Polyisoprenoids profile and composition from selected mangrove associates leaves

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Abstract. This present study describes the polyisoprenoids (polyprenol/dehydrodolichol and dolichol) profile, and composition from selected mangrove associates leaves namely \textit{Amorphophallus paeoniifolius}, \textit{Borassus flabellifer}, \textit{Finlaysonia maritima}, and \textit{Pandanus tectorius}. The occurrence and distribution of polyisoprenoids were investigated using a convenient two-plate thin layer chromatography method. The polyisoprenoid profile in the leaves was determined and classified into two types. Type-I, having domination of dolichols over polyprenols (100\%) was found in \textit{P. tectorius}. These dolichols occurred one dolichol family (C\textsubscript{60}–C\textsubscript{95}). Type-II, displaying the existence of both dehydrodolichols and dolichols, was confirmed in \textit{A. paeoniifolius}, \textit{B. flabellifer}, and \textit{F. maritima}. Dolichol contents were slightly more abundance found than polyprenols (in the ratio of approximately 60\%:40\%) in these three species. Polyprenols and dolichols with the chain length of C\textsubscript{80}–C\textsubscript{90}, respectively, detected in \textit{A. paeoniifolius}. Ficaprenol (C\textsubscript{50}–C\textsubscript{70}) was only found in \textit{B. flabellifer}. Dolichols also occurred longer-chains in \textit{B. flabellifer} (C\textsubscript{90}–C\textsubscript{105}) and \textit{F. maritima} (C\textsubscript{65}–C\textsubscript{130}). The present study suggested that the patterns of shorter-chain and longer-chain polyprenol, shorter and longer dolichols are regulated in mangrove associates.

1. Introduction
Indonesia has the most extensive mangrove area worldwide, which is 22.6 \% of the world wide overall, with the length of coastline approximately at 95,181 km [1]. Mangrove forests in Bali province mainly distributed in Tanjung Benoa and Forest Park Conservation Area (\textit{Taman Hutan})
Raya) Ngurah Rai, Nusa Lembongan, and National Park (Taman Nasional) Bali Barat [2]. Mangrove plants along with coastal plants are known to produce secondary metabolites including polyisoprenoid alcohols [3-5].

The existence of polyisoprenoids has been described in some plant tissues either in vegetative: green foliage of plants and roots [3-5], and generative: flowers, fruits, and seeds [6-8]. These studies showed the ubiquitous spreading of polyisoprenoids in the Plantae. The occurrence and distribution of polyisoprenoid in mangrove and coastal plants have been reported [3-5]. To obtain more insight into the function and significant polyisoprenoids in mangroves, the detail information on the occurrence of mangroves are entirely required. Therefore present study aimed to analyze the polyisoprenoids (polyrenol and dolichol) profile and composition from selected mangrove associates leave namely A. paeoniifolius, B. flabellifer, F. maritima, and P. tectorius to extend our previous works.

2. Materials and methods

2.1 Chemicals
In this study, the identification of polyisoprenoid has used a standard of dolichols (C₉₀₀-C₉₅), and polyrenols (C₉₀₀-C₁₀₀) as earlier reported [3]. The determination of the family correlating to dehydrodolichols or dolichols was carried out in triplicate independent works. Silica gel 60 TLC glass plates and silica RP-18 HPTLC glass plates were obtained from Merck (Darmstadt, Germany).

2.2 Plant materials
The leaves of A. paeoniifolius (Areceaceae), B. flabellifer (Areceaceae), F. maritima (Asclepiadaceae), and P. tectorius (Pandanaeace) were collected from Taman Hutan Raya Ngurah Rai, Bali, Indonesia, in May 2017. These mangrove associates exist expose to sunlight. The mean temperature in the month of the sampling was 30-32°C with a mean value of humidity 75-77%. All of the samples were maintained in freezer until used.

2.3 Separation of polyisoprenoid alcohols
The protocol for the separation of dolichols from dehydrodolichols as earlier reported [5]. Briefly, the leaves of fou rspecies were oven-dried at 65-70°C for 48 h. The shriveled tissue (5 g each) was wrinkled into a fine grain and submerged in chloroform/methanol (2/1, v/v) solvent for two days. The crude lipid extract of leaves was thensaponified and dissolved with hexane, and this organic solvent was dried-up and treated with hexane.

2.4 Investigation by two-plate thin layer chromatography
Separation of polyrenol from dolichol was investigated by two-plate TLC [5-6]. This procedure involved the development of two different plates, firstly, a silica gel plate was done with toluene-ethyl acetate (9:1) solvent system of as earlier reported [6]. The TLC plate was then developed vertically to move polyrenol or dolichol to the central area of the reversed-phase plate.

The secondly, the reversed-phase plate was carried out with acetone for about 60 min. The spots of dehydrodolichols, dolichols, and standard solutions are distinguished and then characterized and imaged with iodine vapour. The enhanced chromatographic was scanned with a Canon E-470 printer. The polyisoprenoid was detected by the association of movement on a plate with that of standards of dolichol or dehydrodolichol that were used in reversed-phase run. The dehydrodolichols and dolichols on chromatogram plates were measured using ImageJ version 1.46r [9], with dolichol and dehydrodolichol standards as criterions.

3. Results and Discussion

3.1. Polyisoprenoid profile and composition
The exploration for polyisoprenoids compound from the leaves of A. paeoniifolius, B. flabellifer, F. maritima, and P. tectorius in Bali province, Indonesia was performed using 2D-TLC [3-4] resulted in
the clear separation of polyprenols and dolichols concerning the carbon chain length. Tables 1-2 summarize the measurable examination of polyisoprenoids and polyprenols and dolichols profile and composition with the carbon-chain lengths given each species. The quantity of TL was the largest in *F. maritime* leaves (932.2 mg g\(^{-1}\) dw) and the minimum in *P. tectorius* (541.8 mg g\(^{-1}\) dw). By contrast, the amount of PI was the highest in *P. tectorius* (50.2 mg g\(^{-1}\) dw), the minimal concentration of PI was in *F. maritime* (5.6 mg g\(^{-1}\) dw). The comparable findings on TL and PI contents of the present study were also have been reported for North Sumatran coastal leaves [5] and *Nephellium lappaceum* various tissues [6]. The TL and PI values in coastal and mangrove associates plants presented in this study were higher than those described from major and minor components of mangrove plants [3-4].

**Table 1. Polyisoprenoids contents in four mangrove associate leaves**

| Species        | Tissue   | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in TL | % of PI |
|----------------|----------|--------------|--------------|------------|------------|---------|---------|
| A. paeonifolius| leaves   | 669.3±13.8   | 12.8         | 5.5        | 7.3        | 2.0     | 0.8     |
| B. flabellifer | leaves   | 579.4±12.4   | 33.0         | 12.1       | 20.9       | 5.7     | 2.1     |
| F. maritima    | leaves   | 932.2±14.6   | 5.6          | 2.1        | 3.5        | 0.6     | 0.2     |
| P. tectorius   | leaves   | 541.8±17.5   | 50.2         | nd         | 50.2       | 9.2     | nd      |

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

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

| Species        | Tissue | Polyprenol (C\(_{43}\)) | Dolichol |
|----------------|--------|-------------------------|----------|
| A. paeonifolius| leaves | 80 85 90                | 80 85 90 |
| B. flabellifer | leaves | 50 55 60 65 70          | 50 55 60 70 75 80 85 90 95 100 105 |
| F. maritima    | leaves | 0 90 95 100             | 110 115 120 125 130 |
| P. tectorius   | leaves | 60 65 70 75 80 85 90 95 |

3.2. Analysis polyisoprenoid by two-plate TLC

The profile of dehydrodolichols and dolichols in the leaves of *A. paeonifolius*, *B. flabellifer*, *F. maritima*, and *P. tectorius* were categorized as earlier defined [3-6] into two types (I and II) (Tables 1-2). In type-I, the majority of dolichols over dehydrodolichols (100%) was detected in *P. tectorius* leaves (Table 2, Figure 1D). These dolichols occurred one dolichol family (C\(_{80}-\)C\(_{90}\)). Type-II, showing the existence of both dehydrodolichols and dolichols, was observed in the leaves of *A. paeonifolius*, *B. flabellifer*, and *F. maritima*.

Dolichol contents were faintly more plenty found than polyprenols (around 60%-40%) in these three species (Figure 1A-C). No type-III leave, the dominating polyprenols over dolichols was not detectable. Polyprenols and dolichols with the carbon chain-length of C\(_{80}-\)C\(_{90}\), respectively, detected in *A. paeonifolius* (Figure 1A). Ficaprenol (C\(_{50}-\)C\(_{70}\)) was only found in *B. flabellifer* (Figure 1B). Dolichols also happened longer-chains in *B. flabellifer* (C\(_{50}-\)C\(_{105}\)) and *F. maritima* (C\(_{65}-\)C\(_{130}\)). It is interesting to note that Bombiprenone(C\(_{43}\)) was detected in *A. paeonifolius* leaves and *F. maritime* leaves (Table 2, Figure 1A-C).

The dominance of dolichols over polyprenols have been reported in mangrove plants leaves from Okinawa and North Sumatra [3-4]. These study suggested that the significant polyisoprenoids were dolichols, not polyprenols. By contrast, the presence of both polyprenols and dolichols are more likely occurred in mangrove associates including coastal plants [5] and this study. Furthermore, in the plant world (mainly green foliage tissues), polyprenols are plentifully identified compare to dolichols [6-8, 10-11]. The present study, therefore, reveals the occurrence of shorter and longer polyprenols, shorter and longer dolichols are modulated in the plant kingdom, including in mangrove associates.
Figure 1. Two plate-TLC chromatograms of hexane extract from the leaves of A. paeoniifolius (A), B. flabellifer (B), F. maritime (C), and P. tectorius (D). The carbon numbers indicate the carbon-chain length of polyisoprenoid alcohols.

Recently it has been reported that salinity changes the polyisoprenoid contents in four salt-secretor and non-salt-secretor mangroves [12]. The alteration of polyisoprenoids including in polyprenols, dolichols, and bombiprenone as well. Bombiprenone has occurred in various tissues such as leaves [3-5, 12], roots [3-5], flowers [6], and fruits [8].

4. Conclusion
The present study confirmed the profile and composition of polyisoprenoids in A. paeoniifolius, B. flabellifer, F. maritime, and P. tectorius from Bali province, Indonesia. The present study also indicated that the pattern of shorter-chain and longer-chain polyprenol, shorter and longer dolichols are regulated in mangrove associates.
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