

Polyisoprenoid-based chemotaxonomy and cluster analysis of mangrove reproductive tissues from North Sumatra, Indonesia

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Manuscript received: 8 July 2025. Revision accepted: 9 October 2025.

Abstract. Basyuni M, Pulungan W, Al Mustaniroh SS, Baba S. 2025. Polyisoprenoid-based chemotaxonomy and cluster analysis of mangrove reproductive tissues from North Sumatra, Indonesia. *Asian J Nat Prod Biochem* 23: 110-121. This study investigates the chemotaxonomic significance of polyisoprenoids—specifically polyprenols and dolichols—in the reproductive organs (flowers and fruits) of fourteen mangrove species from North Sumatra, Indonesia. Employing Two-Dimensional Thin-Layer Chromatography (2D-TLC) as a high-resolution analytical tool and Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering as a classification approach, we profiled the polyisoprenoid compounds in flowers. We classified them into three types based on their relative abundance. Type I, with a predominance of dolichols over polyprenols (more than ninefold), was observed in *Barringtonia asiatica*, *Lumnitzera racemosa*, *Ricinus communis*, and *Scyphiphora hydrophyllacea*. Type II, with the occurrence of both polyprenols and dolichols, was observed in *Acanthus ilicifolius*, *Excoecaria agallocha*, *Hibiscus tiliaceus*, *Melastoma candidum*, and *Sonneratia alba*. Type III, with a predominance of polyprenols over dolichols, was observed in *Avicennia officinalis*, *Bruguiera sexangula*, *Rhizophora apiculata*, and *Rhizophora mucronata*. However, in the fruits, a type-I distribution was observed in nine species. In comparison, five species, *Acanthus ilicifolius*, *A. officinalis*, *H. tiliaceus*, *Lumnitzera littorea*, and *M. candidum*, corresponded to a type II distribution and were not type III. Quantitative analyses revealed that dolichols were dominant in 64% of the fruit tissues, while floral tissues showed more diversity. Hierarchical cluster analysis using UPGMA grouped species based on polyisoprenoid similarity, demonstrating their value as chemotaxonomic markers. These findings provide a significant foundation for mangrove conservation strategies by linking biochemical markers to species resilience in the coastal ecosystem, inspiring potential new approaches to conservation.

Keyword: 2D-TLC, chemotaxonomy, generative tissues, mangrove, polyisoprenoid

Abbreviations: 2D-TLC: Two-Dimensional Thin Layer Chromatography, Dol: Dolichols, HPTLC: High Performance Thin Layer Chromatography, Pol: Polyprenols, Poly: Polyisoprenoids, TL: Total Lipids, UPGMA: Unweighted-Pair Group Method with Arithmetic mean

INTRODUCTION

Mangroves are among the most productive and ecologically significant coastal ecosystems, supporting biodiversity, shoreline stability, and climate regulation, particularly in tropical and subtropical regions (Friess et al. 2020; Kasihw et al. 2024). Accurate identification of mangrove species is critical for conservation planning, especially in the face of habitat loss and climate stress. The integration of chemical profiling, such as polyisoprenoid analysis, with ecological data could enhance biodiversity monitoring and reveal species-specific adaptation strategies (Umoh 2020; Upadhyay et al. 2025). This is particularly important in Southeast Asia, a major hotspot for mangrove diversity and degradation pressure (Friess et al. 2020).

In addition to their ecological roles, mangrove plants are increasingly recognized as rich sources of phytochemical compounds, including secondary metabolites with potential pharmacological applications (Bibi et al. 2019; Upadhyay et al. 2025). Among these are polyisoprenoids, a class of long-chain isoprene compounds including polyprenols and dolichols, which are linear polymers of five-carbon

isoprene units found across all domains of life (Swiezewska and Danikiewicz 2005; Surmacz and Swiezewska 2011; Basyuni et al. 2017a).

One of the abundant secondary metabolites in mangrove plants is polyisoprenoid alcohol. Polyisoprenoid alcohols are linear polymers of five-carbon chain units that are present in almost all living cells. Long-chain polyisoprenoids are present in various plant tissues (Swiezewska and Danikiewicz 2005). Polyisoprenoid alcohol (dolichol and polyprenol) is found in all living organisms, from bacteria to mammals/animals (Surmacz and Swiezewska 2011). Polyisoprenoids are found in cells and tissues in a variety of different forms. Polyisoprenoids are present in plants with different chain lengths, including increasing age (tissue aging) (Swiezewska and Danikiewicz 2005; Basyuni et al. 2017b), salinity stress (Basyuni et al. 2021), tissue differences (Tateyama et al. 1999; Surmacz and Swiezewska 2011), light (Bajda et al. 2005; Swiezewska and Danikiewicz 2005), and temperature (Salvador-Castell et al. 2020). Polyisoprenoids are present as free alcohols or esterified with acetate or fatty acids (Gelder et al. 2021).

Chemotaxonomy, which involves using chemical constituents for taxonomic classification, has become a valuable complementary tool alongside morphological and molecular methods (Basyuni et al. 2017a). This approach is especially important in mangrove research, where traditional identification techniques may fail due to convergent traits among unrelated taxa. Polyisoprenoids, including polyprenols and dolichols, are long-chain isoprenoid alcohols that play essential roles in cellular processes such as glycoprotein synthesis and maintaining membrane integrity (Basyuni et al. 2021).

In plants, polyisoprenoids are involved in various biological functions, particularly in response to environmental stressors such as salinity, light, and tissue aging (Swiezewska and Danikiewicz 2005; Basyuni et al. 2017a). The biosynthesis and accumulation of polyisoprenoids can vary with tissue type and developmental stage (Tateyama et al. 1999; Surmacz and Swiezewska 2011; Rizkiany et al. 2021). However, beyond their biochemical function, their distribution among plant taxa shows potential as chemotaxonomic markers, especially in complex and poorly resolved genera like *Rhizophora* and *Bruguiera* (Bandaranayake 2002). Recent studies have emphasized the need to explore secondary metabolites, including polyisoprenoids, for better species delimitation and ecological function interpretation within mangrove ecosystems (Upadhyay et al. 2025; Mali et al. 2023).

Despite their widespread presence, little is known about how these compounds are distributed in mangrove reproductive structures. Therefore, this study aims to investigate the occurrence and chemotaxonomic significance of polyisoprenoid profiles in the generative tissues—flowers and fruits—of fourteen mangrove species in North Sumatra, Indonesia. By employing Two-Dimensional Thin-Layer Chromatography (2D-TLC) and hierarchical cluster analysis, we evaluated the variation in polyprenol and dolichol contents to explore their potential roles in species differentiation and adaptation, as well as the further roles of polyisoprenoids.

MATERIALS AND METHODS

Plant materials

Reproductive organs (flowers and fruits) from 14 carefully selected mangrove species were collected from Lubuk Kertang and Pulau Sembilan, North Sumatra, Indonesia, during the peak fruiting season. The selection of these species was based on their abundance, reproductive activity, and ecological distribution, ensuring a comprehensive study. The harvested flowers and fruits were harvested from healthy, mature plants and immediately preserved in cool, dark conditions for laboratory analysis. Fourteen mangrove species were selected based on their abundance, reproductive activity, and ecological distribution. Flowers and fruits were harvested during the peak reproductive season (May-August) as shown in Figure 1, with three individual plants per species being sampled, and tissues were analyzed separately. Habitat characteristics, such as tidal exposure and salinity,

were recorded at the time of collection. All samples were transported on ice and stored at -20°C until further analysis to minimize metabolic degradation.

Extraction

Polyisoprenoid compounds were extracted via a modified protocol from Swiezewska and Danikiewicz (2005) and Basyuni et al. (2017a). Fresh plant tissue (approximately 2 g) was homogenized and subjected to lipid extraction with chloroform-methanol (2:1, v/v). The extract was partitioned and washed with aqueous solutions to isolate the lipid fraction, which was then saponified and purified as previously reported (Basyuni et al. 2021).

Polyprenol and dolichol profiles were analyzed via 2D-TLC. Silica gel plates were developed in two solvent systems to resolve polyisoprenoid compounds. Identification was based on chromatography with authentic standards and confirmed by visualization via iodine vapor staining. The enhanced chromatograph was imaged and alphanumerically scanned with an Epson L360 series printer.

Two-Dimensional Thin Layer Chromatography (2D-TLC) analysis

Polyisoprenoid profiles were analyzed via 2D-TLC on silica gel RP-18 HPTLC plates (Merck). The plates were first developed in toluene:ethyl acetate (9:1 v/v), then in acetone to separate polyprenols and dolichols on the basis of polarity and to resolve compound families.

Visualization was achieved via the use of iodine vapor. Polyprenols and dolichols were identified on the basis of their R_f values relative to those of authentic standards and by their characteristic yellow-brown coloration upon iodine exposure. The polyprenols and dolichols that were traced on RP-18 HPTLC plates were measured with dolichol and polyprenol specifications according to previously reported criteria (Basyuni et al. 2017b). Chromatograms were visualized using iodine vapor and documented via an Epson L360 scanner. Quantitative measurements were performed by densitometry.

Quantification and cluster analysis

Quantification of polyisoprenoids was performed by densitometry, using a standard solution prepared with dolichol or polyprenol as internal standards for two-dimensional TLC. A standard curve was generated by correlating iodine-stained band intensities with concentrations of dolichol on a reverse-phase C18 plate.



Figure 1. Generative organs were collected from mangrove trees

The polyisoprenoid types were classified into three categories on the basis of the predominance of the compound class: Type-I (dolichol dominant), Type-II (both present), and Type-III (polyprenol dominant). Cluster analysis was performed on subsets of flower and fruit data. In this analysis, 62 variables, each representing polyprenols and dolichols from the flowers and fruits of 14 species, were log-transformed (base 10) as previously described (Basyuni et al. 2017a, b). From these data, dendrograms representing the flower and fruit data were drawn by clustering analysis via the Unweighted-Pair Group Method with Arithmetic mean (UPGMA) and Multivariate Statistical Package (MVSP) 3.22 (Kovach Computing Service). The choice of Euclidean distance as the measure to quantify dissimilarity in polyisoprenoid abundance profiles was significant, as it is a widely accepted measure in cluster analysis.

RESULTS AND DISCUSSION

Polyisoprenoid profiles

The analysis of polyisoprenoid content in the reproductive organs of 14 mangrove species revealed distinct patterns, allowing for classification into three chemotypes. Table 1 presents the relative abundance or presence of polyprenols and dolichols in the flowers of 14 mangrove species, which are categorized into three distinct types. These findings were based on the relative dominance of dolichols and polyprenols.

Type I dolichol-dominant were found in the flowers of *Barringtonia asiatica* (L.) Kurz., *Lumnitzera racemosa* Willd., *Ricinus communis* L., and *Scyphiphora hydrophyllacea* C.F.Gaertn.. These species exhibited a greater than ninefold greater abundance of dolichols than of polyprenols. This profile suggests a strong shift toward dolichol biosynthesis, possibly associated with tissue aging, specific physiological roles in floral development, or species-specific genetic control (Surmacz and Swiezewska 2011; Tholl 2015; Rizkiyani et al. 2021).

Type II (mixed profile) included *Acanthus ilicifolius* L., *Excoecaria agallocha* L., *Hibiscus tiliaceus* L., *Melastoma candidum* D. Don, and *Sonneratia alba* Sm., which presented relatively balanced levels of both polyprenols and dolichols. This balanced occurrence could indicate a dual role of both compounds in cellular processes, such as stress tolerance or active membrane remodeling in flowers (Basyuni et al. 2017a). Type III (polyprenol-dominant) were identified in *Avicennia officinalis* L., *Bruguiera sexangula* (Lour.) Poir., *Rhizophora apiculata* Blume, and *Rhizophora mucronata* Lam., where polyprenols outnumbered dolichols.

Interestingly, in the fruit tissues, 64% of the species exhibited a slight dominance of dolichols over polyprenols, although the diversity was less pronounced than that in flowers. This result indicates a shift in polyisoprenoid metabolism compared with that in floral tissues (Rizkiyani et al. 2021).

Figure 2 displays 2D-TLC profiles of polyisoprenoid extracts from mangrove flowers. The chromatograms reveal distinct banding patterns, allowing the differentiation of

polyprenol- and dolichol-rich species. The polyisoprenoid profile derived from: *Acanthus ilicifolius* L. flowers, *A. officinalis* flowers, *B. asiatica* flowers, *B. sexangula* flowers, *E. agallocha* flowers, *H. tiliaceus* flowers.

Figure S1 displays the diversity of polyisoprenoid alcohols extracted from mangrove fruits. The 2D-TLC spots are marked with carbon chain lengths (C60 to C140), differentiating polyprenols (typically shorter chains) and dolichols (longer chains with a saturated alpha isoprene unit). *Excoecaria agallocha* shows a notably wide range (up to C140), indicating high polyisoprenoid complexity (Figure 2.E). Both polyprenols and dolichols are present in several species, suggesting diverse biosynthetic capabilities.

Figure S2 presents a similar polyisoprenoid profile from: *Lumnitzera littorea* (Jack) Voigt flowers, *L. racemosa* flowers, *M. candidum* flowers, *R. communis* flowers, depicting comparative intensities and mobility differences, which reflect tissue-specific polyisoprenoid accumulation.

Figure S3 summarizes the chemical diversity across tissues and species, reinforcing the findings from Table 1, specifically dolichols and polyprenols, which are known to play critical roles in membrane fluidity, protein glycosylation, and antioxidant defense. Dolichols are key intermediates in N-linked glycoprotein biosynthesis and are essential for protein folding and stress signal transduction in plants (Banerjee et al. 2017). In mangroves, which are exposed to high salinity and oxidative stress, the accumulation of dolichols could serve as a protective adaptation, helping to stabilize membrane-associated processes during reproduction (Basyuni et al. 2017a).

Figure S1 shows the two-dimensional TLC separation of polyisoprenoids from six mangrove fruit species. The results revealed diverse carbon chain lengths, especially in *E. agallocha*, which presented the richest dolichol profile among the groups. This diversity could reflect ecological adaptations to saline environments or functional roles in stress tolerance.

Figure 3 highlights differences in polyisoprenoid profiles between mangrove, coastal, and nonmangrove (mangrove associates) species. *Lumnitzera* fruits are high-molecular-weight dolichols typical of mangrove species. In contrast, *M. candidum* and *R. communis* present a more limited polyisoprenoid composition, suggesting a possible link between environmental stress and isoprenoid diversity (Basyuni et al. 2021).

Figure 4 presents polyisoprenoid profiles from Rhizophoraceae and related mangroves. The *Rhizophora* species maintain consistent dolichol chain lengths, whereas *S. alba* produces a wider spectrum, including shorter-chain dolichols. These biochemical patterns could reflect their physiological roles in saline environments and reproductive tissue development. *Rhizophora apiculata* and *R. mucronata* present mid- to long-chain dolichols (C85-C105), which are consistent with their structural or stress-protective functions. *Scyphiphora hydrophyllacea* displays dolichols from C70 to C95. *Sonneratia alba* is unique in that it contains both short- and long-chain dolichols (C50 to C90), indicating a complex isoprenoid biosynthesis pathway.

Table 1. Carbon-chain lengths and type of polyprenol and dolichol occurring in 14 Indonesian mangrove generative organ*

Species	Tissue	Polyprenol	Dolichol	Type
<i>A. illicifolius</i>	flower	80 85 90 95	80 85 90 95 100	II
<i>A. officinalis</i>	flower	60 65		III
<i>B. asiatica</i>	flower		65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140	I
<i>B. sexangula</i>	flower	40 45 50 55 60 65		III
<i>E. agallocha</i>	flower	80 85 90 95	80 85 90 95	II
<i>H. tiliaceus</i>	flower	55 60 65 70 75 80 85	55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140	II
<i>L. littorea</i>	flower	70 75 80 85 90 95 100 105 110 115 120	70 75 80 85 90 95 100 105 110 115 120	II
<i>L. racemosa</i>	flower		65 70 75 80 85 90 95	I
<i>M. candidum</i>	flower	75 80 85 90	70 75 80 85 90	II
<i>R. communis</i>	flower		75 80 85 90 95 100 105 110 115 120	I
<i>R. apiculata</i>	flower	45 50 55 60 65 70		III
<i>R. mucronata</i>	flower	45 50 55 60 65		III
<i>S. hydrophyllacea</i>	flower		65 70 75 80 85 90 95 100 105 110 115 120	I
<i>S. alba</i>	flower	70 75 80 85 90 95 100 105	65 70 75 80 85 90 95 100 105 110 115 120 125	II
<i>A. illicifolius</i>	fruit	75 80 85	75 80 85 90	II
<i>A. officinalis</i>	fruit	70 75 80 85 90 95 100 105 110 115 120 125 130 135 140	95 100 105 110 115	II
<i>B. asiatica</i>	fruit		75 80 85 90 95 100 105	I
<i>B. sexangula</i>	fruit		90 95 100 105 110 115 120	I
<i>E. agallocha</i>	fruit		70 75 80 85	I
<i>H. tiliaceus</i>	fruit	60 65 70 75 80	60 65 70 75 80 85 90 95 100 105 110	II
<i>L. littorea</i>	fruit	75 80 85 90 95 100 105 110 115 120	70 75 80 85 90 95 100 105 110 115 120 125 130 135 140	II
<i>L. racemosa</i>	fruit		45 50 55 60 65 70 75 80 85	I
<i>M. candidum</i>	fruit	70 75 80 85 90 95	70 75 80 85 90 95 100	II
<i>R. communis</i>	fruit		80 85 90	I
<i>R. apiculata</i>	fruit		85 90 95 100	I
<i>R. mucronata</i>	fruit		90 95 100 105	I
<i>S. hydrophyllacea</i>	fruit		70 75 80 85 90 95	I
<i>S. alba</i>	fruit		50 55 60 65 70 75 80 85 90	I

Note: *The numbers refer to the carbon-chain length of the polyisoprenoid alcohols

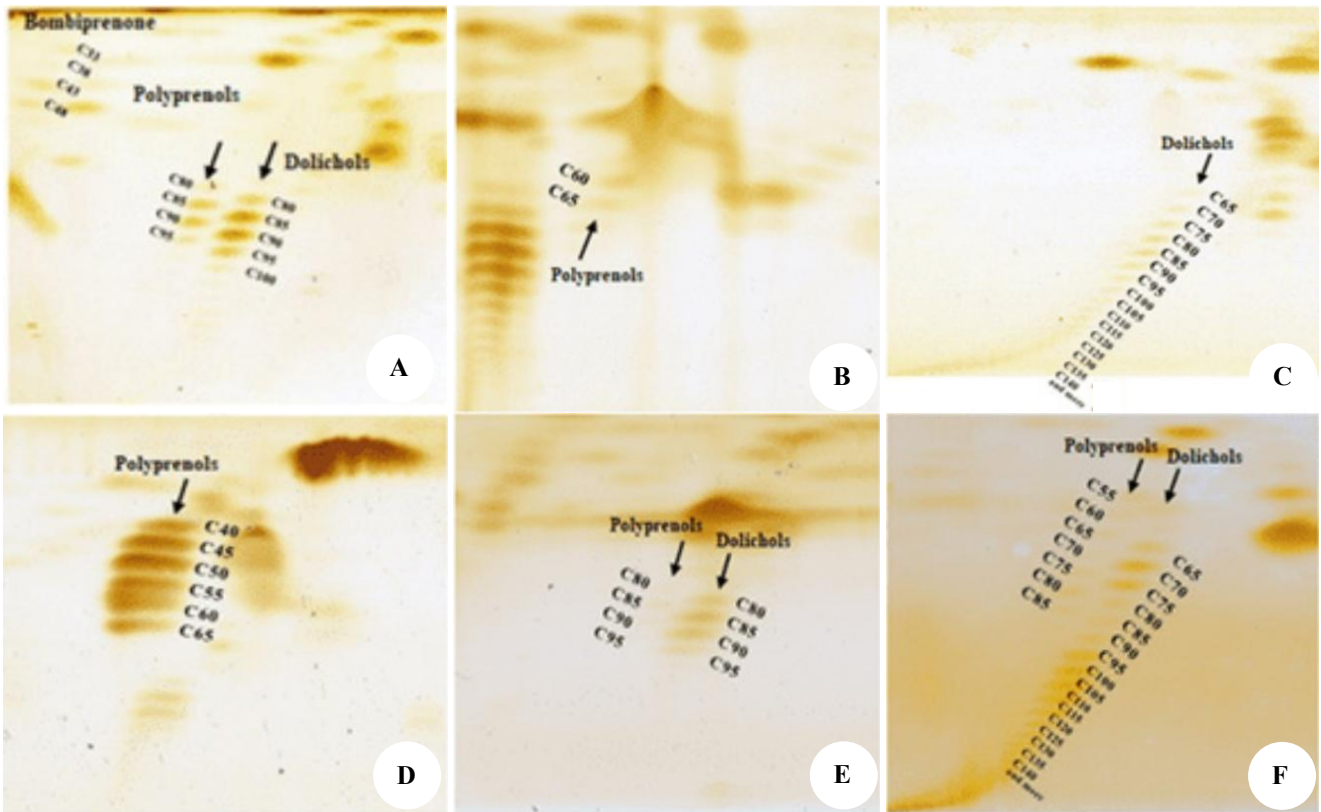


Figure 2. 2D-TLC chromatograms profile of hexane extracts of polyisoprenoid from: A. *Acanthus illicifolius* flowers, B. *Avicennia officinalis* flowers, C. *Barringtonia asiatica* flowers, D. *Bruguiera sexangula* flowers, E. *Excoecaria agallocha* flowers, F. *Hibiscus tiliaceus* flowers. The number shows the carbon chain length of polyisoprenoid alcohols

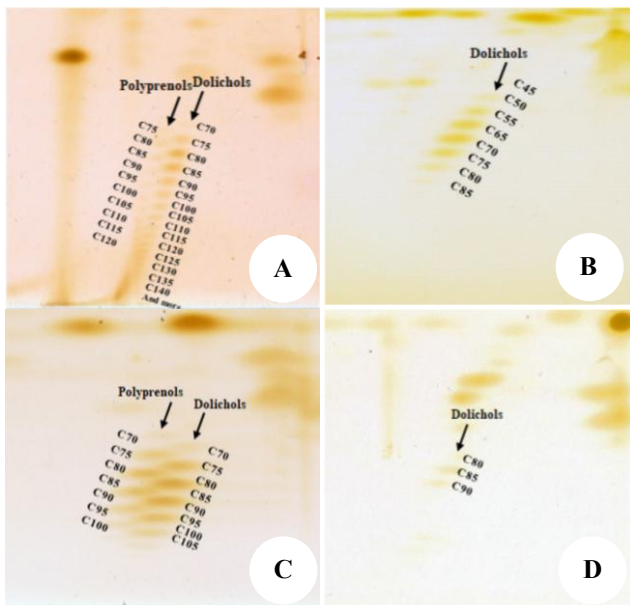


Figure 3. 2D-TLC chromatograms of hexane extracts of polyisoprenoid from: A. *Lumnitzera littorea* fruit, B. *Lumnitzera racemosa* fruit, C. *Melastoma candidum* fruit, D. *Ricinus communis* fruit. The number shows the carbon chain length of polyisoprenoid alcohols

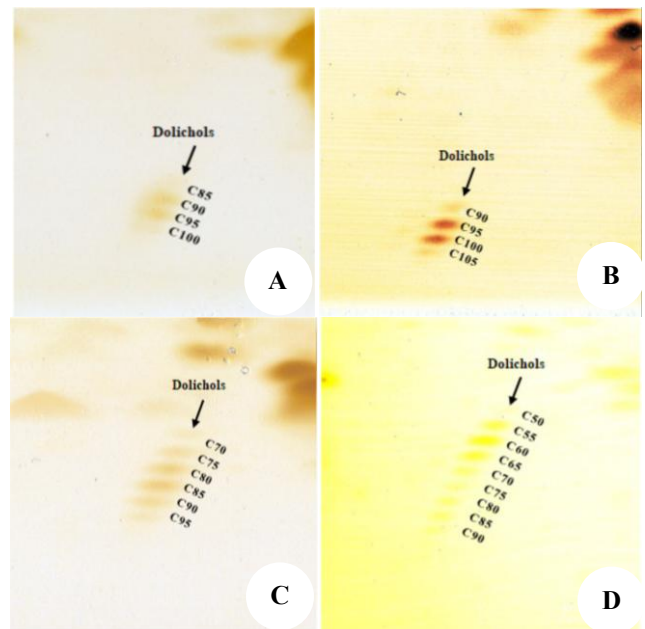


Figure 4. 2D-TLC chromatograms of hexane extracts of polyisoprenoid from: A. *Rhizophora apiculata* fruit, B. *Rhizophora mucronata* fruit, C. *Sciphiphora hydrophyllacea* fruit, D. *Sonneratia alba* fruit. The number shows the carbon chain length of polyisoprenoid alcohols

The dominance of dolichols in Type I species could suggest a greater investment in protein glycosylation machinery during floral development, potentially to maintain reproductive viability under harsh environmental conditions (Basyuni et al. 2017b). Conversely, the prevalence of polyprenols in Type III species could reflect a greater role in modulating membrane properties and interacting with other lipid-based signaling pathways, especially during pollen or seed development.

The intermediate profile observed in Type II species suggests a transitional metabolic state or a dual-functional need for both glycosylation and membrane stability. This balance might be critical for species that inhabit ecotones—zones where salinity, inundation, and light availability fluctuate more dramatically.

Previous studies have demonstrated that polyisoprenoid chain length and type vary across species, organs, and even developmental stages (Tateyama et al. 1999; Basyuni et al. 2021). For example, in the study by Basyuni et al. (2017a, b), longer-chain dolichols were more abundant in roots. In comparison, flowers and leaves presented a broader range of both dolichol and polyprenol types. These observations align with the tissue-specific distributions observed in the present study and support the theory that polyisoprenoid diversity is under adaptive selection in mangrove systems.

The interspecific variation observed in polyisoprenoid content and composition may indicate genetic and ecological differences among mangrove taxa. These biochemical markers could complement traditional morphological classification and provide reliable tools for distinguishing species and assessing populations, especially where hybridization and phenotypic plasticity make field identification challenging (Nakamura 2019; Mali et al. 2023; Upadhyay et al. 2025).

Quantitative analysis of polyisoprenoids

Table 2 presents the quantitative distribution of Total Lipids (TL), polyisoprenoids (Poly), polyprenols (Pol), and dolichols (Dol) in the generative organs (flower and fruit) of 14 mangrove species from North Sumatra. Values were given in mg/g Dry Weight (dw), including standard deviations for total lipids. The percentage contribution of polyprenols and dolichols to both total lipid and total polyisoprenoids was also shown. Two main types of polyisoprenoid alcohols have been described with respect to the OH-terminal (α -) isoprene unit. These include dolichols (α -saturated, Figure 5, Structure 1) and polyprenols (α -unsaturated, Figure 5, Structure 2).

The highest total lipid content was recorded in the flower of *S. hydrophyllacea* (774±30 mg/g dw). Flowers generally exhibited higher lipid content than fruits across species (Table 2). The highest total polyisoprenoid content was found in the flower of *H. tiliaceus* (103.0 mg/g dw), followed by *S. alba* (94.2 mg/g dw) and *L. littorea* (72.8 mg/g dw). In most samples, polyisoprenoids were dominated by either polyprenols or dolichols, but rarely both. The % contribution of dolichols to total polyisoprenoids was highest in *S. alba* flower (85.2%) and *H. tiliaceus* flower (82.7%). In contrast, several species showed 100% contribution from either polyprenols or dolichols.

Table 2 presents the relative abundance or presence of polyprenols and dolichols in the fruits of the same mangrove species. These findings are significant as they suggest that polyisoprenoid biosynthesis is regulated at the organ-specific level, possibly in relation to developmental function and the stress response (Basyuni et al. 2017a). This distribution also implies that fruits tend to have more dolichols, suggesting a potential developmental or protective role during fruit formation or seed maturation (Basyuni et al. 2021). These are linear isoprenoid alcohols that play important biological roles in membrane structure and glycoprotein biosynthesis. Their relative proportions can reflect metabolic, taxonomic, or ecological differences among species. This result therefore provides insight into species-specific and organ-specific variation in lipid metabolism, potentially reflecting different physiological roles of polyisoprenoids in mangrove reproduction tissue and stress adaptation (Basyuni et al. 2017a).

Chemotaxonomic relevance

The cluster analysis effectively grouped mangrove species on the basis of similarities in their polyisoprenoid profiles from generative tissues (Figures 6, 7). Species sharing similar polyisoprenoid patterns—such as those high in dolichols or polyprenols—were placed in proximity within the dendrogram, revealing distinct chemotaxonomic relationships (Yu et al. 2023). This dendrogram illustrates the hierarchical clustering of the fourteen mangrove species on the basis of the composition and abundance of polyprenols and dolichols in their flower tissues. Clustering was performed using UPGMA and Euclidean distance as the similarity metric. Three distinct clusters are observed, representing the predominance of dolichol (Type I), mixed profile (Type II), and polyprenol (Type III) types across species. This clustering highlights the taxonomic potential of polyisoprenoid signatures in differentiating mangrove species at the reproductive organ level.

Dolichols and polyprenols were selected due to their critical roles in membrane integrity, protein glycosylation, and stress response. Their varying chain lengths and relative abundance provide chemotaxonomic insight not offered by other minor isoprenoid derivatives (Menezes et al. 2021). While other compounds, such as sterols and terpenoids, may be present, the consistent detectability, functional importance, and reproducibility of polyisoprenoids make them suitable chemotaxonomic markers.

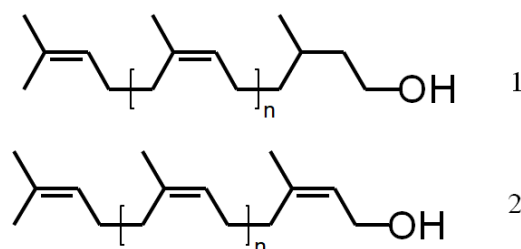


Figure 5. Structure of dolichol (1) and polyprenol (2)

Table 2. Quantitative analysis of polyisoprenoids and dolichols in the generative organs of North Sumatran mangroves in Indonesia

Species	T	TL (mg/g dw)	Poly (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in TL			% in Poly	
						Poly	Pol	Dol	Pol	Dol
<i>Acanthus ilicifolius</i> L.	Flower	118±17	20.4	9.0	11.3	17.2	7.6	9.6	44.3	55.7
<i>Avicennia officinalis</i> L.	Flower	622±29	28.2	28.2	nd	4.5	4.5	nd	100.0	nd
<i>Barringtonia asiatica</i> (L.) Kurz	Flower	690.1±30	41.9	nd	41.9	6.1	nd	6.1	nd	100.0
<i>Bruguiera sexangula</i> (Lour.) Poir.	Flower	603±60	25.9	25.9	nd	4.3	4.3	nd	100.0	nd
<i>Excoecaria agallocha</i> L.	Flower	603±69	52.8	26.0	26.8	8.8	4.3	4.5	49.2	50.8
<i>Hibiscus tiliaceus</i> L.	Flower	558±20	103.0	17.8	85.2	18.4	3.2	15.3	17.3	82.7
<i>Lumnitzera littorea</i> (Jack) Voigt	Flower	611±40	72.8	21.7	51.1	11.9	3.6	8.4	29.9	70.1
<i>Lumnitzera racemosa</i> Willd.	Flower	657±50	27.4	nd	27.4	4.2	nd	4.2	nd	100.0
<i>Melastoma candidum</i> D. Don	Flower	610±25	34.3	9.0	25.3	5.6	1.5	4.2	26.2	73.8
<i>Ricinus communis</i> L.	Flower	708±30	14.5	nd	14.5	2.0	nd	2.0	nd	100.0
<i>Rhizophora apiculata</i> Blume	Flower	648±7	35.5	35.5	nd	5.5	5.5	nd	100.0	nd
<i>Rhizophora mucronata</i> Lam.	Flower	618±25	17.9	17.9	nd	2.9	2.9	nd	100.0	nd
<i>Scyphiphora hydrophyllacea</i> C.F.Gaertn.	Flower	774±30	28.11	nd	28.11	3.6	nd	3.6	nd	100.0
<i>Sonneratia alba</i> Sm.	Flower	609±40	94.2	13.9	80.3	15.5	2.3	13.2	14.8	85.2
<i>Acanthus ilicifolius</i>	Fruit	630±30	29	5.9	23.1	4.6	0.9	3.7	20.3	79.7
<i>Avicennia officinalis</i> L.	Fruit	548±63	32.8	22.4	10.4	6.0	4.1	1.9	68.3	31.7
<i>Barringtonia asiatica</i> (L.) Kurz	Fruit	568±7	21.8	nd	21.8	3.8	nd	3.8	nd	100.0
<i>Bruguiera sexangula</i> (Lour.) Poir.	Fruit	539±46	18.71	nd	18.71	3.5	nd	3.5	nd	100.0
<i>Excoecaria agallocha</i> L.	Fruit	596±55	29.2	nd	29.2	4.9	nd	4.9	nd	100.0
<i>Hibiscus tiliaceus</i> L.	Fruit	535±24	24.9	11.8	13.1	4.7	2.2	2.5	47.4	52.6
<i>Lumnitzera littorea</i> (Jack) Voigt	Fruit	563±28	91.43	18.09	73.34	16.2	3.2	13.0	19.8	80.2
<i>Lumnitzera racemosa</i> Willd.	Fruit	585±60	31.3	nd	31.3	5.3	nd	5.3	nd	100.0
<i>Melastoma candidum</i> D. Don	Fruit	582±39	35.1	13.7	21.4	6.0	2.4	3.7	39.0	61.0
<i>Ricinus communis</i> L.	Fruit	657±40	13	nd	13	2.0	nd	2.0	nd	100.0
<i>Rhizophora apiculata</i> Blume	Fruit	566±49	16.6	nd	16.6	2.9	nd	2.9	nd	100.0
<i>Rhizophora mucronata</i> Lam.	Fruit	543±3	23.4	nd	23.4	4.3	nd	4.3	nd	100.0
<i>Scyphiphora hydrophyllacea</i> C.F.Gaertn.	Fruit	584±6	24.7	nd	24.7	4.2	nd	4.2	nd	100.0
<i>Sonneratia alba</i> Sm.	Fruit	478±21	21.8	nd	21.8	4.6	nd	4.6	nd	100.0

Note: nd: Not detected, total lipids expressed as a fraction of a crude lipid estimated gravimetrically and represented as the mean ± SD (n=3). T: Tissue, TL: Total Lipids, Poly: Polyisoprenoid, Pol: Polyphenol, Dol: Dolichol

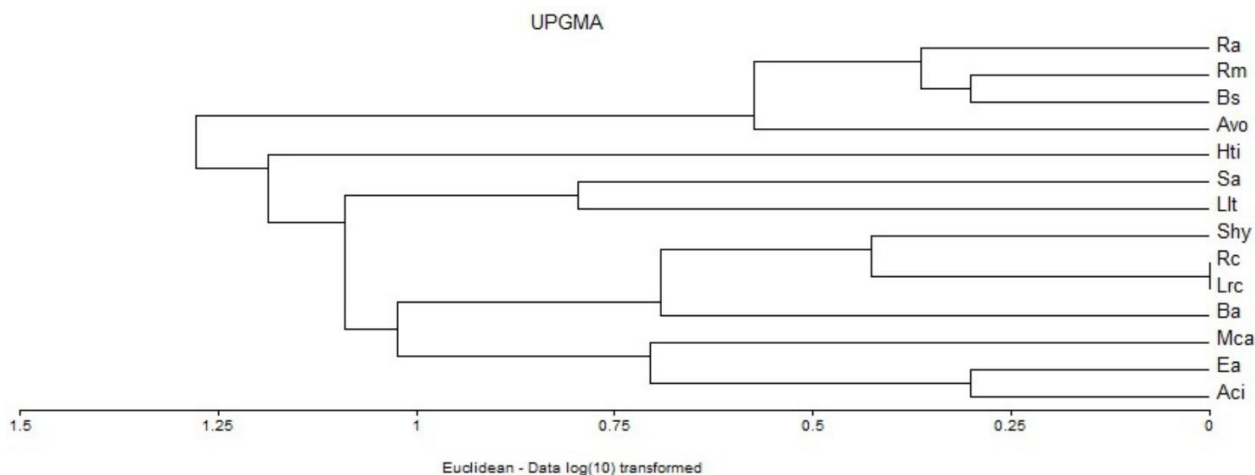


Figure 6. Hierarchical cluster analysis (UPGMA) of polyisoprenoid profiles in mangrove flowers. Ra: *Rhizophora apiculata*, Rm: *Rhizophora mucronata*, Bs: *Bruguiera sexangula*, Avo: *Avicennia officinalis*, Hti: *Hibiscus tiliaceus*, Sa: *Sonneratia alba*, Lt: *Lumnitzera littorea*, Shy: *Scyphiphora hydrophyllacea*, Rc: *Ricinus communis*, Lrc: *Lumnitzera racemosa*, Ba: *Barringtonia asiatica*, Mca: *Melastoma candidum*, Ea: *Excoecaria agallocha*, Aci: *Acanthus ilicifolius*

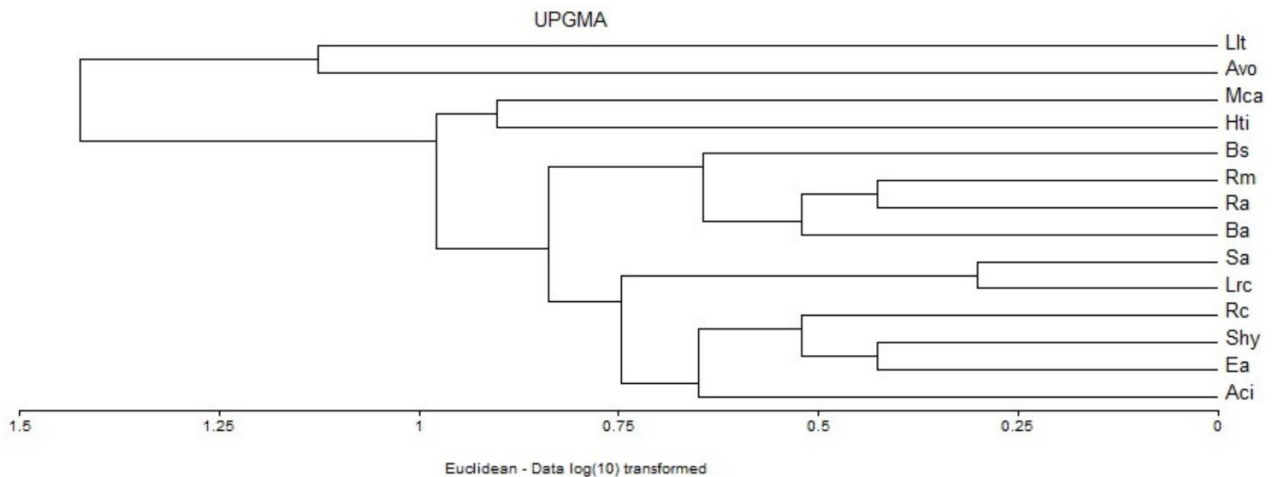


Figure 7. Hierarchical cluster analysis (UPGMA) of polyisoprenoid profiles in mangrove fruits. Llt: *Lumnitzera littorea*, Avo: *Avicennia officinalis*, Mca: *Melastoma candidum*, Hti: *Hibiscus tiliaceus*, Bs: *Brguiera sexangula*, Rm: *Rhizophora mucronata*, Ra: *Rhizophora apiculata*, Ba: *Barrington asiatica*, Sa: *Sonneratia alba*, Lrc: *Lumnitzera racemosa*, Rc: *Ricinus communis*, Shy: *Scyphiphora hydrophyllacea*, Ea: *Excoecaria aggallocha*, Aci: *Acanthus ilicifolius*

These findings indicate that polyisoprenoid composition is not only tissue-specific but also species-dependent, serving as a robust biochemical criterion for mangrove classification. Cluster analysis on the basis of polyisoprenoid composition successfully grouped species with taxonomic proximity, confirming the chemotaxonomic potential of these metabolites. These findings support previous work by Basyuni et al. (2017a), where polyisoprenoid profiles were used to cluster 14 North Sumatran mangrove species into meaningful genus-based groups.

Moreover, the discovery of polyprenyl acetones—novel derivatives identified in *Sonneratia caseolaris* (L.) Engl. and *Xylocarpus granatum* J.Koenig (Basyuni et al. 2017b)—opens new perspectives on the chemical diversity within mangrove taxa. Although not the focus of this study, such findings suggest that the scope of polyisoprenoid-based taxonomy can be extended beyond polyprenol and dolichol profiling.

These patterns are consistent with previous work by Basyuni et al. (2017a), where clustering on the basis of isoprenoid concentration distinguished species more effectively than morphology alone. Chemotaxonomic classification using polyisoprenoids is a promising tool for resolving taxonomic ambiguity among mangrove species, particularly in hybrid zones or areas with overlapping morphological traits (Umoh 2020; Basyuni et al. 2021; Mali et al. 2023).

The observation of distinct chain length distributions (e.g., C70-C120 for dolichols and C60-C140 for polyprenols) among species adds another layer of specificity. In Okinawa mangroves, *L. racemosa* presented a bimodal distribution of long-chain polyprenols, which could be replicated in our study for specific flower tissues (Basyuni et al. 2017b). These features further increase the taxonomic utility of polyisoprenoid profiling. This finding supports the use of polyisoprenoid signatures as chemotaxonomic markers, aligning with prior work in medicinal and woody plants

where isoprenoid profiling has revealed meaningful evolutionary patterns.

The clustering patterns derived from polyisoprenoid profiles generally correspond to known phylogenetic relationships, reinforcing the taxonomic relevance of this approach. Moreover, the alignment of chemotypes with ecological zones indicates a connection between isoprenoid metabolism and environmental adaptation. For instance, species from high-salinity or frequently inundated zones exhibited higher dolichol accumulation, supporting their role in osmotic stress mitigation and ecological resilience (Alongi 2015).

By incorporating ecological and botanical insights, this study positions chemotaxonomy as a valuable tool for biodiversity assessment and monitoring, and plant taxonomy. As mangrove habitats face growing pressures from sea-level rise, land conversion, and climate change, biochemical approaches play a crucial role in tracking species dynamics, stress responses, and conservation outcomes, complementing morphological and molecular data (Friess et al. 2020).

Adaptive physiology

Mangroves thrive under conditions of fluctuating salinity, tidal inundation, and oxidative stress. Polyisoprenoid diversity may reflect evolved biochemical strategies to ensure reproductive resilience. For example, dolichol accumulation during flower development may stabilize protein glycosylation under osmotic stress, whereas polyprenol-rich flowers may support flexible physiological responses (Basyuni et al. 2018, 2021).

Furthermore, the tissue-specific distribution of polyisoprenoids observed in this study mirrors the PCA-derived gene expression variability in *Eucommia ulmoides* Oliv., where fruit tissues were enriched in MEP-linked gene expression, and stems showed MVA dominance (Lipko et al. 2023). This regulation suggests that mangroves, such as *E. ulmoides*, exhibit spatial and developmental

coordination of isoprenoid pathway activity. The presence of long-chain polyprenyl alcohols in photosynthetically active tissues such as leaves and flowers also suggests their involvement in photosynthetic membrane maintenance, redox signaling, or stress adaptation, which are essential for survival in saline, sun-exposed intertidal zones (Surmacz and Swiezewska 2011; Basyuni et al. 2017a).

In contrast, polyprenol-rich species might utilize more dynamic, stress-responsive pathways. For example, their role in modulating lipid bilayers, possibly through interactions with sterols and phospholipids, may provide reproductive organs greater flexibility under osmotic or thermal stress (Swiezewska and Danikiewicz 2005; Surmacz and Swiezewska 2011).

Furthermore, a recent study by Zhao et al. (2024) in *E. ulmoides* highlighted the tissue-specific coexpression of isoprenoid biosynthesis genes, supporting the idea that differential pathway activation could underlie the observed polyisoprenoid patterns in mangroves. These findings suggest a molecular basis for the developmental and ecological regulation of isoprenoid biosynthesis in plants, which is likely parallel in mangrove species.

Finally, the presence of novel isoprenoid derivatives, such as polyprenyl acetones found in *X. granatum* and *S. caseolaris* (Basyuni et al. 2017b), suggests that mangrove polyisoprenoid metabolism is not only diverse but also chemically innovative. These compounds may play additional functional roles in defense, signaling, or reproduction, thereby opening avenues for future biochemical and pharmacological studies (Bibi et al. 2019; Upadhyay et al. 2025).

Limitations and future work

This study has several limitations, as it focused solely on generative tissues (flowers and fruits) of mangroves. Other organs, such as leaves, stems, and roots, which are known to exhibit different isoprenoid profiles, have not been analyzed by Basyuni et al. (2017a, b, 2018, 2021), which may limit the comprehensive understanding of whole-plant chemotaxonomic variation. Furthermore, the work did not integrate genetic or transcriptomic data (e.g., isoprenoid pathway gene expression), which could provide a molecular basis for the observed chemical patterns and strengthen the link between phenotype chemotaxonomy and genotype in mangrove taxonomy. Although this study relied primarily on 2D-TLC, future work should incorporate spectroscopic techniques such as UV-Vis and FTIR to confirm compound identity and structure, particularly for distinguishing structurally similar isoprenoid alcohols. Given the ecological and conservation importance of mangrove forests, the findings of this study contribute to a broader framework of integrative biodiversity monitoring. By combining remote sensing, ecological surveys, and biochemical profiling, including polyisoprenoids, future conservation strategies can more precisely identify priority areas for protection and restoration (Friess et al. 2020; Umoh 2020).

Conclusion, this study revealed significant variation in the polyisoprenoid profiles—specifically dolichols and polyprenols—across the reproductive tissues of fourteen

mangrove species from North Sumatra, Indonesia. The classification into three chemotypes (dolichol-dominant, polyprenol-dominant, and mixed) reflects both species-specific and tissue-specific metabolic regulation. Flowers tended to exhibit greater diversity in isoprenoid composition than fruits did, suggesting a dynamic biochemical adjustment during reproductive development. The biochemical signatures identified herein contribute to mangrove biodiversity assessment, help species delimitation, and may support conservation programs by providing molecular insight where morphological traits overlap or are environmentally plastic. This study establishes polyisoprenoids as reliable chemotaxonomic markers for mangroves, helping conservation efforts in biodiverse areas. The findings also demonstrate that polyisoprenoid profiles are useful chemotaxonomic markers, with clustering patterns generally aligning with known phylogenetic relationships. The occurrence of distinct chain length distributions and compound dominance indicates ecological adaptation, possibly linked to reproductive success under saline, oxidative, and tidal stress conditions. This expands the scope of polyisoprenoid studies to include mangrove reproductive tissues. These findings support the hypothesis that these compounds play essential physiological and evolutionary roles. Future work should focus on integrating genomic data to unravel the genetic basis of polyisoprenoid diversity.

ACKNOWLEDGEMENTS

This research is financed by the Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan, Indonesia, through grants from the Ministry of Education, Culture, Research, and Technology 2024.

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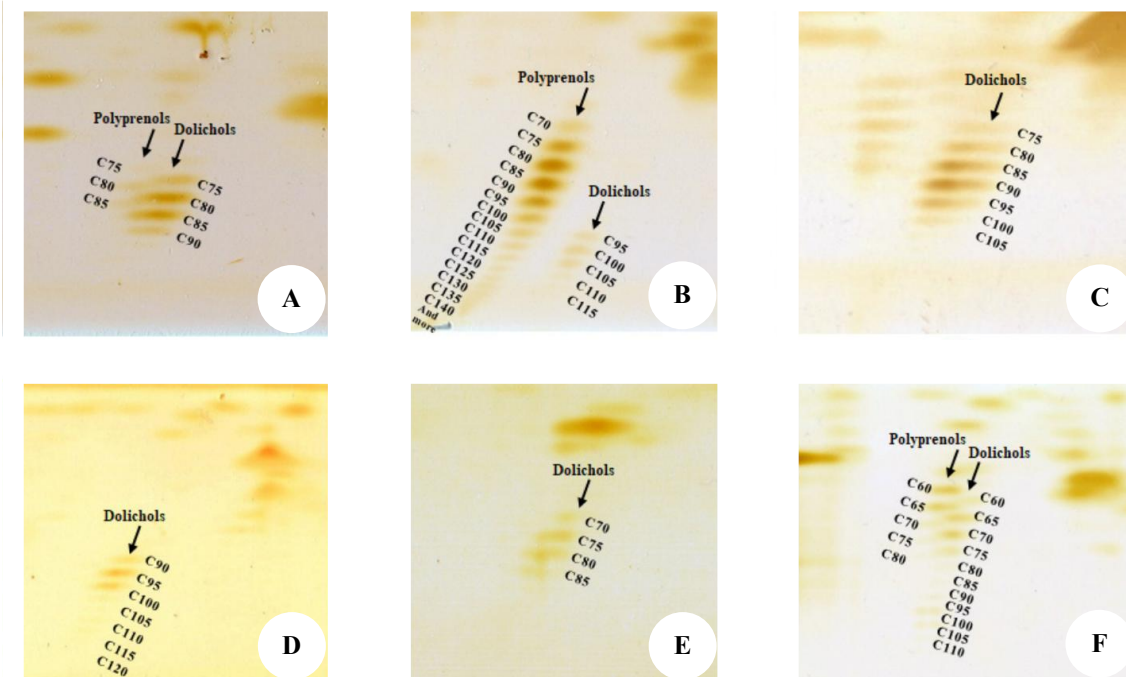


Figure S1. 2D-TLC chromatograms profile of hexane extracts of polyisoprenoid from: A. *Acanthus illicifolius* fruit, B. *Avicennia officinalis* fruit, C. *Barringtonia asiatica* fruit, D. *Bruguiera sexangula* fruit, E. *Excoecaria agallocha* fruit, F. *Hibiscus tilliaceus* fruit. The number shows the carbon chain length of polyisoprenoid alcohols

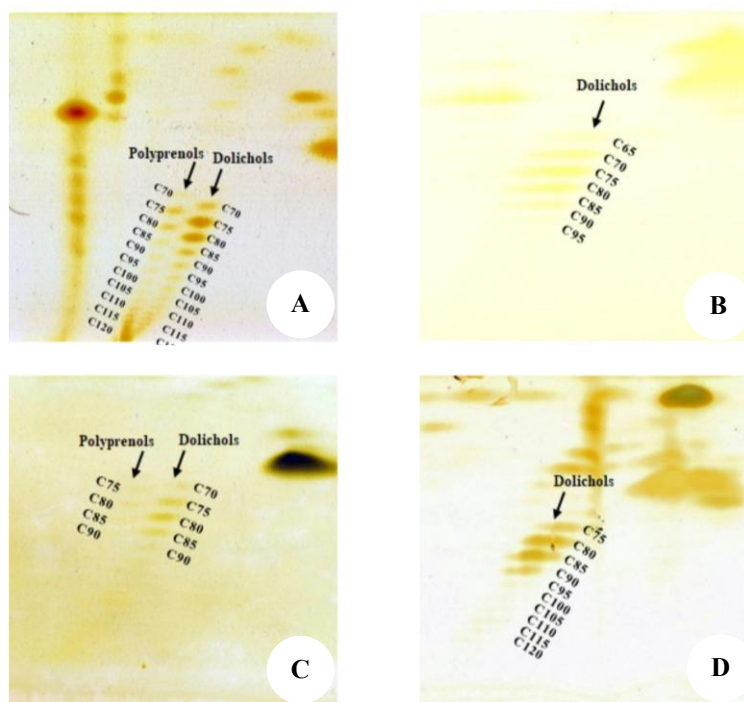


Figure S2. 2D-TLC chromatograms profile of hexane extracts of polyisoprenoid from: A. *Lumnitzera littorea* flowers, B. *Lumnitzera racemosa* flowers, C. *Melastoma candidum* flowers, D. *Ricinus communis* flowers. The number shows the carbon chain length of polyisoprenoid alcohols

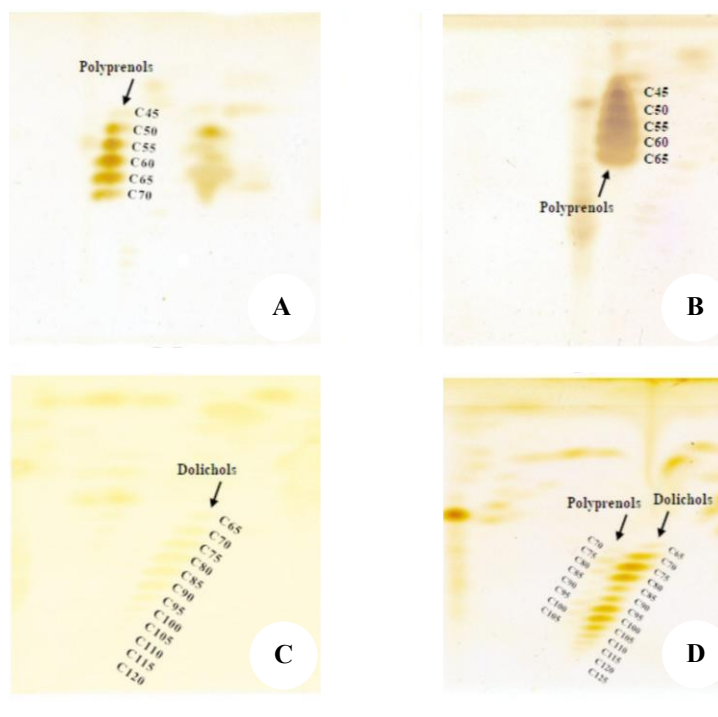


Figure S3. 2D-TLC chromatograms of hexane extracts of polyisoprenoid from: A. *Rhizophora apiculata* flower, B. *Rhizophora mucronata* flower, C. *Sciphiphora hydrophyllacea* flower, D. *Sonneratia alba* flower. The number shows the carbon chain length of polyisoprenoid alcohols