Polyisoprenoids Composition from *Araucaria heterophylla* and *Casuarina equisetifolia* Leaves

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Abstract: This current work examines the polyisoprenoids (dehydrodolichol or polyprenol and dolichol) profiling and conformation from Norfolk Island pine *Araucaria heterophylla* (Araucariaceae) and Australian pine *Casuarina equisetifolia* (Casuarinaceae). The pattern and structure of polyisoprenes were determined by two-plate thin layer chromatography (2P-TLC). The polyisoprene pattern in the leaves was found and categorized into two categories. Group-I, showing a majority of dolichols over dehydrodolichols was detected in *A. heterophylla*. These dolichols showed as one longer dolichol tribe (C_{40}–C_{110}). Group-II, exhibiting the incidence of the pair polyprenols and dolichols, was traced in *C. equisetifolia*. Dolichol concentrations were faintly extra richness detected comparing to dehydrodolichols (approximately 54%:46%) in this species. Dolichols with chain length of C_{75}–C_{95} and shorter dehydrodolichol (C_{50}–C_{55}) were detected in *C. equisetifolia*. This study suggested that different pattern of ficaprenols, shorter-chain and longer dolichols are modulated in both pine species.

1 INTRODUCTION

Higher plants are renowned to generate secondary metabolites containing polyisoprenes or polyisoprene. The occurrence and profile of polyisoprenoids has been demonstrated in numerous organs tropical and subtropical plants (Jankowski et al., 1994; Tateyama et al., 1999; Skorupinska-Tudek et al., 2008; Surmacz and Swiezewska, 2011; Basyuni et al., 2016, 2017, 2018a; Arifiyanto et al., 2017; Basyuni and Wati, 2017; Sagami et al., 2018). These papers showed the ubiquitous composition and occurrence of polyisoprenoids in the flora.

Several works have been revealed for biological and pharmacological properties of plant species such as Norfolk Island pine *Araucaria heterophylla* (Araucariaceae) and Australian pine *Casuarina equisetifolia* (Casuarinaceae) (Aslam et al., 2013; Elkady et al., 2018). It has been reported that the resin isolate of *A. Heterophylla* depicted antiulcerogenic activity in opposition to ethanol-activated stomach ulcers in Sprauge Dawely rats (Abdel-Sattar et al., 2009). Furthermore, this extract showed variable cytotoxic activities contrary to the breast (MCF7) and colon (HCT116) row of cancer cells (Abdel-Sattar et al., 2009). Elkady et al., (2018) reported that chemical mark and antiproliferative influence of important oils of *A. heterophylla*. Likewise, *C. equisetifolia* has been shown to have potential antibacterial activity (Parekh et al., 2006). The physiological significance of *C. equisetifolia* has been playing an essential role in reply to cold pressure (Li et al., 2017).

To get more understanding into the critical role of polysioprenoids in plant species, the feature data on the pattern and distribution of polyisoprenoids from plant species are entirely needed. Thus the current report proposed to determine the

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dehydrodolichol and dolichol pattern and conformation from *A. heterophylla* and *C. equisetifolia* extending our prior studies.

2 MATERIALS AND METHODS

2.1 Chemicals

A standard combination of dolichols (C_{90}-C_{95}) and dehydrodolichols (C_{55}-C_{65}) as prior applied (Basyuni et al., 2016) was applied to classify the polyisoprenes in the experiment. The recognition of the tribe linking to dehydrodolichols or dolichols was performed with three triplicates.

2.2 Plant Materials

The leaves of Norfolk Island pine *Araucaria heterophylla* (Araucariaceae) and Australian pine *Casuarina equisetifolia* (Casuarinaceae) were collected from Universitas Sumatera Utara campus in October 2017. Both species usually are rising in forthright sunlight. In the month of compilation, the average temperature was 31 ºC with ordinary moisture of 76%. samples were placed at -20ºC prior to utilization.

2.3 Extraction of Polyisoprene

A protocol for the isolation of polyisoprene as already depicted (Basyuni et al., 2018a,b). Simplely, the leaves of two species were placed on oven at 75°C for 1-2 days. The drained organ (4-6 g) was blended in and suppressed in chloroform/methanol then saponified and re-suspended in hexane.

2.4 Determination by Two-plate Thin Layer Chromatography (2P-TLC)

To determine polyisoprenoid profile, two approaches were carried out: first-plate TLC (1P-TLC) and two-plate TLC (2P-TLC) as formerly reported (Basyuni et al., 2017). The polyisoprenoid tribe was analyzed by the evaluation of progress on TLC with the accurate examples of dolichol or dehydrodolichol. The polyisoprenoids were calculated using ImageJ ver. 1.46r (Schneider et al., 2012).

3 RESULTS AND DISCUSSION

3.1 Polyisoprene Pattern and Distribution

The investigation for polyisoprenes derived the leaves of *A. heterophylla* and *C. equisetifola*, from North Sumatra, Indonesia was done using 2D-TLC (Basyuni et al., 2016; Basyuni et al., 2017) lead to the vibrant parting of dehydrodolichols and dolichols involving to the carbon chain length. Tables 1-2 recapitulate the quantitative determination of polysioprenoids and dehydrodolichols and dolichols pattern and configuration with the carbon-chain lengths provided every species. The amount of TL was the major in *C. equisetifolia* leaves and the lowest in *A. heterophylla.* In contrast to this observation, the measure of PI was the uppermost in *A. heterophylla* (8.8 mg g^{-1}dw), the bottommost concentration of PI was in *C. equisetifolia* (6.3 mg g^{-1}dw).

The analogous outcomes for TL and PI concentrations were described for North Sumatran coastal leaves (Basyuni et al., 2018a). On the other hand, The TL and PI contents in true and associate mangrove forests reported in the work were lower than that studied derived from true and mangrove associate species (Basyuni et al., 2016; Basyuni et al., 2017; Basyuni et al., 2018a, b).

Table 1: Pattern and composition of polyisoprenes in two leaves

Plant	Organ	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in TL			% in PI		Group
						Pl	Pol	Dol	Pol	Dol	
A. heterophylla	Leaves	9.0	8.8	nf	8.8	7.8	Nf	7.8	Nf	100	I
C. equisetifolia	Leaves	9.1	6.3	2.9	3.4	2.8	4.0	3.4	46.0	54.0	II

nd= not found, TL = Total lipids, PI = Polyisoprenes, Pol = Polyprenols/Dehydrodolichols, Dol = Dolichols. Results are displayed as an average of three repeatation examines.

Tabel 2: Dehydrodolichol and dolichol carbon-chain lengths in two plant photosynthetic organs

Plant	Organ	Dehydrodolichol	Dolichol
A. heterophylla	Leaves		40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
C. equisetifolia	Leaves	50 55	75 80 85 90 95

3.2 Analysis Polyisoprene by 2P-TLC

The physical categories of dehydrodolichols and dolichols in both species were grouped as earlier termed (Basyuni et al., 2016, 2017, 2018a) into two groups (I and II). Group-I, possessing dominance of dolichols over dehydrodolichols (>90%) was discerned in *A. heterophylla* leaves. Group-II is depicting the occurrance of the pair dehydrodolichol and dolichols, was verified in the leaves of *C. equisetifolia*.

Dolichols occurred one dolichol family (C40-C110) detected in *A. heterophylla*, while shorter dehydrodolichol type (as called ficaprenol) happened in *C. equisetifolia* (C50-C55) and dolichol with a carbon chain length of C75-C95 (Table 2).

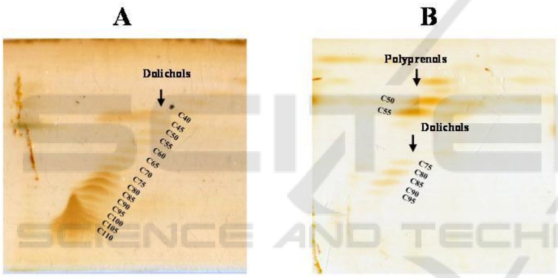

Figure 1. 2P-TLC chromatograms of samples from the leaves of *A. heterophylla* (A) and *C. equisetifolia* (B). The carbon numbers indicate the polyisoprene carbon chain length.

It is important to observe that significant of dolichols over dehydrodolichols have been described in coastal plant and mangroves plants (Basyuni et al., 2016, 2017, 2018a). The presence and distribution of both compounds namely dehydrodolichols and dolichols were characterized of oil palm (*Elaeis guineensis*) leaves and non-mangrove plant species (terrestrial plants or dry land forests) (Basyuni et al., 2018b; Basyuni and Wati, 2017, 2018).

4 CONCLUSION

This report shed light on the pattern and distribution of polyisoprenes in *A. heterophylla* and *C. equisetifolia* from North Sumatra province, Indonesia. The obtainable work specified that the formation of shorter-chain dehydrodolichols, shorter-chain dolichols and longer dolichols are conducted in higher plants.

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