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

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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 polyprenols (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 equisetifolia*, *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*, *Ricinus 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 polysioprenoid 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

Abbreviations: 2D-TLC: Two-Dimensional Thin Layer Chromatography, Bom: Bombiprenone, Dol: dolichol, dw: dry weight, MVSP: Multivariate Statistical Package, PI: polyisoprenoid, Pol: polyprenol, TL: total lipid, UPGMA: Unweighted-Pair Group Method with Arithmetic mean

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 (Tomlinnson, 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 eukaryotes and prokaryotes (Swiezewska and Danikiewicz 2005; Skrorupinska-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 polyprenol (α-unsaturated) and dolichol (α-saturated) compounds (Figure 1). The occurrence of polyisoprenoids has been reported in tropical and sub-tropical 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 tiliaceus* L. (Malvaceae), *Melastoma candidum* D. Don (Melastomataceae), *Morinda citrifolia* L. (Rubiaceae), *Pandanus odoratissimus* (Pandanaceae), *Pongamia pinnata* (L.) Pierre (Fabaceae), *Ricinus communis* Linn. (Euphorbiaceae), *Scyphiphora hydrophyllacea* 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. tiliaceus*, *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_{90}-C_{105}) standard compounds was isolated from horse testicles together with a mixture of polyprenol (C_{90}-C_{100}) from *Malus* sp. (Swiezewska and Danikiewicz 2005). A mixture of dolichol (C_{95}-C_{110}) standards derived from skipjack tuna livers (Ishiguro et al. 2014) was also used in this study. The identification of the family corresponding to polyprenols or dolichols was performed in at least three independent experiments. Bombiprenone (C_{43}) (Figure 1), as described by Irvine et al. (1972), was purified by the silica-gel chromatography of unsaponifiable lipids of the $CHCl_3/CH_3OH$ (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 $[M + Na^+]$ ions were detected with m/z 625.53183, corresponding to $C_{43}H_{70}O$ (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 polyisoprenoid alcohols

The procedure for the isolation of polyisoprenoids was performed as previously described (Sagami et al. 1992; Basyuni 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 gravemetrically. 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.

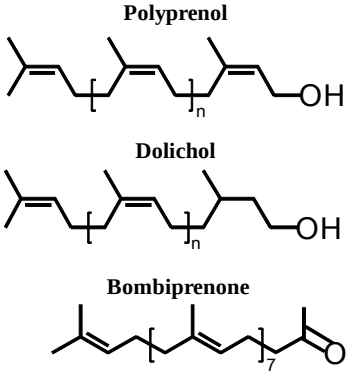

Figure 1. Structure of polyprenol, dolichol, and bombiprenone. n shows the number of internal isoprene residues

Analysis by two-dimensional thin layer chromatography (2D-TLC)

First-dimension TLC was carried out for 45 min on a silica gel glass plate (20 × 3 cm) with a solvent system of toluene-ethyl acetate (9:1) as previously described (Basyuni et al. 2016; 2017). The second-dimension reversed-phase C-18 silica gel TLC was performed with acetone as the solvent for approximately 45 min. The position of the separated polyisoprenoid alcohols being developed by 2D-TLC was identified and visualized with iodine vapour (Basyuni et al. 2017). To determine whether the family corresponds to dolichols or polyprenols, in the case of the one family that was observed on 2D-TLC, a dolichol or polyprenol reference was added to the sample line of the first-dimension TLC and developed with a solvent system, as previously described (Basyuni et al. 2016). The developed chromatographic images were obtained and digitally scanned with a Canon E-400 series printer. The polyisoprenoid family was identified by the comparison of mobility on TLC with that of authentic standards of dolichol or polyprenol that were applied in the second-dimension development. The polyprenols and dolichols that were detected on RP-18 HPTLC plates were semi-quantified using ImageJ ver. 1.46r (Schneider et al. 2012) with dolichol and polyprenol standards as references. The scan chromatogram was grey scale mode analyzed to obtain plot lines and label peaks. This peak area was copied and pasted to the program Microsoft Excel 2010.

Data analysis

Cluster analysis was performed on selected subsets of leaf data consisting of 41 variables, including polyprenols and dolichols from 23 species. In this analysis, 13 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. Two dendrograms representing the leaf and root data were drawn by cluster analysis using the unweighted-pair group method with arithmetic mean (UPGMA) and MVSP (multivariate statistical package) ver. 3.22 (Kovach 2010). Euclidean distance was chosen as the criterion for cluster combination.

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), polyprenols 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*, *M. citrifolia*, *S. hydrophyllacea*, *S. portulacastrum* and *T. catappa* (Table 2). In the leaves of *M. candidum*, *M. citrifolia*, *S. hydrophyllacea*, and *T. catappa*, pol (ficaprenols and longer polyprenols) 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 polyprenols and dolichols, i.e., ficaprenol (C_{60}-C_{65}) and dolichols (C_{75}-C_{90}), as shown in Supplementary Figure 1G. In the leaves of *M. candidum*, *M. citrifolia*, *S. hydrophyllacea*, and *T. catappa*, polyprenols much longer than dolichols (>C_{100} 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 occurence 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 polyprenols only, ficaprenol-like chain length (C_{60}-C_{65}) and that dolichols and longer polyprenols 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 polyprenols 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.

Tabel 1. Occurrence and distribution of polyprenols and dolichols in North Sumatran coastal plants. TL was presented as means of triplicate analyses ± SD. TLs are expressed as a fraction of a crude lipids estimated gravimetrically

Species	Plant code	Tissue	TL (mg/g dw)	Pl (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in total lipid			% in polyisoprenoid		Type
					Pl	Pol			Dol	Pol	Dol	
A. auriculiformis	Aa	Leaves	651±50	28	28	nd	4	4	nd	100	nd	III
B. asiatica	Ba	Leaves	630±26	133	11	122	21	2	19	8	92	I
C. equisatifolia	Ce	Leaves	576±23	65	18	47	11	3	8	28	72	II
C. inophyllum	Ci	Leaves	132±11	25	nd	25	4	nd	4	nd	100	I
*H. tiliaceus**	Ht	Leaves	100±6	14	14	nd	14	14	nd	100	nd	III
M. candidum	Mca	Leaves	589±89	140	95	45	23	16	7	68	32	II
M. citrifolia	Mci	Leaves	611±71	119	58	61	19	9	10	49	51	II
P. odoratatissima	Po	Leaves	556±65	23	nd	23	4	nd	4	nd	100	I
P. pinnata	Pp	Leaves	654±27	12	12	nd	2	2	nd	100	nd	III
R. communis	Rc	Leaves	861±15	20	20	nd	2	2	nd	100	nd	III
S. hydrophyllacea	Sh	Leaves	631±4	138	65	73	22	10	12	47	53	II
S. jamaicensis	Sj	Leaves	658±55	19	nd	19	3	nd	3	nd	100	I
S. portulacastrum	Sp	Leaves	650±27	53	24	29	8	3	5	45	55	II
T. catappa	Tc	Leaves	572±82	65	35	30	11	6	5	54	46	II
A. auriculiformis	Aa	Roots	514±25	54	25	29	11	5	6	47	53	II
B. asiatica	Ba	Roots	556±55	47	nd	47	8	nd	8	nd	100	I
C. equisatifolia	Ce	Roots	516±26	23	nd	23	5	nd	5	nd	100	I
C. inophyllum	Ci	Roots	645±65	29	nd	29	4	nd	4	nd	100	I
H. tiliaaceus	Ht	Roots	517±39	27	nd	27	5	nd	5	nd	100	I
M. candidum	Mca	Roots	546±23	34	15	19	7	3	4	44	56	II
M. citrifolia	Mci	Roots	565±43	23	8	15	5	2	3	35	65	II
P. odoratatissima	Po	Roots	549±147	300	nd	300	54	nd	54	nd	100	I
P. pinnata	Pp	Roots	562±9	25	nd	25	5	nd	5	nd	100	I
R. communis	Rc	Roots	529±19	31	nd	31	6	nd	6	nd	100	I
S. hydrophyllacea	Sh	Roots	603±69	30	nd	30	5	nd	5	nd	100	I
S. jamaicensis	Sj	Roots	538±10	27	nd	27	5	nd	5	nd	100	I
S. portulacastrum	Sp	Roots	523±45	25	nd	25	5	nd	5	nd	100	I
T. catappa	Tc	Roots	555±70	28	nd	28	5	nd	5	nd	100	I

Note: *data was taken from Basyuni et al. 2016. nd = not detected

Cluster analysis of polyisoprenoid data

The cluster 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 fell into two groups (Figure 4). One group was a cluster of nine species including five true mangroves (*Avicennia marina*, *Phempis acidula*, *Lumnitzera racemosa*, *Sonneratia alba*, and *S. hydrophyllacea*). Three mangrove associates (*M. candidum*, *M. citrifolia*, and *T. catappa*) with long-chain polyprenols 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 polyprenols 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. gymnorrhiza*, *K. obovata*, and *R. stylosa*) are included in this group. It is interesting to note that shorter-chain polyprenols (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. tiliaceus*, *H. littolaris*, 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_{80}-C_{95}. The Rhizophoraceae tribe formed a distinct branch consisting of the true mangroves, *K. obovata*, *R. stylosa*, and *B. gymnorrhiza*. 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. littolaris* 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	
B. asiatica	Leaves	o	80 **85** **90** 95	55 60 65 70 **75** **80** **85** 90 95 100 105 110 115 120 125 130 135 140
C. equisetifolia	Leaves	o	75 **80** 85	70 75 **80** **85** 90
C. inophyllum	Leaves			75 80 85
*H. tiliaceus**	Leaves	o	**60** 65	
M. candidum	Leaves	o	45 50 55 60 65 **70** **75** **80** 85 90 95 100 105 110 115 120 125 130 135 140	50 55 60 65 **70** **75** 80 85 90 95 100 105 110 115
M. citrifolia	Leaves		60 65 70 **75** **80** 85 90 95 100 105 110 115 120 125 130 135 140	60 65 70 **75** **80** **85** 90 95 100 105 110 115 120 125
P. pinnata	Leaves		**60** 65	
P. odoratatissima	Leaves			75 80 **85** **90** 95
R. communis	Leaves		**60** 65	
S. hydrophyllacea	leaves	o	**60** 65 70 75 **80** **85** 90 95 100 105 110 115 120 125 130 135 140	60 65 70 **75** **80** **85** 90 95 100
S. jamaicensis	Leaves			75 80 **85** **90** **95** 100
S. portulacastrum	Leaves		**60** 65	75 **80** **85** 90
T. catappa	Leaves	o	70 75 **80** **85** 90 95 100 105 110 115 120 125 130 135 140	70 75 80 **85** **90** **95** 100 105 110 115 120
A. auriculiformis	Roots		**55** 60	80 **85** 90
B. asiatica	Roots			75 80 **85** **90** 95 100
C. equisetifolia	Roots			75 80 **85** **90** 95
C. inophyllum	Roots			80 **85** 90
H. tiliaceus	Roots			75 80 **85** **90** 95
M. candidum	Roots		80 **85** 90	75 **80** **85** 90
M. citrifolia	Roots	o	50 **55** 60	50 55 60 65 70 75 80 **85** **90** 95 100
P. odoratatissima	Roots			80 **85** 90
P. pinnata	Roots			80 **85** **90** 95
R. communis	Roots			80 **85** 90
S. hydrophyllacea	Roots			65 70 75 80 **85** **90** **95** 100
S. jamaicensis	Roots			70 75 **80** **85** 90
S. portulacastrum	Roots	o		70 75 80 85 **90** **95**
T. catappa	Roots			75 **80** **85** 90

Note: *The numbers refer to the carbon-chain length of the polyisprenoid alcohols. Bom: Bombiprenone. The chain length of the main polyisoprenoid alcohols is indicated in bold. O = detected.

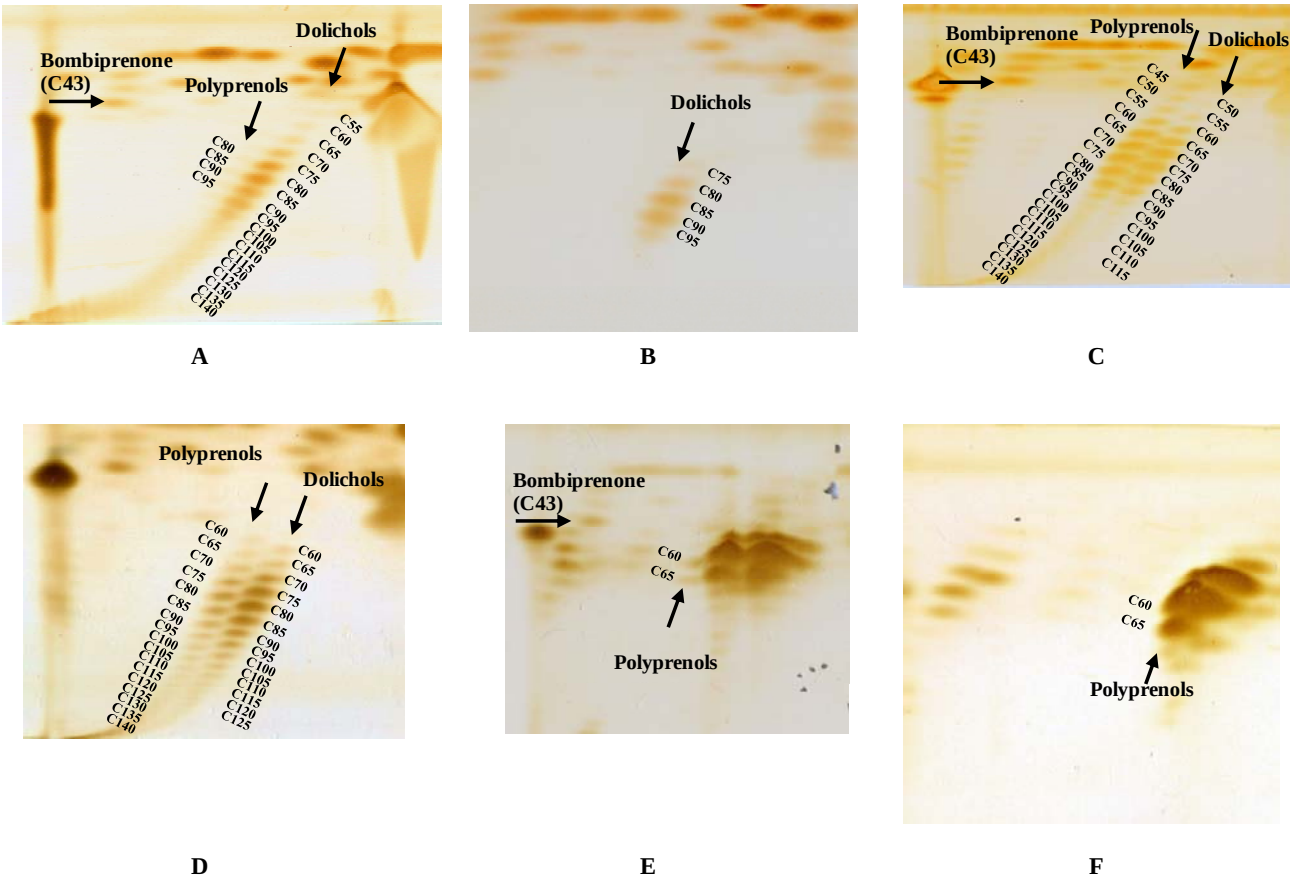

Figure 2. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *B. asiatica* leaves, B. *P. odoratissimus* leaves, C. *M. candidum* leaves, D. *M. citrifolia* leaves, E. *A. auriculiformis* leaves, and F. *R. communis* leaves. The number indicates the carbon-chain length of the polyisoprenoid alcohols

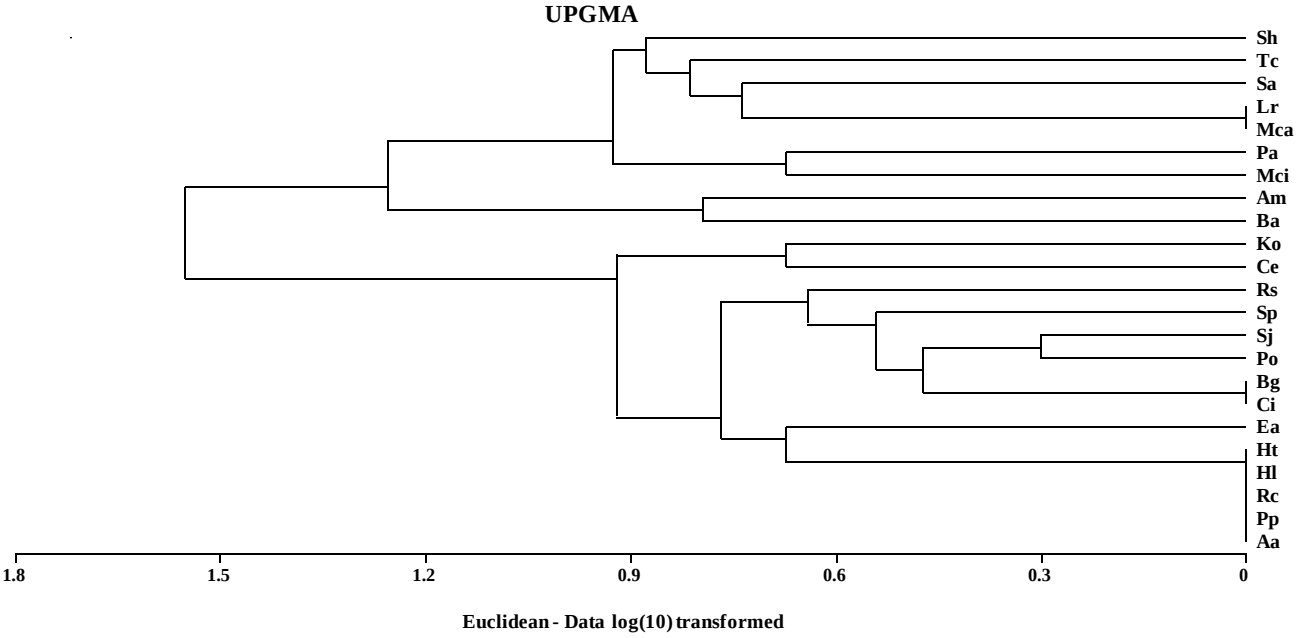

Figure 4. Dendrogram depicting the similarities of species based on carbon-chain length leaves data of polyisoprenoids by log (10) transformation using the Euclidean distance of 13 North Sumatran coastal plants and 10 Okinawan mangrove species. UPGMA: unweighted-pair group method with arithmetic mean. For species name, see Table 1. Am, *Avicennia marina*; Bg, *Bruguiera gymnorrhiza*; Ea, *Excoecaria agallocha*; Hl, *Heritiera littoralis*; Ht, *Hibiscus tiliaceus*; Ko, *Kandelia obovata*; Lr, *Lumnitzera racemosa*; Pa, *Pemphis acidula*; Rs, *Rhizophora stylosa*; and Sa, *Sonneratia alba*.

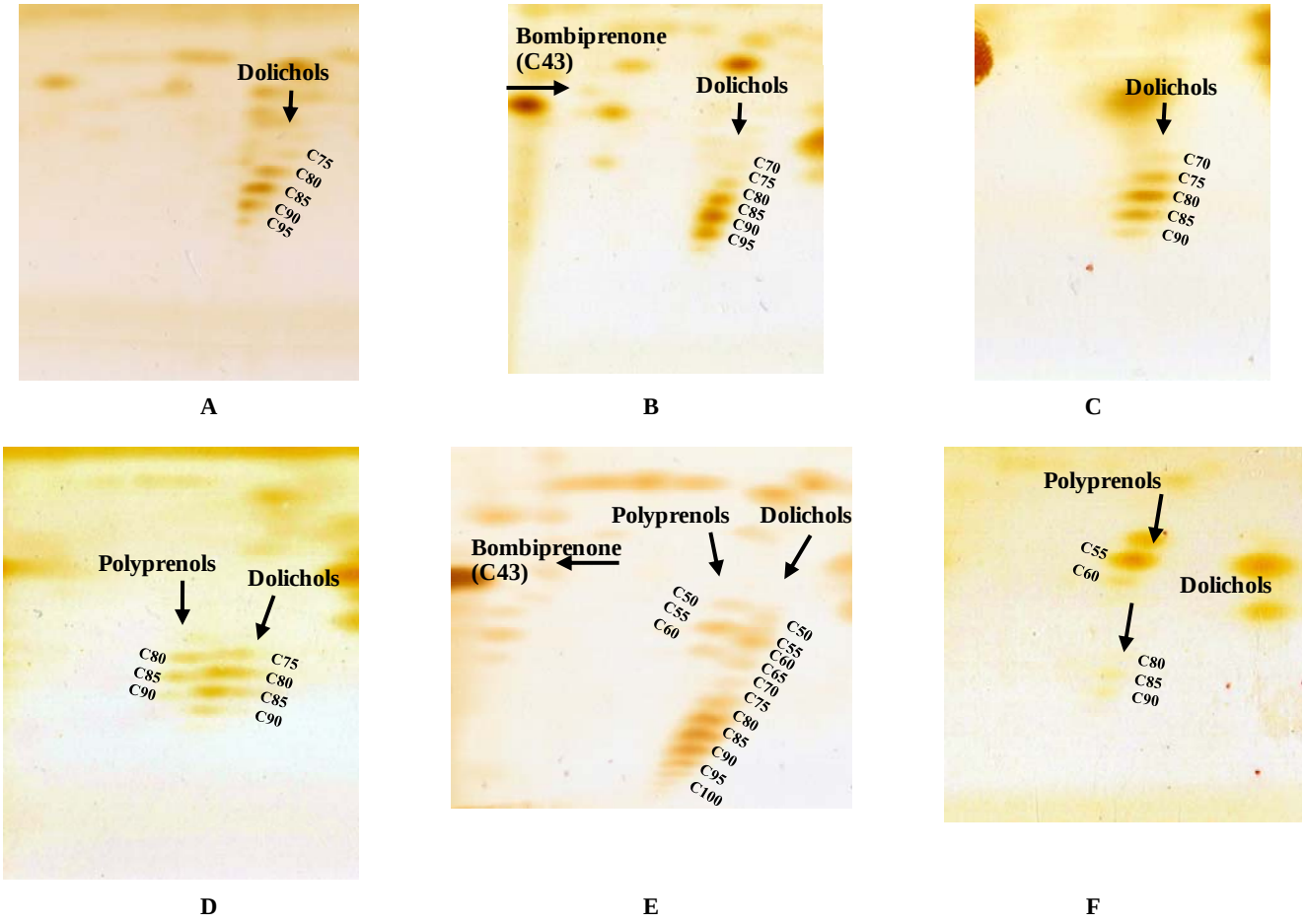

Figure 3. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *H. tiliaceus* roots, B. *S. portulacastrum* roots, C. *S. jamaicensis* roots, D. *M. candidum* roots, E. *M. citrifolia* roots, and F. *A. auriculiformis* roots. The number indicates the carbon-chain length of the polyisoprenoid alcohols

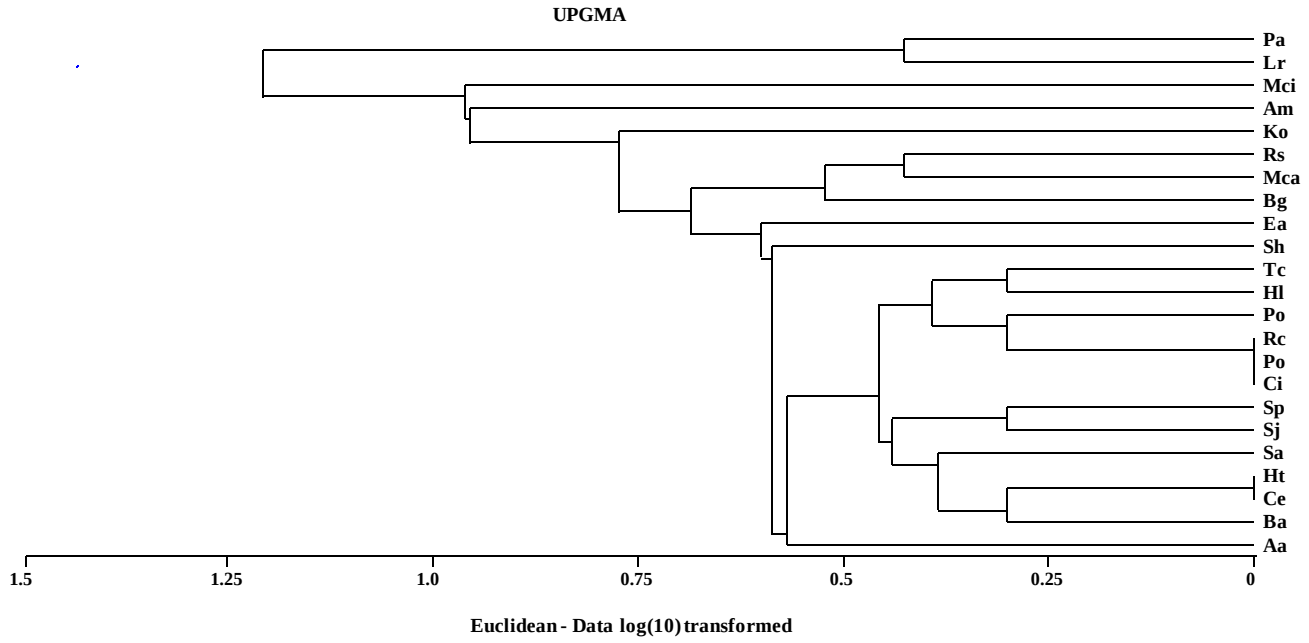

Figure 5. Dendrogram depicting the similarities of species based on carbon-chain length roots data of polyisoprenoids by log (10) transformation using the Euclidean distance of 14 North Sumatran coastal plants and 9 Okinawan mangrove species. For species name, see Table 1 and Figure 4

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 polyprenol families, specifically ficaprenol-type polyprenols (shorter polyprenols) and longer polyprenols, depending on the plants and tissues. The type-III contained only shorter-chain polyprenol, this distribution pattern is similar to the case in the families of Euphorbiaceae, Lauraceae, Magnoliaceae, and Moraceae (Swiezewska et al. 1994; Skorupinska-Tudek et al. 2003).

Two polyprenol 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 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, 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 polyprenols 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 polyprenols (ficaprenols; medium and longer prenols). 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* (C_{65}-$_{130}$), *Lumnitzera racemosa* (C_{60}-C_{140}), *Phempis acidula* (C_{50}-C_{140}), *Sonneratia alba* (C_{65}-C_{130}), *Acanthus ilicifolius* (C_{60}-C_{125}), *Aegiceras corniculatum* (C_{60}-C_{140}), *S. caseolaris* (C_{50}-C_{120}), *A. auriculiformis* (C_{55}-C_{140}), *M. candidum* (C_{50}-C_{115}), *M. citrifolia* (C_{60}-C_{140}), and *T. catappa* (C_{70}-C_{120}). 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 Danikiewicz 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 polyprenols, longer-chain polyprenols, 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 polyprenols may not play an important role in coastal plants, although the function of polyprenols 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 polyprenol 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 *C. equisetifolia* (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 support 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 polyprenols 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 polyprenols 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 polyprenol reductases in coastal plant leaves, which catalyze the conversion of polyprenol to dolichol and corresponds to the SRD5A-3 protein in animals, differs from those of other coastal plants in reduction activity (Pattisom and Amtmann 2009; Jozwiak et al. 2015). 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

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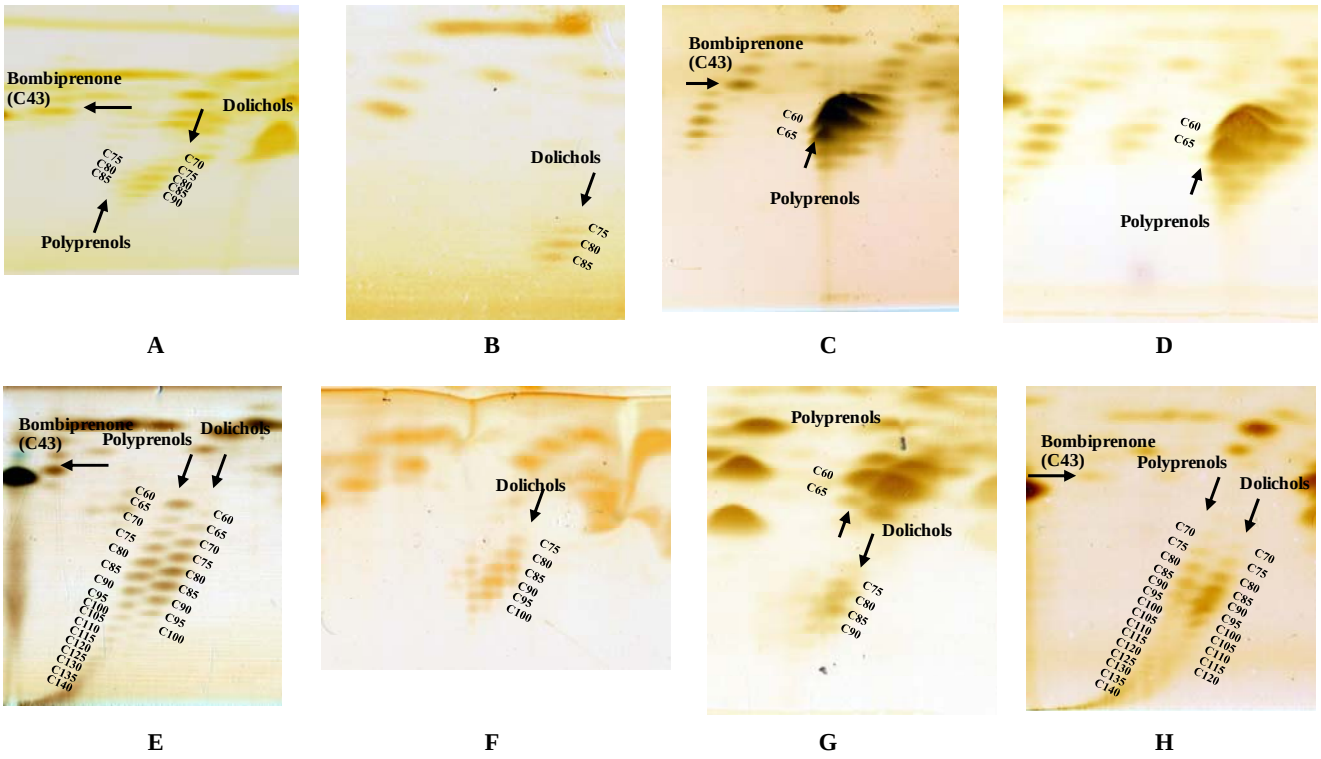

Figure S1. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *C. equisetifolia* leaves, B. *C. inophyllum* leaves, C. *H. tiliaceus* leaves, D. *P. pinnata* leaves, E. *S. hydrophyllacea* leaves, F. *S. jamaicensis* leaves., G. *S. portulacastrum* leaves, and H. *T. catappa* leaves. The number indicates the carbon-chain length of the polyisoprenoid alcohols

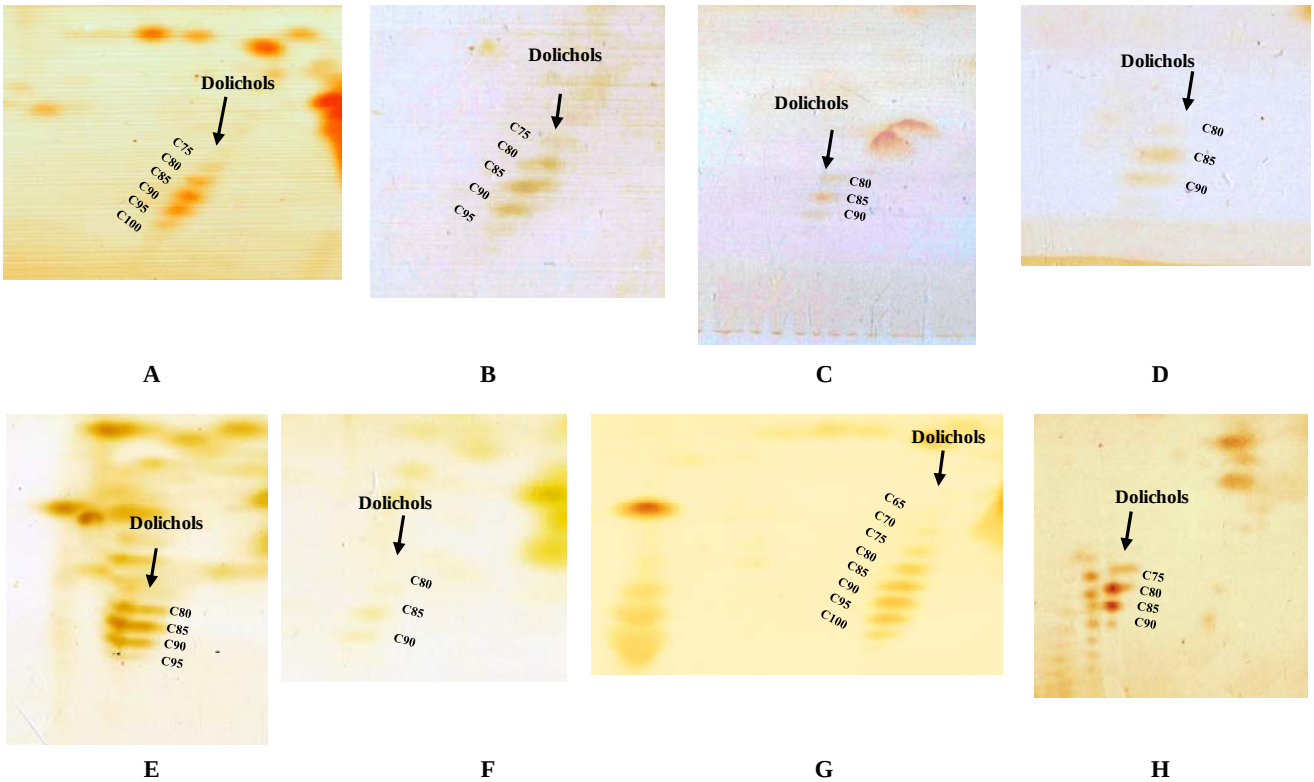

Figure S2. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from: A. *B. asiatica* roots, B. *C. equisetifolia* roots, C. *C. inophyllum* roots, D. *P. odoratissimus* roots, E. *P. pinnata* roots, F. *R. communis* roots, G. *S. hydrophyllacea* roots, and H. *T. catappa* roots. The number indicates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