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Distribution, occurrence, and cluster analysis of new polyprenyl acetones and other polyisoprenoids from North Sumatran mangroves

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Abstract

Background. Mangrove forests have long been known as a source of phytochemical compounds producing various secondary metabolites. Despite the ubiquitous diversity of polyisoprenoids in the plant kingdom, few studies have focused on the distribution of polyisoprenoids in mangrove plants. The present study describes the distribution and occurrence of a new class of prenyl derivates – polyprenyl acetone as well as other polyisoprenoids in fourteen species of Indonesian mangroves, with an emphasis on chemotaxonomic importance.
Material and methods. The leaves and roots of fourteen North Sumatran mangroves were analyzed using two-dimensional thin layer chromatography and electrospray ionization mass spectrometry.
Results. In the leaves, the distribution of several types of polyprenyl acetones, polyprenols, and dolichols was detected and classified into types: type-I, having a predominance of dolichols over polyprenols (more than nine-fold), was observed in *Acrostichum aureum* (younger leaves), *Avicennia alba*, *Av. lanata*, *Av. officinalis*, *Bruguiera parviflora*, *Ceriops tagal*, *Nypa fruticans*, and *Rhizophora mucronata*; type-II, having the presence of both polyprenols and dolichols, was observed in *Acanthus ilicifolius*, *Acr. aureum*, *B. cylindrica*, and *R. apiculata*; type-III having a predominance of polyprenols over dolichols (more than nine-fold), was not observed in any North Sumatran mangroves; type-IV, having the presence of both polyprenyl acetones and dolichols, was observed in *Aegiceras corniculatum*; type-V, having the presence of polyprenyl acetones, polyprenols, and dolichols, was observed in *Sonneratia caseolaris* and *Xylocarpus granatum*. In the roots, type-I distribution was observed in *Ae. corniculatum*, *Av. alba*, *Av. lanata*, *Av. officinalis*, *B. parviflora*, *C. tagal*, *N. fruticans*, *R. apiculata*, *R. mucronata*, *S. caseolaris*, and *X. granatum*. Type-II distribution was observed in *Ac. ilicifolius*, *Acr. aureum*, and *B. cylindrica*. Type-III, -IV, and -V distributions were not observed in mangrove roots. Cluster analysis demonstrated that polyisoprenoid patterns in the leaves and roots form distinct separation into appropriate genera and tribe, suggesting that mangrove polyisoprenoids are chemotaxonomically significant.
Conclusions. The major polyisoprenoid alcohols in Indonesian mangroves were found to be dolichols rather than polyprenols. The diversity of polyisoprenoids in both leaves and roots of mangroves may provide chemotaxonomic marker. The discovery of a new class of polyprenyl acetone is the first report from mangrove plants.

Keywords: Chemotaxonomic marker, dolichol, Indonesian mangroves, polyprenol, two-dimensional thin layer chromatography

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Introduction

Mangrove forests are widespread in the inter-tidal zone of tropical and sub-tropical regions and have long been known as a source of phytochemical compounds producing various secondary metabolites (Bandaranayake, 2002; Basyuni et al., 2007a; Patra & Thatoi, 2011). Indonesia has the largest mangrove area, comprising 22.6% of the total global mangrove area (Giri et al., 2011). The lipid and isoprenoid content of Indonesian mangroves has been previously reported (Basyuni et al., 2012a; 2013). Polyisoprenoid alcohols are secondary metabolites that constitute a group of hydrophobic polymers widely distributed among living organism both in eukaryotes and prokaryotes (Swiezewska & Danikiewicz, 2005; Skorupinska-Tudek et al., 2008). Two main types of polyisoprenoid alcohols have been described with respect to the OH-terminal (α-) isoprene unit. These include polyprenols (α-unsaturated) and dolichols (α-saturated) compounds (Fig. 1, structure 1 and 2, respectively).

The occurrence of polyisoprenoids have been reported in bacteria (Wolucka et al., 1994), yeast (Grabinska & Palamarczyk, 2002), fungi (Wojtas et al., 2004), animals (Chojnacki & Dallner, 1988; Sagami et al., 1993; Rezanka & Votruba, 2001; Ishiguro et al., 2014), and plants (Swiezewska et al., 1994; Rezanka & Votruba, 2001; Chouda & Jankowski, 2005). Despite the ubiquitous diversity of polyisoprenoids in the plant kingdom, the physiological roles in plants are poorly understood, particularly in mangroves species. Long-chain rubber-like polyisoprenoids occur in mangrove *Lumnitzera racemosa* (Skoczylas et al., 1994). In addition, the content of polyisoprenoids altered in tissue or organ with stage of development and upon abiotic or biotic stress (Daniels & Hemming, 1990; Swiezewska et al., 1994; Tateyama et al., 1999; Zhang et al., 2008; Bajda et al., 2009; Baczewska et al., 2014).

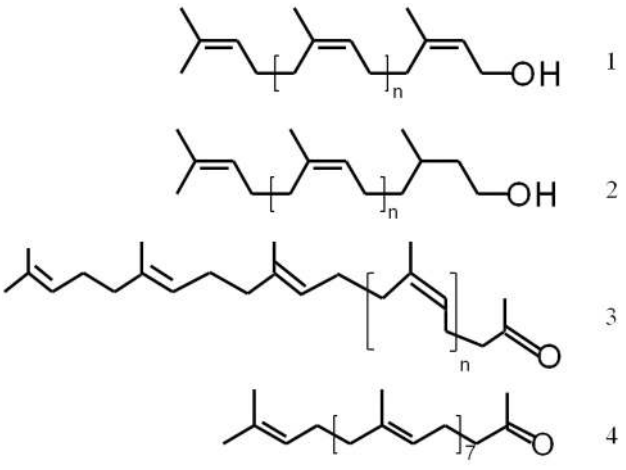

Fig. 1. Structure of polyprenol (1), dolichol (2), polyprenyl acetone (3), and bombiprenone (4). *n* shows the number of internal isoprene residues

A number of studies have shown that the distribution of lipids as well as polyisoprenoids may serve as clear chemotaxonomic markers (Hogg & Gillan, 1984; Roslinska et al., 2002; Sun et al., 2010). These studies demonstrated that lipid, isoprenoid, and polyisoprenoid compounds exhibit a distinct character and pattern that can be used to distinguish plants, including mangroves, into systematic genera and families. To get more insight into the physiological and chemotaxonomic significance of polyisoprenoids, it is important to obtain information on the occurrence of these compounds. However, few studies have focused on the distribution of polyisoprenoids in mangrove plants. Recently, the first identification on the diversity of polyisoprenoids in ten Okinawan mangroves has been reported (Basyuni et al., 2016). The present study on North Sumatran mangroves extends the previous work and describes the distribution and occurrence of a new class of polyisoprenoid derivatives, polyprenyl acetones (Fig. 1 structure 3), as well as other polyisoprenoids in fourteen species of Indonesian mangroves, with an emphasis on chemotaxonomic importance.

Materials and methods

Chemicals

Standard mixture of dolichols (C_{90}–C_{105}) and polyprenols (C_{90}–C_{100}) were isolated from horse testicles and *Malus sp*., respectively (Swiezewska & Danikiewicz, 2005). These mixtures were generously provided by Dr. Ewa Swiezewska and were used to identify the polyisoprenoids that were detected in this study. Silica-gel 60 TLC glass plates and reversed-phase silica RP-18 HPTLC glass plates were obtained from

Merck (Darmstadt, Germany). All other chemicals and solvents were of reagent grade and obtained from Merck. Identification of the polyprenyl acetone family from *Sonneratia caseolaris* leaves was performed by mass spectrometry equipped with electrospray ionization (ESI, Burker Daltonix). The identification of the family corresponding to polyprenols, dolichols, or polyprenyl acetones was performed in at least three experiments. The bombiprenone family (Fig. 1 structure 4), as described by Irvine et al. (1972), was purified using silica-gel chromatography of non-saponifiable lipids from the $CHCl_3/CH_3OH$ (2:1) extract of dry perilla leaves. The structure of bombiprenone ($C_{43}H_{70}O$) was confirmed with the aid of ESI/MS - *m/z* 625.53183, sodiated ion $[M + Na]^+$ was recorded.

Plant materials

The leaves and roots of fourteen mangrove tree species thriving on Pulau Sembilan, North Sumatra, Indonesia were collected in April 2014: *Acanthus ilicifolius* L. (Acanthaceae), *Acrostichum aureum* L. (Pteridaceae), *Aegiceras corniculatum* (L.) Blanco (Myrsinaceae), *Avicennia alba* Blume (Acanthaceae), *Av. lanata* Ridley (Acanthaceae), *Av. officinalis* L. (Acanthaceae), *Bruguiera cylindrica* Blume (Rhizophoraceae), *B. parviflora* (Roxb.) Wight & Arn. ex Griffith (Rhizophoraceae), *Ceriops tagal* (Perr.) C.B. (Rhizophoraceae), *Nypa fruticans* Wurmb (Arecaceae), *Rhizophora apiculata* Bl. (Rhizophoraceae), *R. mucronata* Lam. (Rhizophoraceae), *S. caseolaris* (L.) Engl. (Sonneratiaceae) and *Xylocarpus granatum* K.D. Koen. (Meliaceae). The age of the green leaves was estimated to be approximately 2–5 months, whereas younger leaves were estimated to be one month old. Mangrove plants grew naturally with exposure to natural sun light. The average temperature in the month of collection was 29 °C with an average humidity of 74%. All of the fresh samples were kept at −20 °C until use.

Preparing and purifying polyprenyl acetone compounds from *S. caseolaris* leaves

The dried leaves of *S. caseolaris* (4.3 g) were cut into small pieces and placed in 30 ml of chloroform/methanol (2/1, v/v) solvent for 48 h. The cell wall debris insoluble to CM21 was removed by filtration through No. 2 filter paper (Advantec, Tokyo, Japan). The lipid extract of the leaves was saponified after adding 4 mL of a mixture containing KOH (0.45 g), ethanol (2 mL), and H_2O (2 mL) at 65 °C for 24 h. Non-saponifiable lipids were extracted with hexane and the hexane extract (30 mg) was applied to a silica-gel 60 column (2.0 x 14 cm) that was equilibrated with toluene as previously reported (Ishiguro et al., 2014). The solvent was initially removed and then the solid was resuspended. Two partially purified preparations (Fractions 8 and 20) containing putative polyprenyl acetones and dolichols, respectively, and analyzed by electrospray ionization mass spectrometry (ESI/MS).

Isolation of polyisoprenoid alcohols

The procedure for the isolation of polyisoprenoids was performed as previously described (Sagami et al., 1992; Basyuni et al., 2016). The leaves and roots were dried at 60–75 °C for 1–2 days. The dried tissue (3–7 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 cell wall debris insoluble to CM21 was removed as described previously (Basyuni et al., 2016). The lipid extract of the leaves and roots was saponified in 4 mL at 65 °C for 24 h in 50% ethanol containing 2 M KOH. The non-saponifiable lipids of each tissue were extracted with hexane and the organic solvent was evaporated and re-dissolved in hexane. The leaf (50–100 mg) and root (150–200 mg) extracts were applied to each TLC plate.

Analysis by two-dimensional thin layer chromatography

First-dimension TLC was carried out for 50–60 min on a silica-gel glass plate (20 × 3 cm) with a solvent system of toluene-ethyl acetate (9:1) as previously described (Sagami et al., 1992; Basyuni et al., 2016). The second-dimension reversed-phase C-18 silica gel HPTLC was performed with acetone as the solvent for approximately 30–40 min. The position of the separated polyisoprenoid alcohols by two-dimensional silica gel TLC were identified and visualized with iodine vapor. To determine whether the family corresponded to dolichols or polyprenols, dolichol or polyprenol reference standards were added to the sample line of the first-dimension TLC and developed with a solvent system as previously described (Basyuni et al., 2016). Developed chromatographic images were obtained and digitally scanned with a Canon MG6100 series printer. The polyisoprenoid family was identified through the comparison of mobility on TLC with that of authentic standards of dolichol or polyprenol that were applied in the second-dimensional development. The polyprenols and dolichols that were detected on RP-18 HPTLC glass plates were quantified using ImageJ 1.46r (Schneider et al., 2012), with dolichol and polyprenol standards as references.

Cluster analysis

Cluster analysis was performed on selected subsets of leaf data consisting of 61 variables, including

polyprenyl acetones, polyprenols, and dolichols from 24 species. In this analysis, 14 species were from this study and 10 species were from Basyuni et al. (2016); all data were log (10) transformed. For root data, 31 variables of polyprenols and dolichols from 23 species [14 species from this study and 9 species from Basyuni et al. (2016)] were also log (10) transformed. From these data, dendrograms representing the leaf and root data, respectively, were drawn by clustering analysis using the unweighted-pair group method with arithmetic mean (UPGMA) and MVSP (multivariate statistical package) 3.22 (Kovach Computing Service). Euclidean distance was chosen as the criterion for cluster combination.

Results

Purification and identification of a new family of polyprenyl acetones

A new family of polyisoprenoid compounds in *Ae. corniculatum*, *S. caseolaris*, and *X. granatum* was detected, the structure was similar although not identical to bombiprenone (Fig. 2B, 3C, 3D, Supplementary Fig. 2F). This family as expected may correspond to cis-type polyprenyl acetones, which migrate faster than the trans-polyprenyl acetones such as bombiprenone on a normal phase TLC. The polyprenyl acetones and dominating dolichol compounds from *S. caseolarisis* leaves were purified by silica-gel column chromatography and RP-18 silica-gel chromatography. Two-dimensional thin layer chromatography of the hexane extract of *S. caseolaris* leaves contained a new class of polyprenyl acetone, bombiprenone, dolichols, and polyprenols (Fig. 2A). There are two fractions of 0.2 mL each were collected. Fraction 8 was identified putatively as cis polyprenyl acetone and bombiprenone

Table 1. Major polyprenyl acetone family found in the *S. caseolaris* leaves

Compounds	Calculation	Experimental
$C_{23}H_{38}O + Na^+$	353.28149	353.28161
$C_{28}H_{46}O + Na^+$	421.34409	421.34408
$C_{33}H_{54}O + Na^+$	489.40929	489.40654
$C_{38}H_{62}O + Na^+$	557.46929	557.46916
$C_{43}H_{70}O + Na^+$	625.53189	625.53186
$C_{48}H_{78}O + Na^+$	693.59449	693.59455
$C_{53}H_{86}O + Na^+$	761.65709	761.65708
$C_{58}H_{94}O + Na^+$	829.71969	829.71988
$C_{63}H_{102}O + Na^+$	897.78229	897.78267

(Fig. 2B) and fraction 20 was identified as pure-dominating dolichol and few polyprenols (Fig. 2C). Fractions 8 and 20 were pooled and evaporated to dryness to yield 1.1 mg and 1.3 mg, respectively. Both samples were analyzed by ESI/MS and polyprenyl acetones (C_{23}–C_{68}) (Supplementary Fig. 1) and dolichols (C_{55}–C_{115}) in ESI mass spectrums were confirmed. In Fraction 20, sodiated molecules with $[M + Na]^+$ ions were detected with *m/z* 1200.0795 (Dol-17) and 1268.1419 (Dol-18). In Fraction 8, there are nine positive ion peaks, which displays in Table 1. Sodiated molecules had $[M + Na]^+$ ions were detected with *m/z* 353.2816 corresponding to $C_{23}H_{38}O + Na^+$ (Pol Ace-23), and subsequent polyprenyl acetones ESI/MS data appear in Table 1 with the highest intensity at peak Pol Ace-43 of the bombiprenone family (trans-type polyprenyl acetones).

Occurrence and profile of polyisoprenoids in mangrove tissues

Fourteen North Sumatran mangrove species for long-chain polyisoprenoids was evaluated by two-dimensional thin layer chromatography (TLC) (Sagami

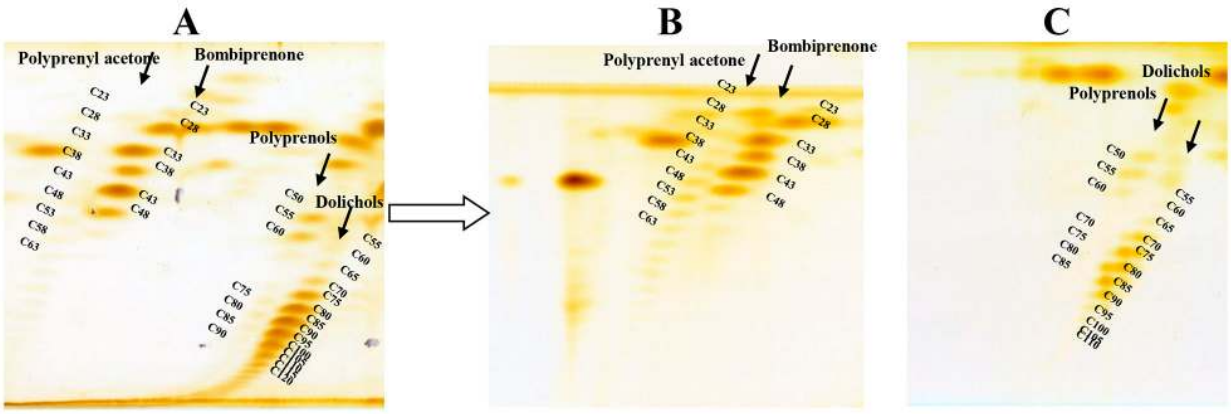

Fig. 2. 2D-TLC hexane extract of *S. caseolaris* leaves obtained by saponification with KOH, contained a new class of polyprenyl acetone, bombiprenone, dolichols, and polyprenols (A). The purification of fraction 8 was identified as new family of polyprenyl acetone and bombiprenone (B). The purification of fraction 20 was identified as dominated dolichols and few polyprenols (C)

et al., 1992; Basyuni et al., 2016). Polyisoprenoids of different chain-lengths were separated into polyprenols, dolichols, and a new class of polyprenyl acetone family. Tables 2 and 3 summarize the analytical results of the occurrence and distribution of the new class of polyprenyl acetones, polyprenols, and dolichols with the carbon-chain lengths given for each family.

The total lipid content of mangrove leaves (Table 2) ranged from 44 to 231 mg g^{-1} dry weight with the lowest and highest weights in *Ac. ilicifolius* and *Acr. aureum*, respectively. The total lipids are expressed as a fraction of a crude lipids estimated gravemetrically. The total lipid content of mangrove roots ranged from 8 to 181 mg g^{-1} dry weight with the lowest and highest contents in *Av. lanata* and *C. tagal*, respectively. The quantity of polyisoprenoids was highest in *B. parviflora* leaves (29.4 mg g^{-1} dry weight) and *N. fruticans* roots (19.2 mg g^{-1} dry weight). The lowest content of polyisoprenoids was in the leaves of *R. mucronata* (4.1 mg g^{-1} dry weight) and the roots of *Acr. aureum* (4.1 mg g^{-1} dry weight) (Table 2).

The structural groups of polyprenols, dolichols, and polyprenyl acetones in the leaves were classified as five types (I, II, III, IV, and V). This classification type based on carbon-chain length and composition of polyprenols, dolichols, and polyprenyl acetones implies to the simply selection group. Type-I, which displays a predominance of dolichols over polyprenols (nine-fold) was observed in *Acr. aureum* (younger leaves), *Av. alba*, *Av. lanata*, *Av. officinalis*, *B. parviflora*, *C. tagal*, *N. fruticans*, and *R. mucronata*. In *Acr. aureum* (younger leaves) and *R. mucronata*, a trace amount of polyprenols with chain-lengths similar to those of dolichols was detected (Supplementary Fig. 1B and 2E, respectively). However, in the leaves

Table 2. Occurrence and distribution of polyprenyl acetones, polyprenols, and dolichols in 14 North Sumatran mangrove species

Species	Plant code	Tissue	TL (mg/g dw)	PI (mg/g dw)	Pol Ace (mg/g)	Pol (mg/g)	Dol (mg/g)	% in total lipid				% in polyisoprenoid			Type
								PI	Pol Ace	Pol	Dol	Pol Ace	Pol	Dol	
Ac. ilicifolius	Aci	leaves	44,3	5,5	nd	2,1	3,4	12,4	nd	4,7	7,7	nd	37,7	62,3	II
Acr. aureum	Acra	leaves	231,1	4,6	nd	2,6	2	2	nd	1,1	0,9	nd	57,4	42,6	II
Acr. aureum		young leaves	67,4	7,7	nd	0,7	7	11,5	nd	1,1	10,4	nd	8,9	91,1	I
Ae. corniculatum	Aec	leaves	97,8	15,9	7,4	nd	8,5	13,1	7,7	nd	5,4	46,5	nd	53,5	IV
Av. alba	Ava	leaves	62,1	5,5	nd	nd	5,5	8,9	nd	nd	8,9	nd	nd	100	I
Av. lanata	Avl	leaves	86,8	14,9	nd	nd	14,9	17,1	nd	nd	17,1	nd	nd	100	I
Av. officinalis	Avo	leaves	92,7	8,4	nd	nd	8,4	9,1	nd	nd	9,1	nd	nd	100	I
B. cylindrica	Bc	leaves	124,2	7,9	nd	3,4	4,5	6,3	nd	2,7	3,6	nd	42,7	57,3	II
B. parviflora	Bp	leaves	186,3	29,4	nd	nd	29,4	15,8	nd	nd	15,8	nd	nd	100	I
C. tagal	Ct	leaves	128,7	27,9	nd	nd	27,9	21,6	nd	nd	21,6	nd	nd	100	I
N. fruticans	Nf	leaves	67,2	10,7	nd	nd	10,7	15,9	nd	nd	15,9	nd	nd	100	I
R. apiculata	Ra	leaves	97	6,1	nd	2,6	3,5	6,3	nd	2,7	3,6	nd	42,8	57,2	II
R. mucronata	Rm	leaves	53,1	4,1	nd	0,4	3,7	7,7	nd	0,7	7	nd	9,8	90,2	I
S. caseolaris	Sc	leaves	65,6	8,8	1	1,2	6,6	13,5	1,6	1,9	10	11,8	13,9	74,3	V
X. granatum	Xg	leaves	96,4	5,1	1,7	2,6	0,8	5,3	1,8	2,7	0,8	33,4	51,1	15,5	V
Ac. ilicifolius		roots	66,1	9	nd	3,9	5,1	13,7	nd	5,9	7,8	nd	43,2	56,8	II
Acr. aureum		roots	11,4	4,1	nd	2,4	1,7	36,3	nd	20,8	15,5	nd	58	42	II
Ae. corniculatum		roots	45,9	11,9	nd	nd	11,9	25,9	nd	nd	25,9	nd	nd	100	I
Av. alba		roots	31,8	4,8	nd	0,1	4,7	15,1	nd	0,3	14,8	nd	2,2	97,8	I
Av. lanata		roots	8,1	4,4	nd	nd	4,4	54,1	nd	nd	54,1	nd	nd	100	I
Av. officinalis		roots	81,8	12,4	nd	1	11,4	15,1	nd	1,2	13,9	nd	7,7	92,3	I
B. cylindrica		roots	25,9	5,2	nd	2	3,2	19,9	nd	7,7	12,2	nd	38,8	61,2	II
B. parviflora		roots	15,8	5,8	nd	0,4	5,4	36,6	nd	2,8	33,8	nd	7,7	92,3	I
C. tagal		roots	181,8	15,4	nd	nd	15,4	8,5	nd	nd	8,5	nd	nd	100	I
N. fruticans		roots	43,5	19,2	nd	nd	19,2	44,2	nd	nd	44,1	nd	nd	100	I
R. apiculata		roots	40,1	6,1	nd	nd	6,1	15,1	nd	nd	15,1	nd	nd	100	I
R. mucronata		roots	52,1	5,4	nd	0,4	5	10,4	nd	0,9	9,5	nd	8,3	91,7	I
S. caseolaris		roots	44,7	12,5	nd	nd	12,5	28	nd	nd	28	nd	nd	100	I
X. granatum		roots	19,4	4,2	nd	0,2	4	21,7	nd	nd	20,6	nd	4,8	95,2	I

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

TL are expressed as a fraction of a crude lipids estimated gravemetrically.

Table 3. Carbon-chain lengths of bombiprenone, polyprenyl acetone, polyprenol, dolichol occurring in 14 North Sumatran mangroves*

Species	Tissue	Bombiprenone	Polyprenyl acetone	Polyprenol	Dolichol
Ac. ilicifolius	leaves	23 28 33 38 43 48		50 55 65 70 75 80 85 90 95 100	65 70 75 80 85 90 95 100 105 110 115 120 125
Acr. aureum	leaves	23 28 33 38 43 48		50 55 60 75 80 85	75 80 85 90
Acr. aureum	young leaves	38 43 48		50 55	75 80 85 90 95 100 105 110
Ae. corniculatum	leaves	23 28 33 38 43 48	23 28 33 38 43 48 53 58 63 68 73 78 83 88 93 98 103 108		60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140
Av. alba	leaves				60 65 70 75 80 85 90 95 100
Av. lanata	leaves	23 28 33 38 43 48			70 75 80 85 90 95 100
Av. officinalis	leaves	28 33 38 43 48		45 50	70 75 80 85 90 95 100
B. cylindrica	leaves	23 28 33 38 43		80 85	75 80 85
B. parviflora	leaves	28 33 38 43 48			80 85 90
C. tagal	leaves				75 80 85
N. fruticans	leaves	23 28 33 38 43 48			75 80 85 90
R. apiculata	leaves	23 28 33 38 43 48		50 55	75 80 85 90 95
R. mucronata	leaves	23 28 33 38 43 48		80 85 90	75 80 85 90 95
S. caseolaris	leaves	23 28 33 38 43 48	23 28 33 38 43 48 53 58 63 68 73 78 83 88	50 55 60 75 80 85 90	50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
X. granatum	leaves		23 28 33 38 43 48 53 58 63 68 73 78 83 88	35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125	60 65 70 75 80 85 90 95 100
Ac. ilicifolius	roots	43 48		35 45 50	70 75 80
Acr. aureum	roots			35 45 50 55 75 80 85	75 80 85 90
Ae. corniculatum	roots				70 75 80 85 90 95 100 105 110
Av. alba	roots			75 80 85 90	60 65 70 75 80 85 90 95 100 105 110
Av. lanata	roots	43 48			70 75 80 85
Av. officinalis	roots	38 43 48		65 70 75 80 85 90	55 60 65 70 75 80 85 90 95 100 105 110
B. cylindrica	roots	23 28 33 38 43 48		75 80 85	70 75 80 85 90
B. parviflora	roots	28 33 38 43		85 90	80 85 90 95
C. tagal	roots				85 90 95
N. fruticans	roots	23 28 33 38 43 48			75 80 85 90
R. apiculata	roots	23 28 33 38 43 48		50 55	75 80 85 90 95
R. mucronata	roots	33 38 43 48		80 85 90	75 80 85 90 95
S. caseolaris	roots				75 80 85 90 95 100
X. granatum	roots	33 38 43 48		75 80	70 75 80 85 90 95 100 105
Ac. ilicifolius	flower	33 38 43 48		80 85 90 95	80 85 90 95 100
Av. lanata	flower				70 75 80 85 90 95 100 105 110

*The numbers refer to the carbon-chain length of the polyisoprenoid alcohols. The chain length of the main polyisoprenoid alcohols in each tissue are indicated in bold.

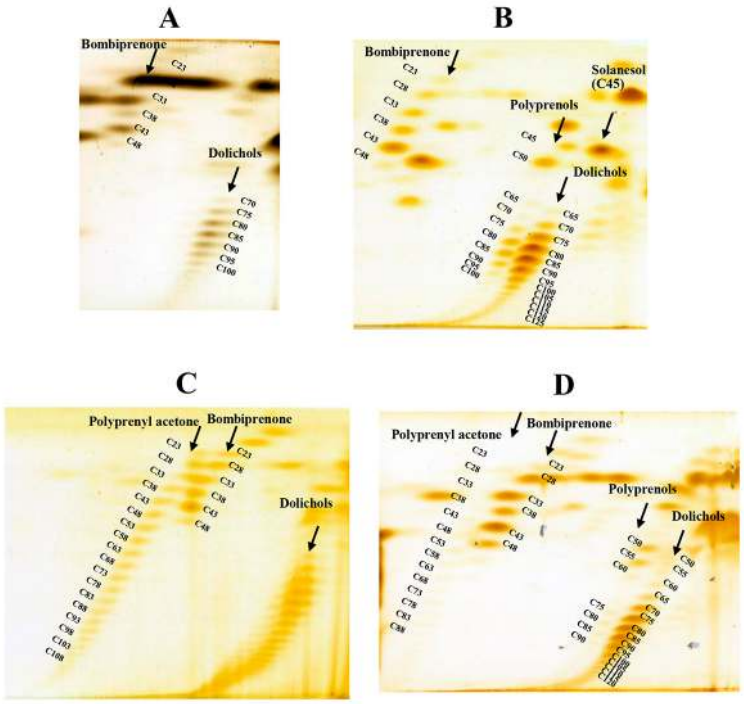

Fig. 3. Two-dimensional thin layer chromatography (2D-TLC) chromatograms of polyisoprenoid hexane extracts from *Av. alba* (A), *Ac. ilicifolius* (B), *Ae. corniculatum* (C), and *S. caseolaris* (D) leaves. The number shows the isoprene units of the polyisoprenoid alcohols

of *Av. alba*, *Av. lanata*, *Av. officinalis*, *B. parviflora*, *C. tagal*, and *N. fruticans* (Fig. 3A, Supplementary Fig. 1C, 1D, 2A, 2B, and 2C respectively), polyprenols with chain-lengths similar to those of dolichols were not detected as these species contained only 100% dolichols (Table 2). Type-II, which displays the occurrence of both polyprenols and dolichols was observed in *Ac. ilicifolius*, *Acr. aureum*, *B. cylindrica*, and *R. apiculata* (Table 2). In *Ac. ilicifolius*, polyprenols (ficaprenols and longer polyprenols) with a chain-length similar to that of dolichols were detected, as shown in Fig. 3B. In *Acr. aureum*, *B. cylindrica*, and *R. apiculata*, chain-lengths differed between polyprenols and dolichols as shown in Supplementary Fig. 1A, 1E, 2D, and Table 3. In *Ac. ilicifolius*, dolichols that were much longer than polyprenols in chain-length were also detected, as shown in Fig. 3B (See Table 3).

As for type-III, the occurrence of polyprenols over dolichols (more than nine-fold), which was observed in the case of Okinawan mangroves (Basyuni et al., 2016), was not observed in this study of North Sumatran mangroves.

In type-IV, the occurrence of both polyprenyl acetones and dolichols was found in *Ae. corniculatum*. In these cases, the chain-lengths of the detected polyprenyl acetone compounds were C_{23}–C_{108}, while those of the dolichols were C_{60}–C_{140}, and the relative percentage of each was 46.5:53.5%, respectively (Fig. 3C and Table 2). In type-V, polyprenyl acetones, polyprenols, and dolichols, were found in *S. caseoalaris* and *X. granatum*. In *S. caseolaris* leaves, the chain-lengths of the detected polyprenyl acetones, polyprenols (shorter and longer) and dolichols were C_{23}–C_{88}, C_{50}–C_{60} (shorter) and C_{75}–C_{90} (longer), and C_{50}–C_{120}, respectively (Fig. 3D). However, in *X. granatum*, a wider range in the chain-lengths of polyprenols was detected [C_{35}–C_{125} (Supplementary Fig. 2F and Table 3)].

In the roots, the predominance of dolichols over polyprenols (more than nine-fold) was observed in *Ae. corniculatum*, *Av. alba*, *Av. lanata*, *Av. officinalis*, *B. parviflora*, *C. tagal*, *N. fruticans*, *R. apiculata*, *R. mucronata*, *S. caseolaris*, and *X. granatum*, similar to that found in the type-I leaves. In *Ae. corniculatum*, *Av. lanata*, *C. tagal*, *N. fruticans*, *R. apiculata*, and *S. caseolaris*, dolichols with no polyprenols were observed (Fig. 4A, 4C, Supplementary Fig 3B, 3C, 3D, and 3F, respectively), although trace amounts of polyprenols were detected in *Av. alba*, *Av. officinalis*, *B. parviflora*, *R. mucronata*, and *X. granatum* (Fig. 4B, 4D, Supplementary Fig 3A, 3E, and Fig 5D, respectively). A significant amount of polyprenols, together with dolichols, was observed in the roots of *Ac. ilicifolius*, *Acr. aureum*, and *B. cylindrica* (Fig. 5A, 5B, and Fig. 5C), similar to type-II leaves. The distribution of both polyprenyl acetones and dolichols and the distribution of polyprenyl acetones, polyprenols, and dolichols, similar to type-IV and -V leaves, respectively, were not observed in any North Sumatran root species.

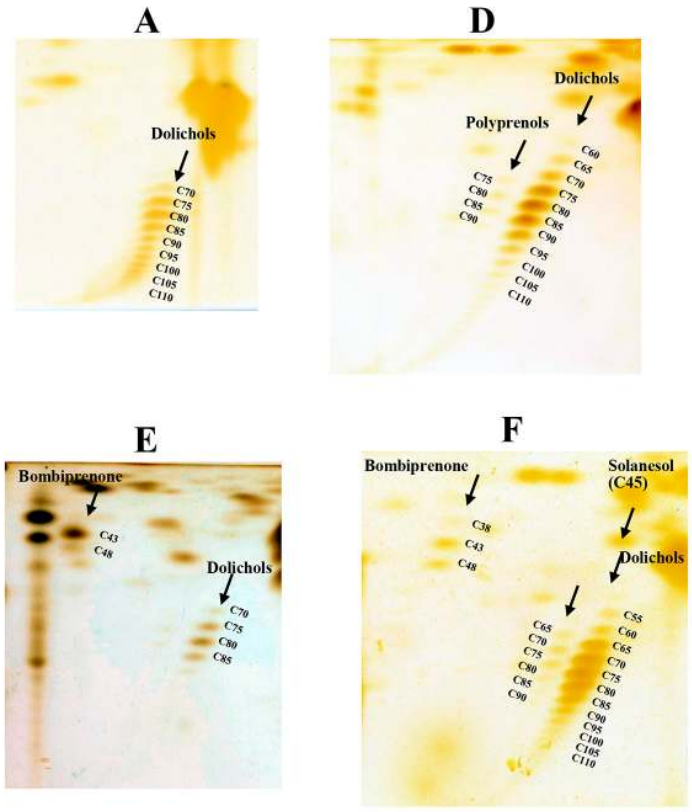

Fig. 4. 2D-TLC chromatograms of polyisoprenoid hexane extracts from *Ae. corniculatum* (A), *Av. alba* (B), *Av. lanata* (C), and *Av. officinalis* (D) roots. The number shows the carbon-chain length of the polyisoprenoid alcohols

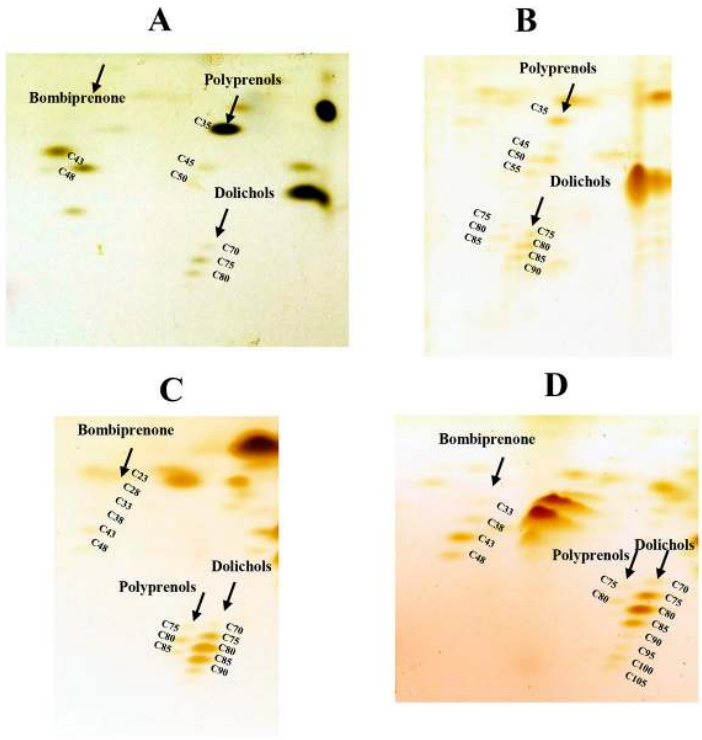

Fig. 5. 2D-TLC chromatograms of polyisoprenoid hexane extracts from *Ac. ilicifolius* (A), *Acr. aureum* (B), *B. cylindrica* (C), and *X. granatum* roots (D). The number shows the isoprene units of the polyisoprenoid alcohols

Cluster analysis of polyisoprenoid data

Cluster analysis using carbon-chain length data for polyisoprenoids were used to construct separate species relationships. Fig. 6 depicts the species relationships from leaf polyisoprenoid carbon-chain lengths from 24 mangrove species. These data revealed that the 24 mangrove species fell into two groups (Fig. 6). One group was a clustering of three species of the new polyprenyl acetone family, namely *X. granatum*, *S. caseolaris*, and *Ae. corniculatum*, which formed one branch that was distinguished from other species. The other group was a clustering of 21 species, including three species (*S. alba*, *P. acidula*, and *L. racemosa*), that showed the occurrence of polyprenols that were much longer than dolichols in chain-length (Basyuni et al., 2016).

Major mangrove species from Rhizophoraceae and Acanthaceae (previously known as Avicenniaceae) tribes were included in this group. It is interesting to note that shorter-chain polyprenols (ficaprenol-type) were detected in *Hi. tiliaceus*, *He. littolaris*, and *E. agallocha* (Basyuni et al., 2016) and also form a distinct

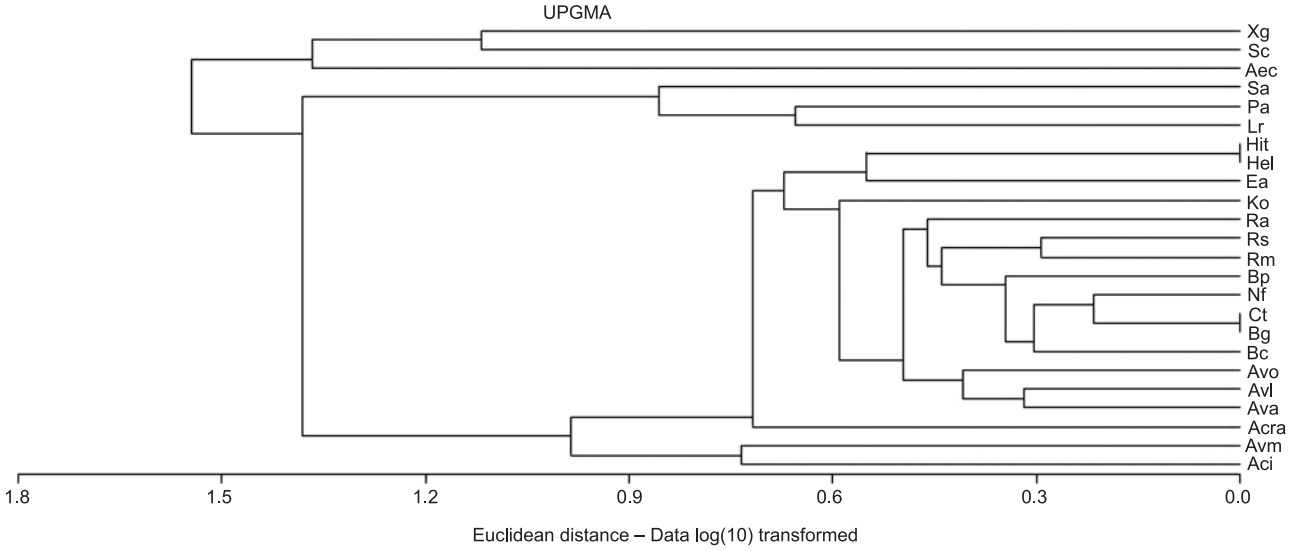

Fig. 6. Dendrogram depicting the relationship among 24 mangrove species of Indonesia and Okinawa from leaf data of the carbon-chain lengths of polyisoprenoids by log (10) transformation using Euclidean distance. For species name, see Table 1. Avm, *Av. marina*; Bg, *B. gymnorrhiza*; Ea, *E. agallocha*; Hel, *He. littoralis*; Hit, *Hi. tiliaceus*; Ko, *K. obovata*; Lr, *L. racemosa*; Pa, *P. acidula*; Rs, *R. stylosa*; and Sa, *S. alba*

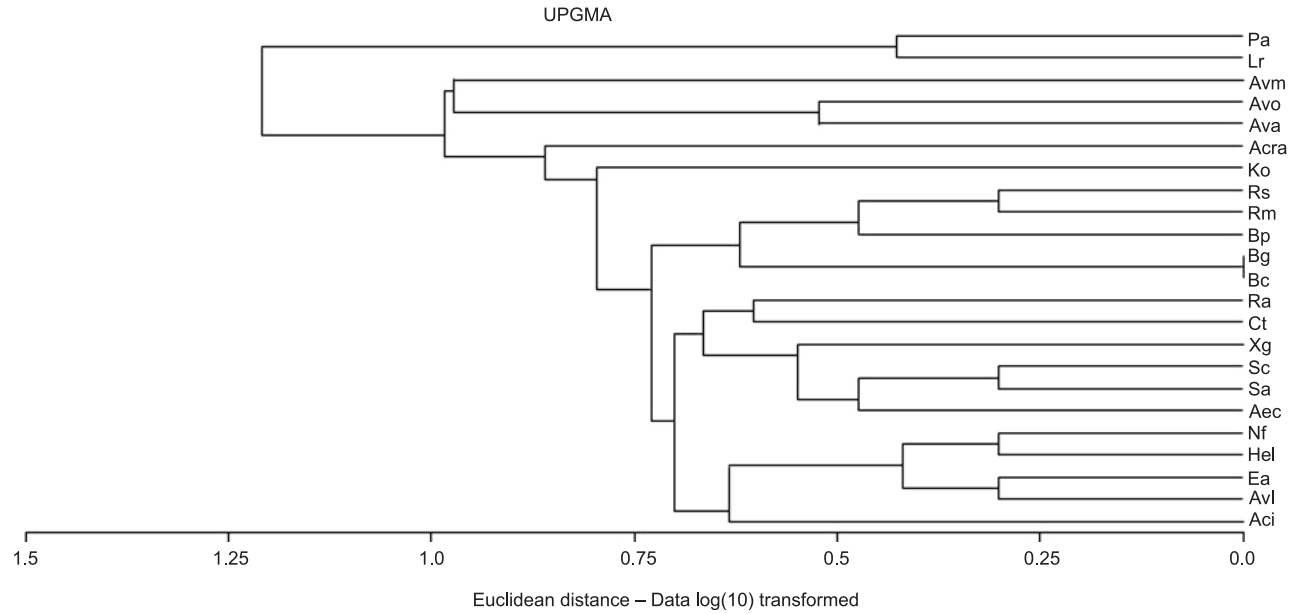

Fig. 7. Dendrogram depicting the relationship among 23 mangrove species in Indonesia and Okinawa from root data of the carbon-chain lengths of polyisoprenoids by log (10) transformation using Euclidean distance. For species name, see Table 2 and Fig. 6

branch in this group (Fig. 6). The Rhizophoraceae tribe comprised of *K. obovata*, *R. apiculata*, *R. stylosa*, *R. mucronata*, *B. parviflora*, *C. tagal*, *B. gymnorrhiza*, and *B. cylindrica*, including *N. fruticans*, join a major group. The Acanthaceae family consists of *Av. officinalis*, *Av. lanata*, *Av. alba*, *Av. marina*, and *Ac. ilicifolius* in addition to *Acr. aureum* and forms another distinct branch (Fig. 6).

The species relationship from the root data of carbon-chain lengths for 23 species also revealed two major groups (Fig. 7). The first group contained only two species, namely *P. acidula* and *L. racemosa*, known to produce longer dolichols (Basyuni et al., 2016). The second group comprised 21 species, including major mangrove species such as Rhizophoraceae, Acanthaceae, and Sonneratiaceae. The Acanthaceae tribe formed two branches; one group contained *Av. marina*, *Av. officinalis*, and *Av. alba*, while another group contained *Av. lanata* and *Ac. ilicifolius*. The Rhizoporaceae tribe formed a distinct branch consisting of *K. obovata*, *R. stylosa*, *R. mucronata*, *B. parviflora*, *B. gymnorrhiza*, *B. cylindrica*, *R. apiculata*, and *C. tagal*. In the case of Sonneratiaceae, which consists of *S. alba* and *S. caseolaris* as well as *He. littoralis* and *E. agallocha*, it forms a clear branch in the clustering analysis.

Discussion

The analysis of polyisoprenoids in the leaves and roots of Indonesian mangroves indicates that the major polyisoprenoid alcohols are dolichols rather than polyprenols. Dolichols were found in all tissues. In the case of Okinawan mangrove leaves, types I, II, and III are found, and in the case of the same mangrove roots, types I and II are found. Whereas as the present study show, in the case of North Sumatran mangrove leaves, types I, II, IV, and V are found, and in the same mangrove roots, types I and II are found. 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. Polyprenols also occurred as one or two polyprenol families, specifically ficaprenol-type polyprenols (shorter polyprenols) and longer polyprenols, depending on the plants and tissues. Two polyprenol families were detected in *Acr. aureum* leaves and roots and *S. caseolaris* leaves. The current results support the previous findings of two polyprenol families in the yellow leaves of *K. obovata* and the leaves of *L. racemosa* and *P. acidula* (Basyuni et al., 2016). In contrast, dolichols occurred as one dolichol family in all tissues observed, with a variety of carbon-chain lengths depending on the mangrove species and tissue. These results, therefore, suggest that the formation of shorter-chain polyprenols, longer-chain polyprenols, and dolichols are independently regulated in mangrove plants.

Dolichols predominated in 19 of 29 mangrove tissues, including the leaves and roots (Tables 2 and 3). Therefore, the occurrence of dolichols in all tissues observed implies that polyprenols may not play an important role in mangrove plants, although the function of polyprenols in the plant world remains obscure. The accumulation of plant polyisoprenoids have been reported to change in ages (Swiezewska et al., 1994; Tateyama et al., 1999; Basyuni et al., 2016), season (Swiezewska et al., 1994), and important for plant responses to biotic and abiotic stress (Zhang et al., 2008; Bajda et al., 2009; Baczewska et al., 2014). Therefore, finding a source of certainly obtainable polysioprenoids should be consideration to the importance of this investigation. Polyprenol reductase may be active in mangrove plant leaves to catalyze the reduction of polyprenol to dolichol and this issue may be specific for mangrove. The apparent predominance of dolichols may be the result of tropical or sub-tropical climatic conditions (Basyuni et al., 2012b; 2016).

In the present study, a new class of polyprenyl acetones was detected together with the all-trans type of polyprenyl acetones such as those of the bombiprenone (C_{43}) family. The compounds are cis-type polyprenyl acetones cannot be concluded, in which the shortest one is geranylneryl acetone with a chain length of C_{23}. However, considering the chromatographic behavior of the new compounds in comparison with the mobility of the bombiprenone family on thin-layer plates, it is expected that these compounds are indeed cis-type polyprenyl acetones.

The occurrence and distribution of polyisoprenoids in mangrove leaves was utilized to discuss the cluster between mangrove species. Cluster analysis using the leaf data of polyisoprenoid carbon-chain lengths revealed that these 24 mangrove species fell into two groups, the polyprenyl acetone mangrove group and the polyprenol-dolichol group. Major mangrove species from the Rhizophoraceae and Acanthaceae tribes were included in the second group. It is very plausible that a distinct compound is responsible for the formation of polyisoprenoids in this species. As a result, the composition of polyisoprenoids may be a reflection of the distribution of tissues in these plants. As shown in Fig. 6 that the circumstance for Rhizophoraceae is in agreement with the previous results on the molecular evolution of the Rhizophoraceae tribe (Basyuni et al., 2007b). *Kandelia* is more similar to *Rhizophora* than to *Bruguiera* or *Ceriops*, even though these genera are originated from the same tribe of Rhizophoraceae. A number of phylogenetic studies of the Rhizophoraceae tribe based on molecular markers and morphological characters support this view (Setoguchi et al., 1999; Schwarzbach & Ricklefs, 2000; Lakshmi et al., 2002).

N. fruticans, a member of true major mangroves clustered with the Rhizophoraceae group.

Rhizophoraceae also form a distinct branch as demonstrated from the root data of the carbon-chain lengths of polyisoprenoids. These eight species, representing four genera (*Kandelia*, *Rhizophora*, *Bruguiera*, and *Ceriops*) of Rhizophoraceae, are characterized by viviparity propagules, which is the most distinguishing feature of mangroves (Lakshmi et al., 2002). These genera are 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, the non-secretor species do have an ultra-filtration mechanism in the roots for excluding salt. Among the other true mangroves analyzed in the present study, the genus Xylocarpus of Meliaceae, represented by *X. granatum* (Fig. 7), was found to be closest to the Rhizophoraceae tribe. Rhizophoraceae and Meliaceae were previously placed under the same order of Myrtales; however, Rhizophoraceae is now under a separate order, the Rhizophorales (Parani et al., 1998).

The possibility of the presence of a new branch of Acanthaceae was considered due to an evolutionary tree of mangrove plants. The reasons for the generation of two branches of *Avicennia* and *Acanthus* are not yet known, although the characterization of polyisoprenoids from other mangroves may provide an explanation. It has been reported that nuclear and chloroplast DNA has placed *Avicennia* as a member of Acanthaceae, whereas it was previously treated as a member of Verbenaceae (Schwarzbach & Ricklefs, 2000).

These findings suggest that the distribution of lipid analysis, including polyisoprenoids, may provide clear chemotaxonomic markers in mangrove leaves and roots allowing the classification into appropriate genera and families. These finding also support previous view that the lipids of mangroves are chemotaxonomically significant (Hogg & Gillan, 1994; Basyuni et al., 2007a; 2007b; 2016).

Conclusions

Collectively, present findings demonstrate that the distribution of a new class of polyisoprenoid derivatives, i.e. polyprenyl acetones, polyprenols and dolichols found in Indonesian mangrove plants varies depending on each tissue. Polyisoprenoid patterns in both the leaves and roots form distinct separation into appropriate genera and tribe, suggesting that mangrove polyisoprenoids are chemotaxonomically significant. Future studies are needed to understand whether dolichols in mangrove plants function as sugar-carrier lipids in the biosynthesis of *N*-glycoproteins (Pattison & Amtmann, 2009). Further experiments are also necessary to clarify the physiological significance of polyisoprenoid alcohols under environmental stresses, such as salinity and light, as well as the molecular cloning of the mangrove polyisoprenoid biosynthesis gene.

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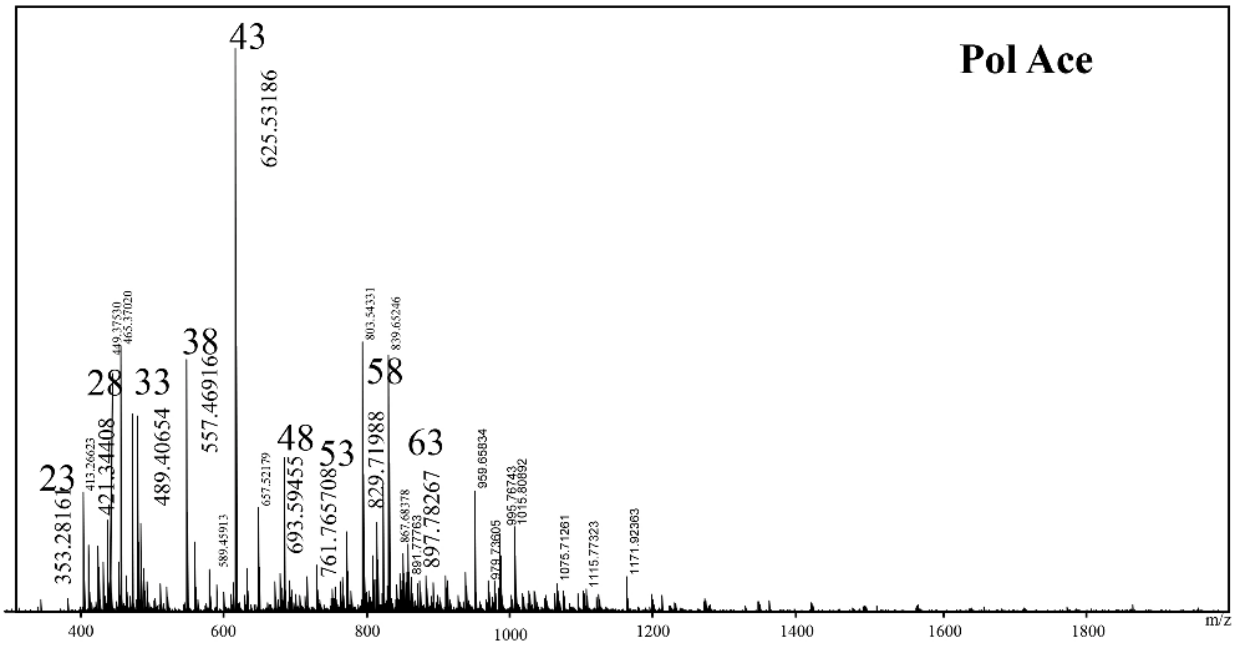
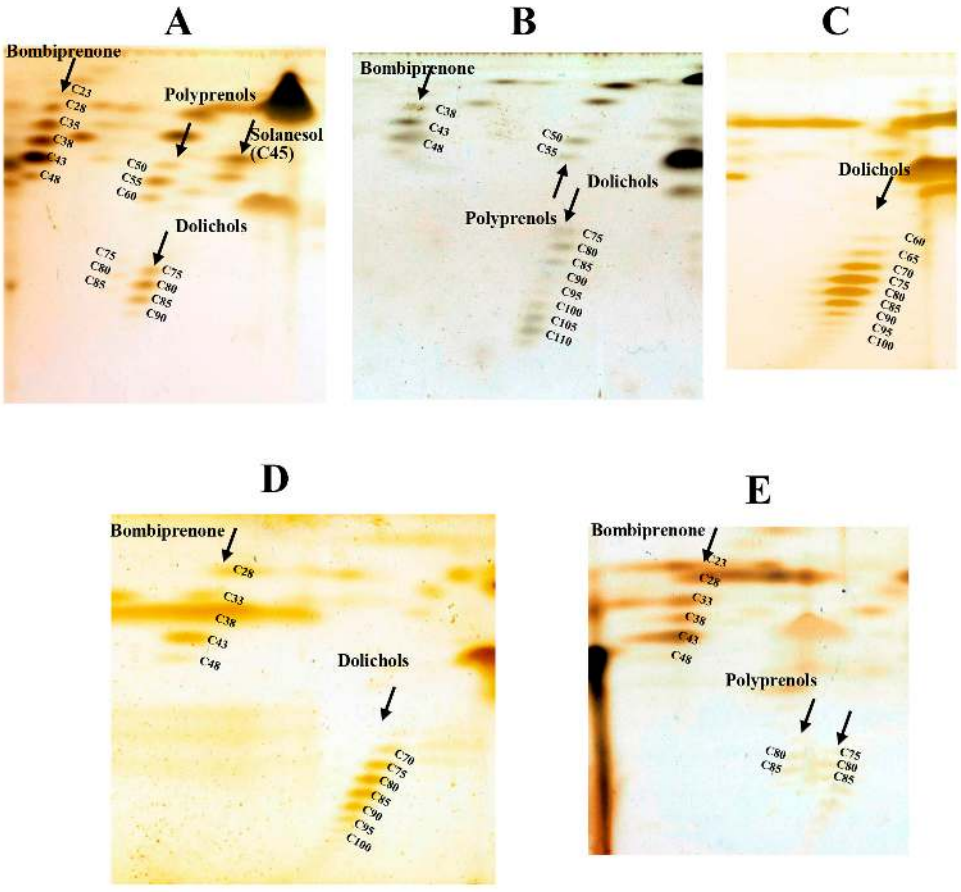
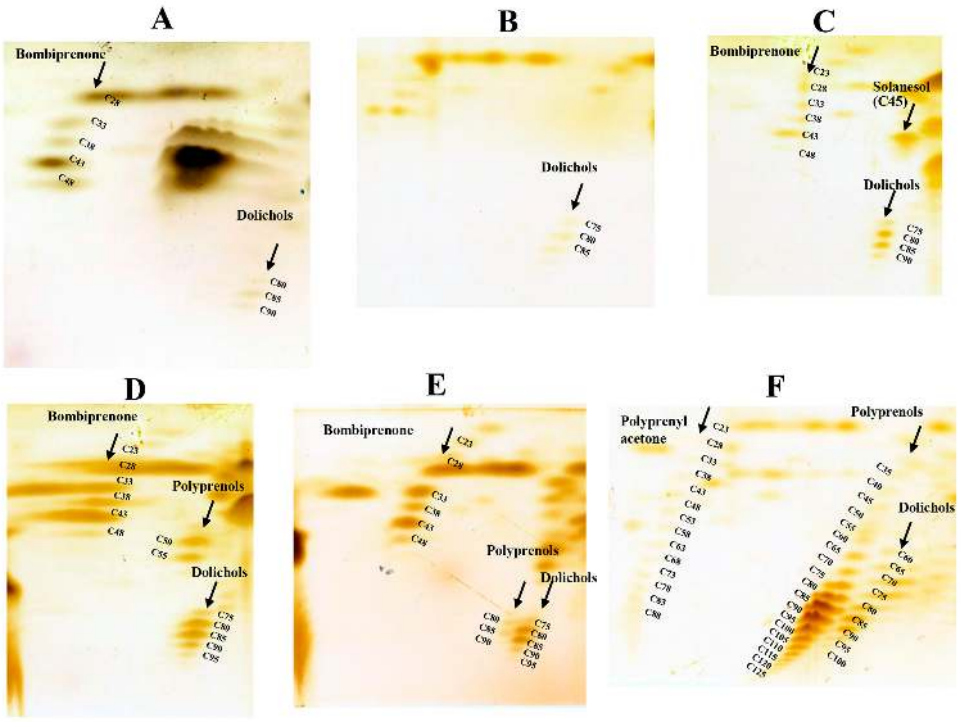


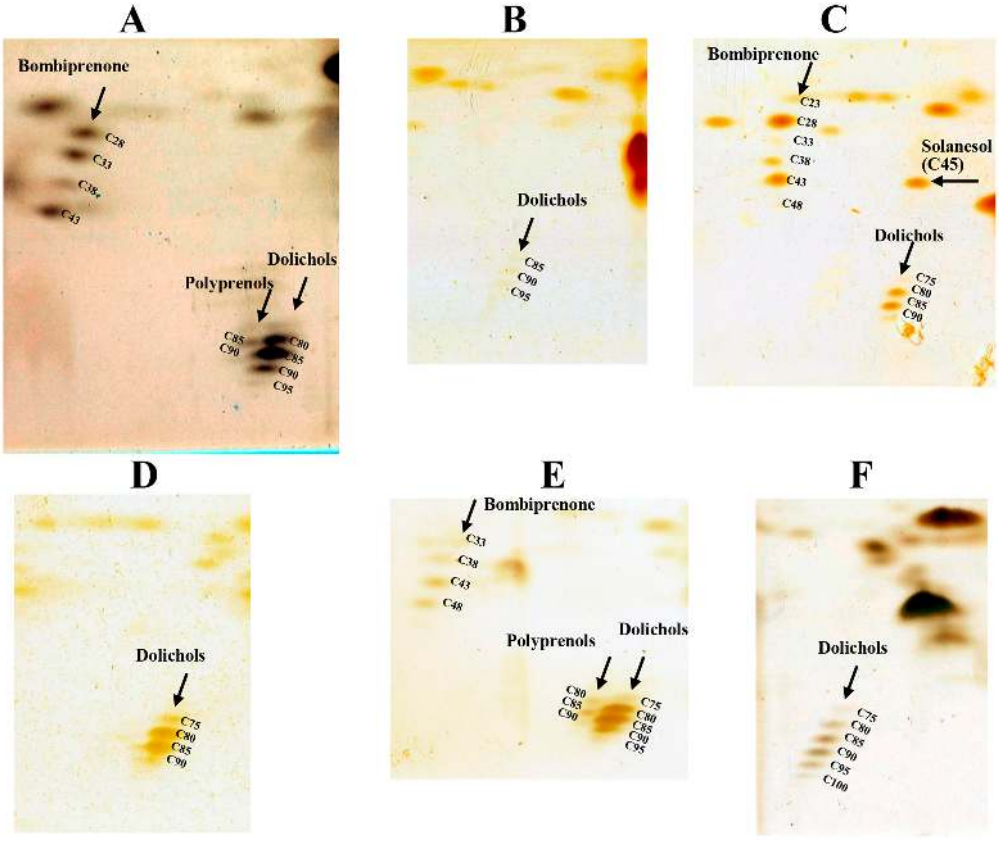
Supplementary Fig. 1. The electrospray ionization spectra of hexane extract of *S. caseoalris* leaves were purified as described in Materials and method section and consist of purified polyprenol acetone (C_{23}–C_{63}). The numbers (23–63) indicates the isoprene units of polyprenol acetone compounds (D)



Supplementary Fig. 2. Two-plate TLC chromatograms of polyisoprenoid hexane extracts from *Acr. aureum* leaves (A), *Acr. aureum* young leaves (B), *Av. lanata* leaves (C), *Av. officinalis* leaves (D), and *B. cylindrica* leaves (E). The number shows the carbon-chain length of the polyisoprenoid alcohols



Supplementary Fig. 3. Two-plate TLC chromatograms of polyisoprenoid hexane extracts from *B. parviflora* (A), *C. tagal* (B), *N. fruticans* (C), *R. apiculata* (D), *R. mucronata* (E), and *X. granatum* (F) leaves. The number shows the carbon-chain length of the polyisoprenoid alcohols



Supplementary Fig. 4. Two-plate TLC chromatograms of polyisoprenoid hexane extracts from *B. parviflora* (A), *C. tagal* (B), *N. fruticans* (C), *R. apiculata* (D), *R. mucronata* (E), and *S. caseolaris* (F) roots. The number shows the carbon-chain lengths of the polyisoprenoid alcohols