The occurrence of polyisoprenoids from the leaves of selected Fabaceae family

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Abstract. This paper describes the search for polyisoprenoid profile and distribution from the leaves of selected economic plants of Fabaceae tribe that distributed in dryland forest, namely Crotalaria anagyroides, Leucaena leucochepala, Samanea saman, and Tamarindus indica. The polyisoprenoids profile was investigated using a convenient two-dimensional thin layer chromatography (2D-TLC). The polyisoprenoid distribution in the leaves was detected and regarded as two types. Type-II, showing the existence of both polyprenols and dolichols, was discovered in C. anagyroides leaves only. Type-III, displaying a predominance of polyprenol over dolichol was found in the leaves of L. leucochepala, S. saman, and T. indicus. By contrast, no type-I, which depicting the dominating dolichol over polyprenol was not identified in this analysis. The present study supported the previous findings on the world leaves that polyprenols but not dolichols are richly found.

1. Introduction

The classification of tropical forest land according to the IPCC (Intergovernmental Panel on Climate Change) into three categories namely dryland forests, swamp forests and mangrove forests. 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 to Langkat Regency [2]. Forest tree species are known to be rich sources of secondary metabolites, mainly from isoprenoid groups [3-6].

Our previous studies have successfully isolated and characterized the polyisoprenoid compounds (>C₃₀) in mangrove and coastal forests [3-5]. Recently, it has been reported that salinity altered the composition and content of both secretory and non-secretory of mangrove seedlings [7]. Nonetheless, distribution of polyisoprenoids from terrestrial plants in North Sumatra is rarely described. A part of dryland forests, the economic plant denotes plants that utilised for human or animal food, resources, medicines, environmental intentions, gene sources for breeding, social goals, as well as ones with dissenting impacts such as poisonous or disease-harboring plants or weeds [8].

Therefore, a deeper insight into the function of polyisoprenoid compounds in dryland forest is required. Here we report the search for polyisoprenoid profile and distribution from the leaves of selected economic plants of Fabaceae tribe that widespread in dryland forests, namely Crotalaria anagyroides, Leucaena leucochepala, Samanea saman, and Tamarindus indica.
2. Materials and Method

2.1 Chemicals
Dolichols (C₆₀-C₈₀) and polyprenols (C₉₀-C₁₀₀) standard as earlier reported [7] was used to classify the polyprenols and dolichols. The determination of the family compound related to polyprenols or dolichols was completed in the triplicate analysis. Silica gel 60 TLC glass plates and silica RP-18 HPTLC glass plates were obtained from Merck.

2.2 Plant materials
The leaves of selected Fabaceae family namely rattlepods (C. anagyroides), horse-tamarind (L. leucochepala), cow tamarind (S. saman), and tamarind (Tamarindus indica) were 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 sampling was 26-28°C with an ordinary humidity of 74-76%. All of the samples were stored in a refrigerator until used.

2.3 Isolation of polyisoprenoid alcohols
The separation of polyprenols from dolichols was done as hitherto delineated [9]. The leaves were placed in the oven at 68-70°C for two days. The preserved tissue (5 g each) was dented into a fine grain and enwrapped as previously reported [3]. The lipid extract of leaves was saponified and the unsaponifiable lipids of each leaf sample were dispersed and re-mixed in hexane.

2.4 Search for polyisoprenoids by two-dimensional thin layer chromatography (2D-TLC)
Search for polyisoprenoids using 2D-TLC was carried out with two steps: First-dimensional TLC and second-dimensional TLC as previously described [7,9]. The polyisoprenoids spots on chromatograms was identified with iodine vapour and scanned with a Canon E-470 printer. The polyisoprenoid family was assessed by the comparing of motility on plate with those of specific standards of dolichol or polyprenol. The number of polyisoprenols and dolichols on chromatogram plates were measured using ImageJ version 1.46r [10], in comparison with dolichol and polyprenol criterion as standards.

3. Results and Discussion

3.1 Profile of polyisoprenoids in four Fabaceae leaves
The survey for polyisoprenoids compound from the leaves of selected Fabaceae tribe namely C. anagyroides, L. leucochepala, S. saman, and T. indicus in the campus of Universitas Sumatera Utara, Medan, Indonesia was performed using 2D-TLC [3-5] led to the discrete separation of polyisoprenols and dolichols relating to the carbon chain length. Tables 1-2 summarize the analytical analysis of polyisoprenoids, polyeprenols and dolichols profile and composition with the carbon-chain lengths specified each species. The quantity of TL was the largest in C. anagyroides leaves (70.4 mg/g dw) and the lowest in L. leucochepala (9.1 mg/g dw). By contrast, the amount of PI was the highest in L. leucochepala (8.1 mg/g dw), the lowest content of PI was in S. saman leaves (5.1 mg/g dw). The comparable results on TL and PI contents of the present study were also have been reported for mangrove leaves from North Sumatra [4], Okinawa [5], and palm oil (Elaeis guineensis) [11]. However, much higher contents of TL and PI have been shown in North Sumatran coastal leaves [2] and Nepheilium lappaceum leaves [9]. The TL and PI values in the present study are close to those defined by major and minor components of mangrove plants [4-5].
Table 1. Polyisoprenoids content in four Fabaceae leaves

| Species          | Tissue | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in TL | % of PI |
|------------------|--------|--------------|--------------|------------|------------|---------|---------|
| *C. anagyroides*  | leaves | 70.4         | 7.1          | 3.6        | 3.5        | 1.0     | 0.5     |
| *L. leucocephala*| leaves | 9.1          | 8.1          | 8.1        | nd         | 89.1    | 89.1    |
| *S. saman*       | leaves | 10           | 5.1          | 5.1        | nd         | 51.0    | 51.0    |
| *T. indicus*     | leaves | 10.8         | 5.9          | 5.9        | nd         | 54.4    | 54.4    |

nd= not detected, TL = Total lipids, PI = Polyisoprenoids, Pol = Polyprenols, Dol = Dolichols, dw = dry weight. Data are served as mean ± SD (n= 3).

Table 2. Chain lengths distribution of polyprenol and dolichol of four Fabaceae leaves

| Species          | Tissue | (C43) | Polyprenol | Dolichol |
|------------------|--------|-------|------------|----------|
| *C. anagyroides*  | leaves | 90    | 60 65 70 75 80 85 | 90 95     |
| *L. leucocephala*| leaves | 90    | 80 85      |          |
| *S. saman*       | leaves | 0     | 45 50 55 60 |          |
| *T. indicus*     | leaves | 0     | 60 65      |          |

3.2 Classification of polyisoprenoid

The organizational groups of polyprenols and dolichols in the leaves of *C. anagyroides*, *L. leucocephala*, *S. saman*, and *T. indicus* were grouped as previously characterized [3-5] into two types (II and III) (Tables 1-2, Figure 1). Type-II, showing the occurrence of both polyprenols and dolichols, was detected in *C. anagyroides* leaves only. Type-III, displaying a predominance of polyprenol over dolichol was found in the leaves of *L. leucocephala*, *S. saman*, and *T. indicus*. By contrast, no type-I, which shows the dominance of dolichol over polyprenol was not identified in this analysis.

The dominating polyprenols over dolichol were characterized of the plant worlds (especially in leaf tissues) [9, 12-15]. Ficaprenol (C45–C65) was found in all leaves studied except (Figure 1A, C-D) and in *L. leucocephala* leaves (Figure 1B). This study suggested that the essential polyisoprenoids were polyprenols, but not dolichols. The present study, therefore, reveals the occurrence of shorter and longer polyprenols, and longer dolichols are modulated in the plant kingdom, including in dryland forests. The apparent predominance of polyprenols may be the result of on the terrestrial plants in the tropical or sub-tropical climatic conditions.

Polyrenols also happened as one or two polyprenol families in the leaves, explicitly ficaprenol-type polyprenols (shorter polyprenols) and longer polyprenols, depending on the plants and tissues [3-5]. However, in this study, polyprenol occurred only in one family. This study well agreed to the previous results on the occurrence of one family polyprenols in Lauraceae family [13], families of Rosaceae, Annonaceae, Euphorbiaceae, Moraceae, and Magnoliaceae [15], and Sapindaceae [16].

The occurrence of two polyprenols families was found in several mangroves leaves, for example, Acanthus ilicifolius, Acrostichum aureum, and Sonneratia caseolaris [4], Kandelia obovata yellow leaves [5], Lumnitzera racemosa and Pemphis acidula [5]. Furthermore, two polyprenols families have been detected in pisifera and tenera type of E. guineensis [11], Taxus baccata (Taxopsida), Agathis robusta (Araucariaceae), Juniperus communis (Cupressaceae), Metasequoia glyptostrok (Taxodiaceae), Podocarpus neriformis (Podocarpaceae) [15].
Recently the derivatives of polyprenols have been reported namely polyprenyl acetone, was detected in North Sumatran mangrove leaves of *S. caseolaris* and *Xylocarpus granatum* (*C*₂₃–*C*₈₈) and ranging from *C*₂₃–*C*₁₀₈ in *Aegiceras corniculatum* [4]. Polyprenyl acetone also has been occurred in *Allium tuberosum, Boehmeria nivea var. nippononivea, Laurus nobilis,* and *Euphorbia supine* [12]. The identification of polyprenol reductase, an enzyme to catalyse of polyprenols converted to dolichols has been described by *K. obovata* with three partial genes [17]. In another hand, a new member of *KoPPRD1* of *K. obovata* joined with plant polyprenol reductase genes such as *Ricinus communis, Ipomoea nil* [18] which widespread in tropical and sub-tropical regions.

![Figure 1](image.png)

**Figure 1.** 2D-TLC chromatograms hexane extracts of polyisoprenoids from *C. anagyroides* (A), *L. leucocephala* leaves (B), *S. saman* leaves (C), and *T. indicus* leaves (D).
4. Conclusions
The current study clarified that the majority of polyisoprenoids from selected Fabaceae leaves are polyprenols but not dolichols. This finding approved the previous facts on dominating polyprenols over dolichols in plant worlds (especially photosynthetic tissues).

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