Diversity and abundance of polyisoprenoid composition in coastal plant species from North Sumatra, Indonesia

MOHAMMAD BASYUNI1*, RIDHA WATI1, HIROSHI SAGAMI2, SUMARDI3, SHIGEYUKI BABA4, HIROSUKE OKU5

1Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Dharma Ujung No. 1 Medan 20155, North Sumatra, Indonesia. Tel./Fax: +62-61-820-1920. *email: m.basyuni@usu.ac.id
2Institute of Multidisciplinary Research for Advanced Material, Tohoku University. 2-1-1 Katahira, Aoba-ku, Sendai, 980-8577, Japan
3Faculty of Pharmacy, Universitas Tjut Nyak Dhien. Jl. Rasmi No. 28 Medan 20123, North Sumatra, Indonesia
4Molecular Biotechnology Group, Tropical Biosphere Research Center, University of the Ryukyus. 1 Senbaru, Nishihara Okinawa 903-0213, Japan
5International Society for Mangrove Ecosystems, Faculty of Agriculture, University of the Ryukyus. 1 Senbaru, Nishihara, Okinawa 903-0213, Japan

Abstract. Basyuni M, Wati R, Sagami H, Sumardi, Baba S, Oku H. 2018. Diversity and abundance of polyisoprenoid composition in plant species from North Sumatra, Indonesia. Biodiversitas 19: 1-11. The distribution and abundance of polypropenols (pol) and dolichols (dol) in the leaves and roots of fourteen coastal plants from North Sumatra, Indonesia were analysed using two-dimensional thin layer chromatography. In the leaves, with respect to the distribution of pol and dol were detected and categorized into three-types. In type-I, the predominance of dol over pol, was observed in Barringtonia asiatica, Calophyllum inophyllum, Pandanus odoratissimus, and Stachytarpheta jamaicensis. In type-II, the presence of both pol and dol, was observed in Casuarina equisetfolia, Melastoma candidum, Morinda citrifolia, Scyphiphora hydrophyllacea, Sesuvium portulacastrum and Terminalia catappa. In type-III, the predominance of pol over dol, was observed in Acacia auriculiformis, Hibiscus tiliaceus, Ricius communis, and Pongamia pinnata. However, in the roots, a type-I distribution was observed in eleven species, while three species, A. auriculiformis, M. candidum, and M. citrifolia, corresponded to a type-II distribution instead of type-III. The diversity of polyisoprenoid composition in the leaves was noted, whereas 79% of root tissues indicated that dol occur more abundantly than pol. The range of the contents of polyisoprenoid was 12-300 mg/g dw. The present study indicated that pol and dol could be useful in the classification of mangroves and other coastal forests and in phylogenetic studies. The diversity and presence of polyisoprenoids in coastal plants suggested that plant polyisoprenoids are chemotaxonomically important.

Keywords: Chemotaxonomy, coastal plant, polyisoprenoid, semi-mangrove, two-dimensional thin layer chromatography

INTRODUCTION

Mangroves are woody plant communities that grow in the intertidal coastal zone in tropical and subtropical climates. Indonesia has the largest area, comprising 22.6% of the world’s mangroves (Giri et al. 2011). Mangrove plants are generally divided into two groups, namely, true or exclusive mangroves and non-exclusive mangroves. The non-exclusive species are mainly distributed in the terrestrial or coastal environment but also within other mangroves and are considered associated mangroves, semi-mangroves, or coastal plants. True mangrove species grow in a limited environment and do not extend into other coastal plant communities (Tomlinson, 1986; Wang et al. 2011). The lipid and isoprenoid content of Indonesian mangroves has been previously reported (Basyuni et al. 2012a; 2013). Polyisoprenoid (PI) alcohols are secondary metabolites that constitute a group of hydrophobic polymers widely distributed among living organisms, both in euksaryotes and prokaryotes (Swiezewska and Danikiewicz 2005; Skrórupinska-Tudek et al. 2008). The occurrence and distribution of polyisoprenoids in 14 true North Sumatran mangroves from Indonesia have been described (Basyuni et al. 2017).

Two main types of polyisoprenoid alcohols have been reported with respect to the OH-terminal (α-) isoprene unit. These include polypropenol (α-unsaturated) and dolichol (α-saturated) compounds (Figure 1). The occurrence of polyisoprenoids has been reported in tropical and subtropical plants (Swiezewska et al. 1994; Basyuni et al. 2016), bacteria (Wolucka et al. 1994), yeast (Grabinska and Palamarczyk 2002), fungi (Wojtas et al. 2004), and animals (Sagami et al. 1992; Rezanska and Votruba 2001; Ishiguro et al. 2014). Despite the ubiquitous diversity of polyisoprenoids in the plant kingdom, their biological role in plants is poorly understood, particularly in coastal plants.

A number of studies have shown that the occurrence and distribution of lipids, as well as polyisoprenoids, may be considered as a plant chemotaxonomic marker (Hogg and Gillan 1984; Swiezewska et al. 1994; Basyuni et al. 2007). These studies demonstrated that lipid, isoprenoid, and polyisoprenoid compounds exhibit a distinct character
and pattern that can be used to distinguish plants, including coastal plants, into systematic genera and families. To get more insight into the biological function and chemotaxonomic significance of polyisoprenoids, it is important to understand the distribution and occurrence of polyisoprenoids in coastal plants. However, few studies have focused on the distribution of polyisoprenoids in coastal plants. The present study on coastal plants extends the previous work on North Sumatran mangroves and describes the distribution and occurrence of polyisoprenoids in fourteen species of North Sumatran coastal plants in Indonesia for the first time, with an emphasis on chemotaxonomic significance.

MATERIALS AND METHODS

Plant materials

The leaves and roots of fourteen coastal plants, including mangrove associates, from Sembilan Island, North Sumatra, Indonesia, were collected in August 2016: *Acacia auriculiformis* Cunn. ex Benth. (Fabaceae), *Barringtonia asiatica* (L.) Kurz (Lecythidaceae), *Calophyllum inophyllum* L. (Guttiferae), *Casuarina equisetifolia* L. (Casuarinaceae), *Hibiscus tiliaeus* L. (Malvaceae), *Melastoma candidum* D. Don (Melastomataceae), *Morinda citrifolia* L. (Rubiaceae), *Pandanus odoratissimus* (Pandanaceae), *Pongamia pinnata* (L.) Pierre (Fabaceae), *Ricinus communis* Linn. (Euphorbiaceae), *Scyphiphora hybrida* Gaertin. f. (Rubiaceae), *Sesuvium portulacastrum* (L.) L. (Aizoaceae), *Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae), and *Terminalia catappa* L. (Combretaceae). 30-40 leaflet samples were collected from single trees of coastal plants. (not clear what this means)

In this study, the classification of coastal plants, including mangrove associates, belong to evergreen plants was derived from a number of reports. *A. auriculiformis* is classified as a coastal plant by (Boland et al. 1990). Baba et al. (2013) classified the following species as coastal beach and dune (coastal) plants, whereas Tomlinson (1986), Kitamura et al. (1997) and Wang et al. (2011) classified them as mangrove associates. These species are *B. asiatica*, *C. inophyllum*, *C. equisetifolia*, *H. tiliaeus*, *M. candidum*, *M. citrifolia*, *P. odoratissimus*, *P. pinnata*, *R. communis*, *S. portulacastrum*, *S. jamaicensis*, and *T. catappa*. Furthermore, *S. hydrophyllacea* was included as a minor element of mangroves (Tomlinson 1986).

All of the fresh samples were kept at -20 °C until use. The age of the leaves was estimated to be approximately 2-5 months. The age of trees was about 2-3 years old. The light exposition of all the analyzed leaves was similar among species and naturally exposure to natural sunlight. The average temperature in the month of the collection was 29 °C with an average humidity of 74%.

Instrumentation

The instrumentation used in this study included a mass spectrometry equipped with electrospray ionization (ESI-MS, Burker Daltonix), chamber chromatography (Sigma-Aldrich), a water bath (Scientific laboratory), and an oven (Memmert).

Chemicals

A mixture of dolichol (C<sub>90</sub>-C<sub>105</sub>) standard compounds was isolated from horse testicles together with a mixture of polyplreno (C<sub>90</sub>-C<sub>100</sub>) from *Malus* sp. (Swiezewska and Danikiewicz 2005). A mixture of dolichol (C<sub>95</sub>-C<sub>110</sub>) standards derived from skipjack tuna livers (Ishiguro et al. 2014) was also used in this study. The identification of the family corresponding to polyplreno or dolichols was performed in at least three independent experiments. Bombiprenone (C<sub>41</sub>) (Figure 1), as described by Irvine et al. (1972), was purified by the silica-gel chromatography of unsaponifiable lipids of the CHCl<sub>3</sub>/CH<sub>3</sub>OH (2:1) extract of dry perilla leaves, and the purified fraction was confirmed by mass spectrometry equipped with electrospray ionization (ESI-MS), sodiated molecules with [M + Na]<sup>+</sup> ions were detected with m/z 625.53183, corresponding to C<sub>41</sub>H<sub>90</sub>O (bombiprenone). Silica gel 60 TLC plates and reversed-phase silica RP-18 HPTLC plates were obtained from Merck. All of the other chemicals and solvents were of reagent grade (Merck).

Procedures

**Isolation of polyplreno alcohols**

The procedure for the isolation of polyplreno compounds was performed as previously described (Sagami et al. 1992; Basugeni et al. 2016; Arifiyanto et al. 2017). The leaves and roots were dried at 60 °C for 2 days. The dried tissue (2 g each) was crushed into a fine powder and immersed in 30 mL of chloroform/methanol (2:1, v/v) solvent for 48 h. The total lipid (TL) extract of the leaves and roots was saponified at 65 °C for 24 h in 50% ethanol containing 2 M KOH. TLs are defined as a fraction of a crude lipids estimated gravimetrically. The unsaponifiable lipids of each tissue sample were extracted with hexane, and the organic solvent was evaporated and re-dissolved in hexane. The leaf (≈100 ug) and root (≈200 ug) extracts were applied to each TLC plate.

**Figure 1.** Structure of polyplreno, dolichol, and bombiprenone. n shows the number of internal isoprene residues
**RESULTS AND DISCUSSION**

**Occurrence and profile of polyisoprenoids in coastal plants**

Table 1 summarizes the quantitative analysis of pol and dol content in fourteen North Sumatran coastal plant leaves and roots. The total lipids are expressed as a fraction of crude lipids gravimetrically estimated. The quantity of total lipid was largest in *R. communis* leaves and *C. inophyllum* roots. The quantity of polyisoprenoids was highest in *S. hydrophyllacea* leaves and *P. odoratissimus* roots. The lowest content of polyisoprenoids was in the leaves of *P. pinnata* and the roots of *M. citrifolia* (Table 1). Chloroform/methanol extract-derived lipids were analysed by 2D-TLC.

Table 2 summarizes the occurrence and distribution of pol and dol with the carbon-chain lengths given for each family. The structural groups of pol and dol in the leaves were classified as previously described (Basyuni et al. 2016; 2017) into three types (I, II, and III). In type-I, the predominance of dol over pol (nine-fold) was observed in *B. asiatica*, *C. inophyllum*, *P. odoratissimus*, and *S. jamaicensis*. In *B. asiatica*, a trace amount of pol with chain-lengths similar to those of dol was detected. Dol that were much longer than pol in chain-length were also found (Figure 2.A).

However, in the leaves of *P. odoratissimus*, *C. inophyllum*, and *S. jamaicensis* (Figure 2.B, and Figures S1.B and S1.F, respectively), polyisoprenoids with chain-lengths similar to those of dolichols were not detected, as these species only contained 100% dolichols (Table 2). In type -II, the occurrence of both pol and dol was observed in *C. equisetifolia*, *M. candidum*, *C. inophyllum*, *S. hydrophyllacea*, *S. portulacastrum* and *T. catappa* (Table 2). In the leaves of *M. candidum*, *C. inophyllum*, *S. hydrophyllacea*, and *T. catappa*, pol (ficaprenols and longer polyisoprenols) with a chain-length similar to that of dol were detected, as shown in Figures 2.C, 2.D, and Figures S1.E and S1.H. In *S. portulacastrum* leaves, chain length differed between polyisoprenols and dolichols, i.e., ficaprenol (C60-C65) and dolichols (C75-C90), as shown in Supplementary Figure 1G. In the leaves of *M. candidum*, *C. inophyllum*, *S. hydrophyllacea*, and *T. catappa*, polyisoprenoids much longer than dolichols (>C100 and more) in chain-length were also detected, as shown in Figures 2.C and 2.D and Figures S1.E and S1.H (See Table 2).

As for type-III, the occurrence of pol over dol (more than nine-fold), which was observed in the case of Okinawan mangroves (Basyuni et al. 2016), was observed also in this study of North Sumatran mangrove associates. Interestingly, as shown in Figures 2.E and 2.F and Figure S1.D, the leaves of species *A. auriculiformis*, *R. communis*, and *P. pinnata* were distinguished from the others in that these species contained shorter-chain polyisoprenols only, ficaprenol-like chain length (C60-C65) and that dolichols and longer polyisoprenols were present in no detectable level.

In the roots, the predominance of dol over pol (more than nine-fold) was observed in eleven species (*B. asiatica*, *C. equisetifolia*, *C. inophyllum*, *H. tiliaceus*, *P. odoratissimus*, *P. pinnata*, *R. communis*, *S. hydrophyllacea*, *S. jamaicensis*, *S. portulacastrum*, and *T. catappa*), similar to that found in the type-I leaves. In these eleven species, it is noteworthy that dol with no pol were observed (Figures 3.A-C, and Figures S2.A-H, respectively). A significant amount of polyisoprenols and dolichols was observed in the roots of three species (*A. auriculiformis*, *M. candidum*, and *M. citrifolia*) (Figures 3.D-F), similar to that in type-II leaves. The distribution of predominance of pol over dol, similar to that in type-III leaves, was not observed in any mangrove root species.
Cluster analysis of polyisoprenoid data

The cluster analysis does not show species relationship; however, it shows the similarities of species based on isoprenoids data. Figure 4 depicts the species similarities from the leaf polyisoprenoid carbon-chain lengths from 23 true mangrove and mangrove associate species. These data revealed that the 23 mangrove species largely fall into two groups (Figure 4). One group was a cluster of nine species including five true mangroves (Avicennia marina, Phemis acidula, Lumnitzera racemosa, Sonneratia alba, and S. hydrophyllacea). Three mangrove associates (M. candidum, M. citrifolia, and T. catappa) with long-chain polyisoprenoids are also included in this group. It is interesting to note that a mangrove associate (B. asiatica) belongs to this group and was close to A. marina. Both species had longer-chain dolichols. On the other hand, this group was a clustering of seven species (L. racemosa, M. candidum, M. citrifolia, P. acidula, S. alba, S. hydrophyllacea, and T. catappa) that showed the occurrence of polyisoprenols that were much longer than dolichols in chain-length (Basyuni et al. 2016).

The other group was a cluster of fourteen species, in which major mangrove associates form this branch (79%). Major mangrove species from Rhizophoraceae tribes were included in this group. Only three true mangroves (the Rhizophoraceae family, B. gymnorhiza, K. obovata, and R. stylosa) are included in this group. It is interesting to note that shorter-chain polyisoprenols (ficaprenol-type) were detected in three mangrove associates (A. auriculiformis, P. pinnata, and R. communis), which are also included in this group. These species, along with H. tiliaeus, H. littorialis, and E. agallocha (Basyuni et al. 2016), also form a distinct branch in this group (Figure 4).

The species similarities from the root data of carbon-chain lengths for 23 species also revealed two major groups (Figure 5). The first group contained only two species, namely, P. acidula and L. racemosa, both true mangrove species known to produce longer dolichols (Basyuni et al. 2016). The second group comprised of 21 species, including major mangrove species such as Rhizophoraceae, Acanthaceae, and Sonneratiaceae. A. marina (Acanthaceae, previously known as Avicenniaceae) formed a branch with K. obovata, possibly due to the similarity of the dolichol carbon-chain length C_{60}-C_{95}. The Rhizophoraceae tribe formed a distinct branch consisting of the true mangroves, K. obovata, R. stylosa, and B. gymnorhiza. In the case of Sonneratiaceae, which consists of only S. alba, it was scattered among mangrove associate branches in the cluster.
analysis. It is noteworthy that the largest branch included 13 mangrove species, where 92% were mangrove associates. Only one species, *S. alba*, was joined with this branch. *H. littoralis* was categorized as a mangrove associate (Wang et al. 2011) when grouped in this branch. However, three mangrove associates, namely, *E. agallocha* (Wang et al. 2011), *M. citrifolia* (Kitamura et al. 1997), and *M. candidum* (Kitamura et al. 1997), were scattered among the true mangroves (Figure 5).

**Discussion**

The analysis of polyisoprenoids in the leaves of Indonesian coastal forests indicates that the occurrence of both polyprenols and dolichols is less prevalent than that of pol or dol. These observations are slightly opposite to the leaves and roots of mangrove forests, where the major polyisoprenoid alcohols are dolichols rather than polyprenols. Dolichols were found in all tissues of North Sumatran mangroves (Basyuni et al. 2017). In the case of Okinawan mangrove leaves, types I, II, and III are found, and in the same mangroves roots, roots types I and II are found (Basyuni et al. 2016). We reported that dolichols were predominant in mangrove leaves and roots (Basyuni et al. 2016; 2017). On the other hand, in the analysis of polyisoprenoids in the leaves of mangrove plants, the major polyisoprenoid alcohols are not polyprenols but dolichols. However, consistent results were obtained in the roots of coastal plants, where 79% of root tissues indicated that dolichols were dominant over pol, as similarly found in mangrove roots in Okinawa and Indonesia (Basyuni et al. 2016; 2017).

**Table 2.** Carbon-chain lengths of polyprenol and dolichol occurring in 14 Indonesian coastal plants*

| Species            | Tissue | Bom (C43) | Polyprenol | Dolichol |
|--------------------|--------|-----------|------------|----------|
| *A. auriculiformis*| Leaves | o 60 65   | 80 85 90 95| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *B. asiatica*      | Leaves | o 80 85 90| 80 85 90 95| 115 120 125 130 135 140 |
| *C. equisetifolia* | Leaves | o 75 80 85| 75 80 85 90| 115 120 125 |
| *C. inophyllum*    | Leaves | o 60 65   | 60 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *H. tiliaceus*     | Leaves | o 45 50 55| 45 50 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *M. candidum*      | Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *M. citrifolia*    | Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *P. pinnata*       | Leaves | o 60 65   | 60 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *P. odoratatissima*| Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *R. communis*      | Leaves | o 80 85 90| 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *S. hydrophyllacea*| Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *S. jamaicensis*   | Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *S. portulacastrum*| Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |
| *T. catappa*       | Leaves | o 75 80 85| 75 80 85 90 95 100 105 110 115 120 125 130 135 140 |

Note: *The numbers refer to the carbon-chain length of the polyisoprenoid alcohols. Bom: Bombiprenone. The chain length of the main polyisoprenoid alcohols is indicated in bold. O = detected.*
Figure 2. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *B. asiatica* leaves, B. *P. odoratissimus* leaves, C. *M. candidum* leaves, D. *M. citrifolia* leaves, E. *A. auriculiformis* leaves, and F. *R. communis* leaves. The number indicates the carbon-chain length of the polyisoprenoid alcohols.

Figure 4. Dendrogram depicting the similarities of species based on carbon-chain length leaves data of polyisoprenoids by log (10) transformation using the Euclidean distance of 13 North Sumatran coastal plants and 10 Okinawan mangrove species. UPGMA: unweighted-pair group method with arithmetic mean. For species name, see Table 1. Am, *Avicennia marina*; Bg, *Bruguiera gymnorrhiza*; Ea, *Excoecaria agallocha*; Hl, *Heritiera littoralis*; Ht, *Hibiscus tiliaceus*; Ko, *Kandelia obovata*; Lt, *Lumnitzera racemosa*; Pa, *Pemphis acidula*; Rs, *Rhizophora stylosa*; and Sa, *Sonneratia alba*.
**Figure 3.** 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *H. tiliaceus* roots, B. *S. portulacastrum* roots, C. *S. jamaicensis* roots, D. *M. candidum* roots, E. *M. citrifolia* roots, and F. *A. auriculiformis* roots. The number indicates the carbon-chain length of the polyisoprenoid alcohols.

**Figure 5.** Dendrogram depicting the similarities of species based on carbon-chain length roots data of polyisoprenoids by log (10) transformation using the Euclidean distance of 14 North Sumatran coastal plants and 9 Okinawan mangrove species. For species name, see Table 1 and Figure 4.
It has been suggested by Tateyama et al. (1999) that the chain length of dolichols varies from tissue to tissue, even in the same species, and appears to form distinct families with dominating molecular species. Pol also occurred as one or two polyprelon families, specifically ficaprenol-type polyprelon (shorter polyprelon) and longer polyprelon, depending on the plants and tissues. The type-III contained only shorter-chain polyprelon, this distribution pattern is similar to the case in the families of Euphorbiaceae, Lauraceae, Magnoliaceae, and Moraceae (Skorupinska et al. 1994; Skorupinska-Tudek et al. 2003).

Two polyprelon families were detected in the leaves M. candidum, M. citrifolia, S. hydrophyllacea, and T. catappa. Our current results, therefore, support our previous findings of two polyprelon families in the yellow leaves of K. obovata and the leaves of L. racemosa and P. acutula (Basyuni et al. 2016). In contrast, dolichols, which are largely detectable in mangroves and may be regarded as typical in mangrove and coastal plant roots, occurred as one dolichol family in all tissues observed, with a variety of carbon-chain lengths depending on the coastal plant species and tissue. These results are in agreement with previously reports that dolichols were highly dominant over polyprelon of the same chain-length in the roots of Hevea brasiliensis (Tateyama et al. 1999), Coluria geoides (Skorupinska-Tudek et al. 2003), and mangroves (Basyuni et al. 2016; 2017). A distinctive feature of polyisoprenoids is their occurrence in leaf tissues as a mixture of homologous, more complicated polyprelon (ficaprenol; medium and longer prelon). In contrast, dolichols in the root family are quite "narrow" (6-8 dolichols) when accumulated in this tissue (Surmacz and Swiezewska 2011). However, as has been reported recently (Basyuni et al. 2016; 2017) and in the present study, dolichols also occurred as longer-chains in several mangrove and coastal leaves, Avicennia marina (C65-C130), Lumnitzeraca racemosa (C60-C140), Phemips acutula (C50-C140), Sonneratia alba (C60-C130), Acanthus ilicifolius (C60-C125), Aegiceras cornulatum (C60-C140), S. caseolaris (C50-C120), A. auriculiformis (C50-C140), M. candidum (C50-C115), M. citrifolia (C60-C140), and T. catappa (C70-C120). In higher plants, the biosynthesis of polyisoprenoids is one of many fascinating reactions found in nature, and their biosynthetic pathway has been shown to be a complicated and divergent system of connections between different cellular metabolisms and metabolic pathways (Swiezewska and Dąbrowski 2005; Skorupinska-Tudek and Swiezewska 2008). Moreover, the occurrence of multiple families of polyisoprenoids in plant tissues, including in mangroves and coastal forests, could be a product of different biosynthetic pathways either simultaneously or sequentially active in a different condition of plants (Chouda and Jankowski 2005). These results, therefore, suggest that the formation of shorter-chain polyprelon, longer-chain polyprelon, shorter-chain dolichols, and longer-chain dolichols are independently regulated in higher plants, including coastal plants.

Dol were predominant in 11 of 14 coastal root tissues and mangrove plants (Basyuni et al 2016; 2017). Therefore, the occurrence of dolichols in the tissues examined implies that polyprelon may not play an important role in coastal plants, although the function of polyprelon in the plant world remains obscure. The apparent predominance of dolichols may be the result of either the coastal or mangrove zone in tropical or sub-tropical climatic conditions (Basyuni et al. 2016).

Cluster analysis using the polyisoprenoid carbon-chain lengths leaf data revealed that the 23 mangrove species fell into two groups: the true mangrove group and the mangrove associates/coastal group. Major coastal species were included in the second group. It is very plausible that the presence polyprelon or dolichol family is responsible for the formation of polyisoprenoids in this group. As a result, the composition of polyisoprenoids may be a reflection of the distribution of tissues in these plants. Our results supported the previous report on the differed reliability between true mangroves and mangrove associates in leaf traits and osmotic properties (Wang et al. 2011). Furthermore, the majority of coastal forests/mangrove associates clustered into one group is in good agreement with the classification by Tomlinson (1986) to distinguish true mangroves from mangrove associates. Coastal plant species in this study generally belong to the Barringtonia formation, which occurs behind the pes caprae formation. These tree species are B. asiatica, T. catappa, M. citrifolia, H. tiliaceus, and E. eugeniaefolia (Baba et al. 2013). Further inland, the shrub P. odoratissimus occurs alongside the trees C. inophyllum and P. pinnata (Baba et al. 2013). These results suggest that the occurrence of polyisoprenoids in leaf tissues served as a plant chemotaxonomic criterion and was effective for the classification of true mangroves and mangrove associates growing in tropical regions.

In this regard, the circumstance of Rhizophoraceae is in agreement with our previous results on the molecular evolution of the Rhizophoraceae family (Basyuni et al. 2007). Kandelia is more similar to Rhizophora than to Bruguiera or Ceriops, even though they originated from the same tribe of Rhizophoraceae. A number of phylogenetic studies on the Rhizophoraceae tribe based on molecular markers and morphological characters suggest this view (Parani et al. 1998; Setoguchi et al. 1999).

Rhizophoraceae also form a distinct branch, as demonstrated from the root data of the carbon-chain lengths of polyisoprenoids. These three species, representing four genera (Kandelia, Rhizophora, Ceriops and Bruguiera) of Rhizophoraceae, are characterized by viviparous propagules, which is the most distinguishing feature of mangroves (Setoguchi et al. 1999; Basyuni et al. 2016). They also belong to non-secretor species based on salinity management and do not have salt glands or salt hairs to remove excess salt (Tomlinson 1986). However, they do have an ultra-filtration mechanism in the roots for excluding salt. The different distribution between polyprelon and dolichols including chain length in this study may reflect on their salt tolerance and zonation (Basyuni et al. 2012b; 2016).
We considered the possibility that the presence of the three branches of coastal plants was due to an evolutionary tree of mangrove plants. The reasons for the generation of this cluster are not yet known, although the characterization of polyisoprenoids from other coastal plants and mangrove associates may provide an explanation. Therefore, the species-specific reproducibility of the polyisoprenoids in leaf and root tissues resulted in its consideration as a chemotaxonomic marker and tissue-specific variation should also be taken into consideration (Swiezewska and Danikiewicz 2005). This present results therefore agreed with previous report on the concept of long-chain polyrenols serve as the chemotaxonomic markers (Roslinka et al. 2002; Basyuni et al. 2016).

These findings suggest that the distribution of lipid analysis, including polyisoprenoids, may provide clear chemotaxonomic markers in mangrove and coastal plant leaves and roots allowing the classification into appropriate genera and families. These findings also support view that the lipids of mangroves are chemotaxonomically significant (Hogg and Gillan 1984; Basyuni et al. 2007a,b; Basyuni et al. 2016). Future studies are needed to understand whether dolichols in mangrove plants function as sugar-carrier lipids in the biosynthesis of N-glycoproteins and whether the existence of polyrenol reductases in coastal plant leaves, which catalyze the conversion of polyrenol to dolichol and corresponds to the SRD5A-3 protein in animals, differs from those of other coastal plants in reduction activity (Pattisom and Amtmann 2005). Further experiments are also necessary to clarify the physiological significance of polyisoprenoid alcohols under environmental stresses.

In conclusion, the present study, together with our previous results on Okinawan and Indonesian mangroves using the 2D-TLC technique, indicated that pol and dol could be useful in the classification of mangroves and other coastal forests and in phylogenetic studies. Simplicity and reproducibility provide this approach with an edge over traditional TLC. Cluster analysis demonstrated that polyisoprenoid patterns in the leaves and roots generally form a separation between true mangroves and coastal plants/mangrove associates, suggesting that plant polyisoprenoids are chemotaxonomically important.

ACKNOWLEDGEMENTS

This work was supported by a BPPTN Research Grant (No. 6049/UN5.1.R/PPM/2016 to MB) from the Universitas Sumatera Utara and partly by an International Research Collaboration and Scientific Publication Grant (No. 017/SP2H/LT/DRPM/II/2016 to MB) from the Directorate for Research and Community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia. The authors are grateful to Dr Ewa Swiezewska (Polish Academy of Sciences) for providing the mixtures of polyisoprenoid standards.

REFERENCES

Arifiyanto D, Basyuni M, Sumardi, Putri LAP, Siregar ES, Risnasari I, Suhaputra I. Occurrence and cluster analysis of palm oil (Elaeis guineensis) fruit type using two-dimensional thin layer chromatography. Biodiversitas 18: 1487-1492.

Baba S, Chan HT, Akkorinkose S. 2013. Useful Products from Mangrove and other Coastal Plants. ISME Mangrove Educational Book Series No. 3. International Society for Mangrove Ecosystems (ISME), Okinawa, Japan, and International Tropical Timber Organization (ITTO), Yokohama, Japan: 2-3.

Basyuni M, Oku H, Baba S, Takara K, Iwasaki H. 2007a. Isoprenoids of Okinawan mangroves as lipid input into estuarine ecosystem. J Oceanogr 63: 601-608.

Basyuni M, Oku H, Tsujimoto E, Baba S. 2007b. Cloning and functional expression of cycloartenol synthases from mangrove species Rhizophora stylosa Griff. and Kandelia candel (L.) Druce. Biosci Biotechnol Biochem 71:1788-1792.

Basyuni M, Putri LAP, Nurainun H, Julayha, Oku H 2012a. Nonsaponifiable lipid composition of four salt-secreter and non-secreter mangrove species from North Sumatra, Indonesia. Makara J Sci 16: 89-94.

Basyuni M, Baba S, Kinjo Y, Putri LAP, Hakim L, Oku H. 2012b. Salt-dependent increase in triterpenoids is reversible upon transfer to fresh water in mangrove plants Kandelia candel and Bruguiera gymnorrhiza. J Plant Physiol 169: 1903-1908.

Basyuni M, Putri LAP, Oku H. 2013. Phytochemical investigation from six mangrove tree species, North Sumatra, Indonesia. Ilmu Kelautan: J Indonesia Mar Sci 18: 157-164.

Basyuni M, Sagami H, Baba S, Iwasaki H, Oku H. 2016. Diversity of polyisoprenoids in ten Okinawan mangrove. Dendrobiology 75: 167-175.

Basyuni M, Sagami H, Baba S, Oku H. 2017. Distribution, occurrence, and cluster analysis of new polypropyl acetones and other polyisoprenoids from North Sumatran mangroves. Dendrobiology 78: 18-31.

Boland DJ, Pinyopusarerk K, McDonald MW, Jovanovic T, Booth TH. 1990. The habitat of Acacia auriculiformis and probable factors associated with its distribution. J Trop For Sci 3:159-180.

Chan HT, Baba S. 2009. Manual on Guidelines for Rehabilitation of Coastal Forests damaged by Natural Hazards in the Asia-Pacific Region. International Society for Mangrove Ecosystems (ISME) and International Tropical Timber Organization (ITTO), Okinawa, Japan: 1-44.

Chouda M, Jankowski W. 2005. The occurrence of polyprenols in seeds and leaves of woody plants. Acta Biochim Pol 52: 243-253.

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J, Duke N. 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecol Biogeogr. 20: 154-159.

Grabinska K, Palamarczyk G. 2002. Dolichol biosynthesis in the yeast Saccharomyces cerevisiae: an insight into the regulatory role of farnesyl diphosphate synthase. FEMS Yeast Res 2: 259-265.

Hogg RW, Gillan FT. 1984. Fatty acids, sterols and hydrocarbons in the leaves of woody plants. Acta Biochim Pol 52: 243-253.

Ishiguro T, Morita-Fujimira Y, Shidoji Y, Sagami H. 2014. Dolichol biosynthesis: The occurrence of epoxy dolichol in skipjack tuna liver. Biochem Biophys Res Commun 451: 277-281.

Jozwiak A, Gutkowska M, Gawarecka K, Surmacz L, Buczkowska A, Lichoecka M, Nowakowska J, Swiezewska E. 2015 POLYPRENOL REDUCTASE2 deficiency is lethal in Arabidopsis due to male sterility. Plant Cell 27: 3336-3353.

Kilamura S, Anwar C, Chaniago A, Baba S. 1997. Handbook of Mangroves in Indonesia - Bali & Lombok. International Society for Mangrove Ecosystems, Okinawa, Japan, pp: 66-94.

Kovach WL. 2010. MVSP - A Multi Variate Statistical Package for Windows, ver. 3.22. Kovach Computing Services, Pentraeth, UK.

Lakshmi M, Parani M, Parida A. 2002. Molecular phylogeny of mangroves IX. Molecular marker assisted intra-specific variation and species relationships in the Indian mangrove tribe Rhizophoraceae. Aquat Bot 74: 201-217.

Parani M, Lakshmi M, Senthilkumar P, Nivedita R, Parida A. 1998. Molecular phylogeny of mangroves V. Analysis of genome
relationships in mangrove species using RAPD and RFLP markers. Theor Appl Genet 97: 617-625.

Pattison RJ, Amtmann A. 2009. N-glycan production in the endoplasmic reticulum of plants. Trends Plant Sci 14: 92-99.

Rezanka T, Votruba J. 2001. Chromatography of long chain alcohols (polyprenols) from animal and plant sources. J Chromatogr A 936: 95-110.

Roslinska M, Walinska K, Swiezewska E, Chojnacki T. 2002. Plant long-chain polyprenols as chemotaxonomic markers. Dendrobiology 47: 41-50.

Sagami H, Kurisaki A, Ogura K, Chojnacki T. 1992. Separation of dolichol from dehydrodolichol by a simple two-plate thin layer chromatography. J Lipid Res 33: 1857-1862.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH image to ImageJ: 25 years of image analysis. Nature Methods 9: 671-675.

Setoguchi H, Kosuge K, Tobe T. 1999. Molecular phylogeny of Rhizophoraceae based on rbcl gene sequences. J Plant Res 112: 443-455.

Skorupinska-Tudek K, Bienkowski T, Olszowska O, Furmanowa M, Chojnacki T, Danikiewicz W, Swiezewska E. 2003. Divergent pattern of polyisoprenoid alcohols in the tissues of Coluria geoides: a new electrospray ionization MS approach. Lipids 38: 981-990.

Skorupinska-Tudek K, Wojcik J, Swiezewska E. 2008. Polyisoprenoid alcohols-recent results of structural studies. Chem Rec. 8: 33-45.

Surmacz L, Swiezewska E. 2011. Polyisoprenoids - Secondary metabolites or physiologically important superlipids? Biochem Biophys Res Commun 407: 627-32.

Swiezewska E, Sasak W, Mankowski T, Jankowski W, Vogtman T, Krajewska I, Hertel J, Skocylas E, Chojnacki T. 1994. The search for plant polyprenols. Acta Biochim Pol 41: 221-260.

Swiezewska E, Danikiewicz W. 2005. Polyisoprenoids: Structure, biosynthesis and function. Prog Lipid Res 44: 235-258.

Tomlinson PB. 1986. The Botany of Mangroves.1st edn, Cambridge University Press, New York, USA: 3-10.

Wang L, Mu M, Li X, Lin P, Wang W. 2011. Differentiation between true mangroves and mangrove associates based on leaf traits and salt contents. J Plant Ecol 4: 292-301.

Wojtas M, Bielicki T, Tateyama S, Sagami H, Chojnacki T, Danikiewicz W, Swiezewska E. 2004. Polyisoprenoid alcohols from the mushroom Lentinus edodes. Chem Phys Lipids 130: 109-115.

Wolucka BA, McNeil MR, de Hoffmann E, Chojnacki T, Brennan PJ. 1994. Recognition of the lipid intermediate for arabinogalactan/arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. J Biol Chem 269: 23328-23335.
Figure S1. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *C. equisetifolia* leaves, B. *C. inophyllum* leaves, C. *H. tiliaceus* leaves, D. *P. pinnata* leaves, E. *S. hydrophyllacea* leaves, F. *S. jamaicensis* leaves, G. *S. portulacastrum* leaves, and H. *T. catappa* leaves. The number indicates the carbon-chain length of the polyisoprenoid alcohols.

Figure S2. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *B. asiatica* roots, B. *C. equisetifolia* roots, C. *C. inophyllum* roots, D. *P. odoratissimus* roots, E. *P. pinnata* roots, F. *R. communis* roots, G. *S. hydrophyllacea* roots, and H. *T. catappa* roots. The number indicates the carbon-chain length of the polyisoprenoid alcohols.