile of *K. obovata* leaves was not similar compared *K. candel* leaves. Phytosterols dominated over triterpenoids in the leaves. However, in case of roots were rather similar, phytosterols were the main components of both *K. obovata* and *K. candel* (Tables 1 and 2).

In the seedlings stage of *K. candel* leaves, the main components were β-amyrin, lupeol and α-amyrin, while the phytosterols were minor composition

(Basyuni et al., 2009). Parallel with the results, phytosterols especially stigmasterol was detected to be the dominant compound in the roots of *K. candel* seedlings (Basyuni et al., 2009, 2012).

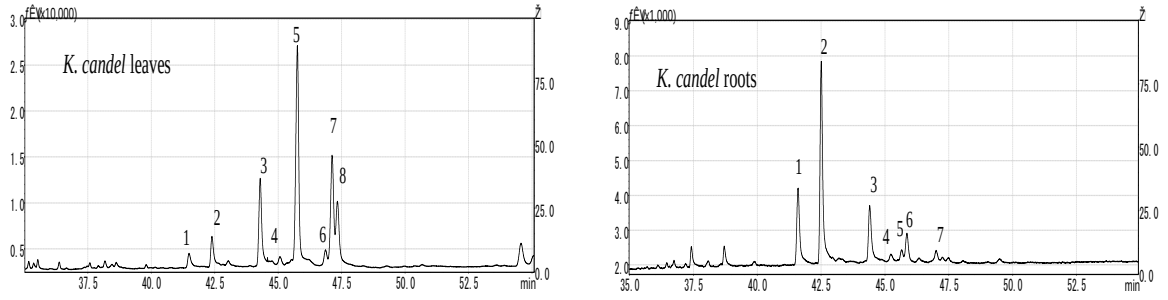

Figure 1: GC-FID profile of *K. candel* leaves and roots. For the compound name, please refer to Table 1.

Furthermore, a bit different composition was shown by *KcMS* multifunctional triterpene synthase from *K. candel*. Lupeol had 50% proportion then followed by β-amyrin and α-amyrin (25%) (Basyuni et al., 2006). The occurrence of triterpenoids (lupeol, β-amyrin, and α-amyrin) as previously reported for fatty acid esters in leaves and roots of *K. candel* seedlings (Oku et al., 2003).

Figure 1 and 2 depict the GC-FID profile of *K. candel* and *K. obovata* leaves and roots. The identification of triterpenoid and phytosterol in the GC profile mainly by the analogy of their retention time on the GC column with those authentic standards and analysis of the mass spectrum (Basyuni et al., 2007, 2012).

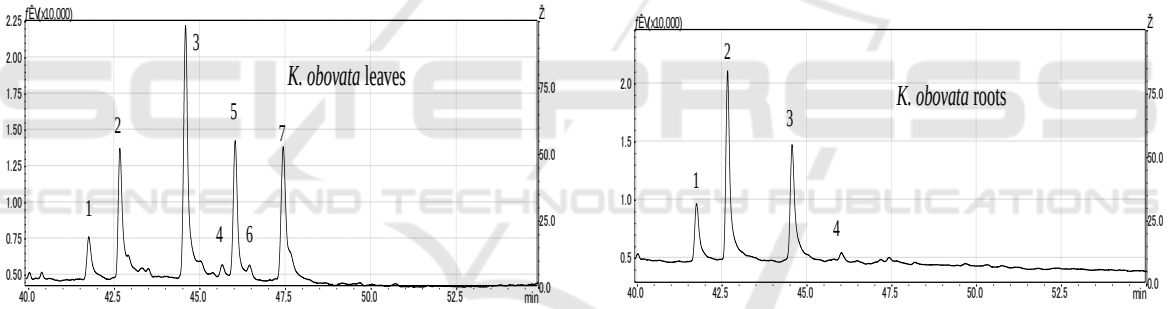

Figure 2: GC-FID profile of *K. obovata* leaves and roots. For the compound name, please refer to Table 2.

4 CONCLUSIONS

Difference isoprenoids composition between *K. candel* and *K. obovata* leaves and roots have been confirmed in this study. In the leaves of *K. candel*, the dominating of β-amyrin, a member of triterpenoid with contrast to the roots, phytosterol dominated the isoprenoid proportional. *K. obovata* leaves and roots, a predominated phytosterol over triterpenoid was found. The present work suggested diversity composition of isoprenoid in both *Kandelia*.

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