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Polyisoprenoids profile and composition from selected plant Sapotaceae family

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Abstract. This present study reports the polyisoprenoids (polyprenol/dehydrodolichol and dolichol) profile, and composition from selected Sapotaceae leaves and roots namely Manilkara zapota, Ma. kauki, and Mimusops elengi. The existence and circulation of polyisoprenoids were investigated using two-dimensional thin layer chromatography (2D-TLC). The polyisoprenoid profile in the leaves and roots were observed and categorised into two types. Type-I, depicting a dominating of dolichols over polyprenols (100%) was detected in Ma. zapota roots only. These dolichols occurred one longer-chain dolichol family (C75-C105). Type-II, expressing the existence of both polyprenols and dolichols, was detected in the leaves of Ma. zapota, Ma. kauki, and Mi. elengi. Similarly, type-II was also found in the roots of Ma. kauki and Mi. elengi. Dolichol concentrations and relative proportions were more abundances found than polyprenols both in the leaves and roots. Polyprenols in the leaves and roots were characterised chain length of C75-C120. By contrast, dolichols occurred shorter and longer-chains in the leaves of Ma. kauki (C55-C140 and more) and Mi. elengi (C45-C140 and more). The present study suggested that the pattern of medium-chain polyprenols, longer-chain polyprenol, shorter dolichols, and longer dolichols occurred in Sapotaceae family.

1. Introduction

The categorization of tropical forest land according to the IPCC (Intergovernmental Panel on Climate Change) was divided into three groups namely dryland forests (terrestrial forests), swamp forests and mangrove forests. Consistent with the data from the Directorate General of Forestry Planning and Environmental Management, Ministry of Environment and Forestry of Indonesia, the forest cover of Indonesia in 2017 is 93.6 million ha. Based on Decree of the Minister of Forestry no. 579/Menhut-II/ 2014 on forest area of North Sumatera Province primary and secondary dry land area consist of 1.894,646.22 ha; this area constitutes 62% of total forest area in North Sumatera [1]. Dryland forests in North Sumatra are concentrated in South Tapanuli Regency, Toba Samosir, Simalungun, Mandailing Natal, Humbang Hasundutan, Padang Lawas, Deli Serdang, Langkat Regency to Medan City [2]. Forest tree species are known to produce secondary metabolites that have phytochemical compounds or biologically active compounds, mainly from isoprenoid and polyisoprenoid groups [3-6].

Because of a broad range of polyisoprenoid activities, the terrestrial forest is regarded as potential natural resources for medicinal properties [3-5]. Selected plant Sapotaceae family widespread in

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terrestrial forests, namely *Manilkara zapota*, *Ma. kauki* and *Mimusops elengi* have been reported to have biological activities, pharmacological properties, and phytochemical compounds [7-9]. For instance, antioxidant and antimicrobial properties have been shown in *Ma. zapota* leaves and *Mi. elengi* leaves [7-8], while *Ma. kauki* as the herbal drug has been shown to reduce the number of cervical cancer cells [9].

The characteristic of polyisoprenoids have been shown in diverse plant organs either in vegetative: leaves and roots [3-6], and generative: flowers, fruits, and seeds [10-11]. These studies disclosed the ubiquitous circulation of polyisoprenoids in the Plantae. Furthermore, some reports have been depicted that plant polyisoprenoids play an essential function in pharmaceutical activities [12-14]. Polyprenols have been reported to avoid toxic liver [12]. Polyprenols also have been described as transmitters of DNA vaccines contrary to influenza virus [13]. By contrast, metabolic syndromes were linked with dolichol biosynthesis also expressed [14]. The important concerning life properties and pharmaceutical activities of *Ma. zapota*, *Mi. elengi*, and *Ma. kauki*, as well as plant polyisoprenoids, have been described. However, the composition and pattern of polyisoprenoid in *Ma. zapota*, *Mi. elengi*, and *Ma. kauki* have not been reported yet. To obtain deep understanding into the biological and pharmacological role of polyisoprenoids, it is critical to have the data on the description of polyisoprenoid in *Ma. zapota*, *Mi. elengi*, and *Ma. kauki*. Here we show the profile and composition of polyisoprenoids from the leaves of selected Sapotaceae family, *Ma. zapota*, *Mi. elengi*, and *Ma. kauki* species.

2. Materials and methods

2.1. Chemicals

A standard mix of dolichols (C_{90}-C_{95}) and polyprenols (C_{90}-C_{100}) as earlier reported [3] was utilised to detect the polyisoprenoids in this report. The description of the family associating to dehydrodolichols or dolichols was done about three different observations. Silica gel 60 TLC glass plates and reversed-phase silica RP-18 HPTLC glass plates were bought from Merck. Entirely from the separate chemicals and solution were of reagent level.

2.2. Plant materials

The leaves and roots of selected Sapotaceae tribe namely *Manilkara zapota. Manilkara kauki* and *Mimusops elengi* collected from the Padang Bulan campus of Universitas Sumatera Utara, Medan, North Sumatra, Indonesia, in October 2017. The mean temperature in the month of the collecting was 28-30 ^{o}C with a regular humidity of 74-76%. All of the samples were stored in a refrigerator until used.

2.3. Extraction of polyisoprenoid alcohols

A manual for the extraction of polyisoprenoids as earlier reported [4-5]. The leaves and roots of three species were desiccated at 70-75°C for two days. The drained organ (5 g each) was homogenized into a fine powder and deeply involved with chloroform/methanol (2/1, v/v) solvent for two days. The lipid extract of leaves and roots were then saponified at 65°C for one day in 50% ethanol including 2 M KOH. The nonsaponifiable lipids of the organ sample isolated with hexane and this organic solvent were dried up and re-dissolved in hexane.

2.4. The investigation by two-dimensional thin layer chromatography (2D-TLC)

Two approaches to investigate polysioprenoids using 2D-TLC: first-dimensional TLC (1D-TLC) was done for roughly 50 min on a silica gel glass plate (20 × 3 cm) with a solvent system of toluene-ethyl acetate (9:1) as earlier reported [6]. The second stage: 2D-TLC reversed-phase C-18 silica gel was done with acetone as the solvent for about one hr as more previously described [10]. The spots of the sample and standard mixtures are detached and being formulated by 2D-TLC was then known and envisaged with iodine vapour. The cleared chromatographic was imaged and scanned with a Canon E-470 series printer. The polyisoprenoid family was observed by the relation of manoeuvrability on TLC with that of authentic standards of dolichol or polyprenol that were employed in 2D development. The

dehydrodolichols and dolichols that were observed on RP-18 HPTLC glass plates were determined using ImageJ version 1.46r [15], with dolichol and polyprenol criterion as evidence.

3. Results and discussion

3.1. Polyisoprenoid profile and composition

The search for polyisoprenoids compound from the leaves and roots of Ma. zapota, Ma. kauki, and Mi. elengi was performed using 2D-TLC [3-4] led the clear separation of polyprenols from dolichols regarding the carbon chain length. Tables 1-3 summarize the quantitative analysis of polyisoprenoids and polyprenols and dolichols pattern and composition with the carbon-chain lengths given each species. The quantity of TL was the largest in *Ma. kauki* leaves (86.8 mg/g dw) and the lowest in *Ma. kauki* roots (78.5 mg/g dw). By contrast, the amount of PI was the highest in *Ma. kauki* roots (15.8 mg/g dw), the lowest content of PI was in *Mi. elengi* (7.3 mg/g dw).

Table 1. Occurrence and distribution of polyisoprenoids in Sapotaceae leaves.

Species	Tissue	TL (mg/g dw)	Pl (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in TL			% in PI		Type
						PI	Pol	Dol	Pol	Dol	
Ma. Zapota	leaves	80.5	10.5	3.4	7.1	13.0	4.2	8.8	32.4	67.6	II
Ma. Kauki	leaves	86.8	12.5	4.7	7.8	14.4	5.4	9.0	37.6	62.4	II
Mi. elengi	leaves	78.5	7.3	2.1	5.2	9.3	2.7	6.6	28.8	71.2	II

TL = Total lipids, PI = Polyisoprenoids, Pol = Polyprenols, Dol = Dolichols. Data are presented as mean of triplicate analyses.

Table 2. Distribution of polysioprenoids in selected Sapotaceae roots.

Species	Tissue	TL (mg/g dw)	Pl (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in TL			% in PI		Type
						PI	Pol	Dol	Pol	Dol	
Ma. zapota	roots	74.4	8.4	nd	8.4	11.3	nd	11.3	nd	100.0	I
Ma. kauki	roots	67.9	15.8	6.0	9.8	23.2	8.8	14.4	38.2	62.4	II
Mi. elengi	roots	68.6	10.4	4.0	6.4	15.1	5.2	9.9	34.4	65.6	II

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

The similar results on TL and PI contents of the present study were also have been reported for major and minor components of Okinawan and North Sumatran mangrove plants [4-5] and various organs of oil palm [11]. However, these TL and PI values are much lower compared to the North Sumatran coastal leaves [3] and *Nephellium lappaceum* various tissues [10]. The present study, therefore, indicated that the TL and PI values in *Ma. zapota, Ma. kauki,* and *Mi. elengi* presented in this study were lower than those reported from mangrove associates [3] and different tissues of *N. lappaceum* [10].

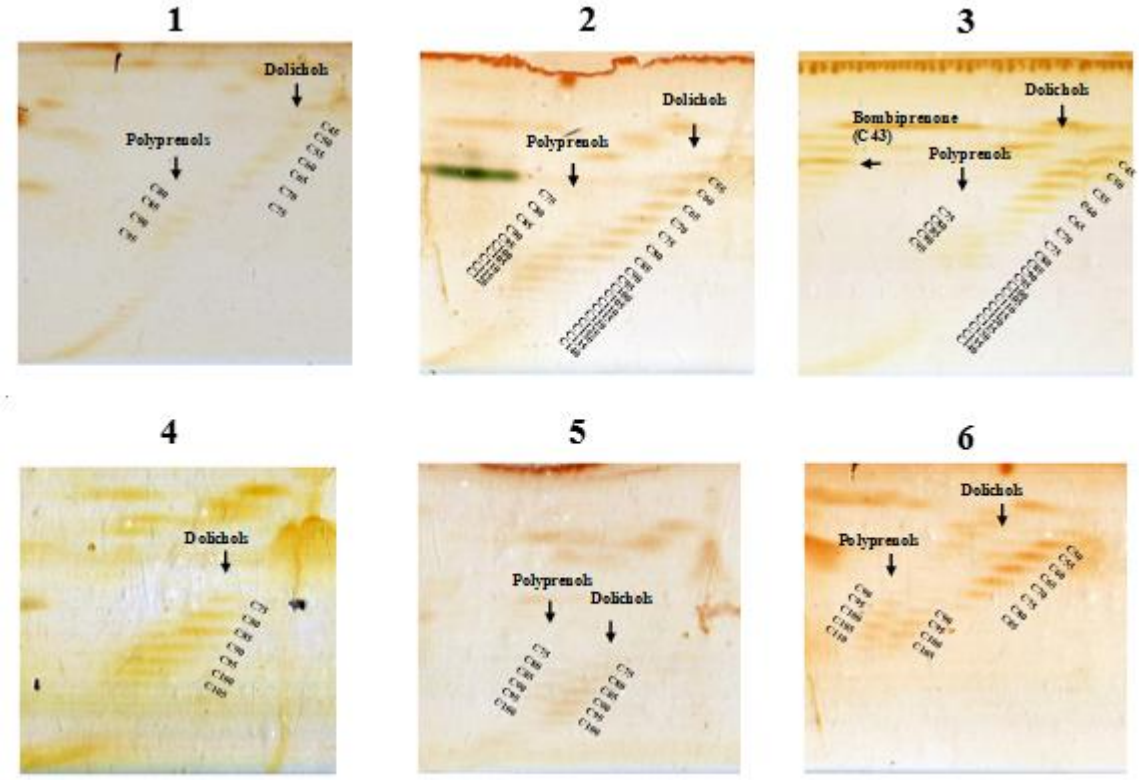

Figure 1. 2D-TLC chromatograms hexane extracts of polyisoprenoids from *Ma. zapota* leaves (1), *Ma. kauki* leaves (2), *Mi. elengi* leaves (3)**,** *Ma. zapota* roots (4), *Ma. kauki* roots (5), and *M. elengi* roots (6).

3.2. Investigation of polyisoprenoid by 2D-TLC

The groups of dehydrodolichols and dolichols in the leaves and roots of *Manilkara zapota, Ma. kauki* and *Mimusops elengi* were categorised as earlier reported [3-6] into two types (I and II) (tables 1-2). In group-I, the dominating of dolichols over polyprenols (100%) was detected in *Ma. zapota* roots (table 3, figure 1.4). These dolichols occurred one dolichol family (C_{75}–C_{105}). Type-II, showing the incidence of both polyprenols and dolichols, was detected in the leaves and roots of *Ma. kauki* and *Mi. elengi*. Type-II was found in the leaves of *Ma. Zapota.*

Dolichol contents were more plenty found than polyprenols (more than 62%:38%) both leaves and roots in these three species (figure 1. 1-6). No type-III, the dominating polyprenols over dolichols was not detectable either in leaves or roots. Polyprenols and dolichols with the chain length of C_{80}–C_{95} and C_{45}–C_{75}, respectively, detected in *Ma. zapota* (figure 1.1). It is noteworthy that dolichols occurred longer-chains in *M. kauki* leaves (C_{55}–C_{140} and more) and *Mi. elengi* leaves (C_{45}–C_{140} and more) (figure. 1.2-3 It is fascinating notable that Bombiprenone (C_{43}) was observed in *M. elengi* leaves only (table 3, figure 1.2). Dolichol (C_{75}–C_{105}) was only found in *Ma. zapota* roots (figure 1.4). Polyprenols were characterized as medium to longer chains; on the other hand, dolichols found much longer chains.

The dominance of dolichols over polyprenols have been reported in mangrove, and coastal plant leaves from Okinawa and North Sumatra [4-5]. These study suggested that the critical polyisoprenoids were dolichols, not polyprenols. By contrast, the presence of both polyprenols and dolichols are more likely occurred in mangrove associates including coastal plants [3], oil palm [11] and this study. Furthermore, in the plant world (mainly green foliage tissues), polyprenols are abundantly detected compare to dolichols [10-11, 15]. The present study, therefore, reveals the occurrence of medium and

longer polyprenols, shorter and longer dolichols are modulated in the plant kingdom, including in-plant selected Sapotaceae, *Ma. zapota*, *Ma. Kaki*, and *Mi.elengi.*

Table 3. Carbon-chain lengths of polyprenol and dolichol occurring in selected Sapotaceae family leaves and roots.

Species	Tissue	(C43)	Polyprenol	Dolichol
Ma. zapota	leaves		80 85 90 95	45 50 55 60 65 70 75
Ma. kauki	leaves		75 80 85 90 95 100 105 110 115 120	55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 and more
Mi. elengi	leaves	o	75 80 85 90 95	45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 and more
Ma. zapota	roots			75 80 85 90 95 100 105
Ma. kauki	roots		75 80 85 90 95 100	75 80 85 90 95 100
Mi. elengi	roots		90 95 100 105 110	50 55 60 65 70 75 80 85 90 95 100 105

Salt stress changes the polyisoprenoid concentrations in four the salt-secreting, and non-salt-secreting mangrove species has been shown recently [6]. The change of polyisoprenoids comprising in polyprenols, dolichols, and bombiprenone as well. Bombiprenone has occurred in various tissues such as leaves [3-6], roots [3-6], flowers [10], and fruits [10]. The abundance of polyisoprenoids in *Ma. zapota*, *Mi. elengi*, and *Ma. kauki* are promising to a non-wood product with pharmacological and biological activities [7-9]. Polyisoprenoids can regulate the rate of cholesterol and avert noxious wounds of the liver [12]. The metabolic diseases were linked with dolichol biosynthesis [14]. These studies suggested the prospect of following beneficial choices for polyisoprenoid biosynthesis [14].

4. Conclusion

Dolichol found more abundance than polyprenols both in the leaves and roots. The selected Sapotaceae plant, polyprenols were characterised as one family. However, dolichols occurred shorter and longer-chains. The existing study suggested that the composition of medium-chain polyprenols, longer-chain polyprenol, shorter dolichols, and longer dolichols happened in Sapotaceae family.

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