Search for polyisoprenoids in the flowers and fruits of selected coastal plants using two-dimensional thin layer chromatography

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Abstract. Coastal plants are recognized to yield secondary metabolites including polyisoprenoid alcohols. Coastal plants have been shown to have biological and phytochemical activities. The present study reports the search for polyisoprenoids composition from flowers and fruits of selected coastal plants, *Amorphophallus paeoniifolius*, *Guetarda speciosa*, and *Jatropha curcas*. A two-dimensional thin layer chromatography (2D-TLC) was used to analyse the composition and occurrence of polyisoprenoid alcohols (polyprenols and dolichols) in coastal plants. The distribution of polyprenols and dolichols in the flowers and fruits were detected and classified into one type only, type-II. Type-II, having the presence of both polyprenols and dolichols, was found in all samples investigated: in the flowers and fruits of *A. paeoniifolius*, *G. speciosa*, and *J. curcas*. It is interesting to note that no dominating dolichols over polyprenols (type-I) or predominance polyprenol over dolichols (type-III) detected in this study. The present study, therefore, suggested the diversity of polyisoprenoids in the generative tissues of tropical coastal plants.

1 Introduction

Indonesia has the most significant mangrove area global, which is 22.6 percent of the worldwide total, with the length of shoreline close to at 95,181 km [1]. Mangrove forests in Bali province primarily widespread in Tanjung Benoa and Forest Park Conservation Area Ngurah Rai, Nusa Lembongan, and National Park Bali Barat [2]. Mangrove species can be separated into true mangroves or exclusive species and mangrove associates also known as non-exclusive or coastal plants [3].

Mangrove plants along with coastal plants are important to yield secondary metabolites including polyisoprenoid alcohols [3-6]. Furthermore selected species, namely *Amorphophallus paeoniifolius*, *Guetarda speciosa*, and *Jatropha curcas* are common mangrove associates in Bali [2]. Phytopharmacological properties have been shown in *A. paeoniifolius*, *G. speciosa*, and *J. curcas* [7-9]. For example, the antioxidant activity of *A. paeoniifolius* correlated to phenolic content [10], antiseizure activities of *G. speciosa* [8], and antimetabolic constituents of *J. curcas* [9]. These studies suggested that these mangrove associates are regarded as medicinal values.

The existence of polyisoprenoids has been referred to various plant tissues either in vegetal: leaves and roots [3-6], and reproductive: flowers, fruits, and seeds [10-

11]. These studies divulged the universal distribution of polyisoprenoids in the plant kingdom. The pattern and spreading of polyisoprenoid in mangrove and coastal plants have been reported [3-6]. It is essential to understand the function of polyisoprenoid various type of tissues, the detail information on the composition and profile of polysioprenoids in generative organs mangroves are entirely required. Therefore present study aimed to investigate the polyisoprenoids (polyprenol and dolichol) pattern and composition from selected coastal plants namely *A. paeoniifolius*, *G. speciosa*, and *J. curcas* flowers and fruits to extend our forgoing works on the exploration of polyisoprenoid from coastal plants.

2 Materials and methods

2.1 Chemicals

A combination of dolichols (C₉₀-C₉₅) and polyprenols (C₉₀-C₁₀₀) standard compounds as hitherto reported [2] was used to recognise the polyisoprenoids in this study. The description of the family concerning polyprenols or dolichols was accomplished in at least three experiments. Silica gel 60 TLC glass plates and reversed-phase silica RP-18 HPTLC glass plates were obtained from Merck (Darmstadt, Germany). All of the other chemicals and

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solvents were of reagent grade.

2.2 Plant materials

The flowers and fruits of selected coastal plants, *Amorphophallus paeoniifolius* (Araceae), *Guettarda speciosa* (Rubiaceae), and *Jatropha curcas* (Euphorbiaceae) were collected from Forest Park Conservation Area Ngurah Rai, Bali, Indonesia, in June 2017. These coastal vegetation are naturally growing to direct sunlight. The mean temperature in the month of the collection was 30 °C with an ordinary humidity of 74%. All of the fresh samples were maintained at -30°C until used.

2.3 Polyisoprenoid alcohols extraction

A manual for the polyisoprenoids extraction was done as previously described [11-12]. The flowers and fruits were dehydrated at 65-70°C for two days. The withered organ (5 g each) was powdered and submerged in 30 ml of chloroform/methanol (2/1, v/v) solvent for two days. The lipid extract of flowers and fruits was treated by saponification at 75°C for one day in 50% ethanol having 2 M KOH. The nonsaponifiable lipids of every tissue sample put in with hexane, and the organic solvent was dispersed and re-dissolved in hexane as earlier described [4]. The fruit (100-150 mg) and flower (150-200 mg) extracts were used to each TLC plate.

2.4 Polyisoprenoids were analysis by two-dimensional thin layer chromatography (2D-TLC)

First-dimensional TLC (1D-TLC) was executed for nearby one h on a silica gel glass plate (20 × 3 cm) with a toluene-ethyl acetate (9:1) solvent as earlier defined [11]. The longitudinal brink of the 1D-TLC plate was 1 cm in width, and the accumulation area of a reversed-phase C-18 TLC was fix by two magnetic bars (4.0 × 1.1 × 0.8 cm) fronting each gel stage. The bound TLC plate was then developed vertically to the 1D to transfer polyprenol or dolichol to the aggregation zone of the reversed-phase TLC plate.

The 2D-TLC was accomplished with acetone as the solvent for roughly 50 min. The marks of plants extract combinations, and standard mixes are dispersed and being generated by 2D-TLC was then recognised and visualised with iodine vapour. The enhanced chromatographic imaged and alphanumerically scanned with a Canon E-470 series printer. The polyisoprenoid family was defined by the association of movement on TLC with those of specific standards of dolichol or polyprenol that were implemented in the 2D improvement. The polyprenols and dolichols that were

traced on RP-18 HPTLC plates were measured with dolichol and polyprenol specifications as criterions.

3 Results and discussion

3.1 Composition and profile of polyisoprenoids in selected coastal plants

The selected coastal plants, namely *A. paeoniifolius*, *G. speciosa*, and *J. curcas* for searching the long-chain polyisoprenoids was investigated by 2D-TLC [3]. Polyisoprenoids of different chain-lengths were divided into polyprenols and dolichols. Tables 1-2 accumulate the analytical results of the spreading of the polyprenols and dolichols with the carbon-chain lengths particular for very species.

TL content of coastal grasses flowers and stems ranged 533 to 888 and 582 to 640 mg/g dw, respectively (Table 1). The quantity of PI was highest in *G. speciosa* flowers (121 mg/g dw) and *A. paeoniifolius* fruits (12 mg/g dw). The lowest content of PI was in the flowers of *A. paeoniifolius* (45 mg/g dw) and the fruits of *J. curcas* (8.3 mg/g dw) (Table 1).

The comparable results on TL and PI contents of this study were also have been earlier well agreed for *Nephellium lappaceum* fruits and flowers [11] and *Hevea brasiliensis* flowers [13]. The TL and PI concentrations in the coastal presented in this study were relatively higher than those shown from major and minor components of mangrove plants [3-5], fruit shell and fruit mesocarp of oil palm [12], and flowers and fruits of *Philesia magellanica* [14] and *Fuchsia magellanica* flowers [14]. These studies suggested the diversity of polyisoprenoid in plant reproductive tissues.

3.2 Polyisoprenoid analyzed by 2D-TLC

The spreading of polyprenols and dolichols in the roots were detected and classified into one group only. Type-II, which has the existence of both polyprenols and dolichols, was found all samples (Fig. 1, Table 2). No detected of group-I, which having to dominate dolichol over polyprenol and group-III, which shows the dominance of polyprenol over dolichol.

It is noteworthy that the presence of both polyprenols and dolichols in the flowers and fruits of selected coastal plants indicated the similar profile of *N. lappaceum* fruits [11], *hybrid tenara* of *Elaeis guineensis* fruit mesocarp and fruit shell from Lame [12].

This present study show opposite result on the plant fruits and fruits that was displaying only polyprenol pattern. It has been depicted that rubber plant flowers only contained 100% polyprenols (C₈₀-C₁₂₀) [13]. These results were paralleled with previous findings on *P. magellanica* flowers and fruits having only polyprenols (C₆₅-C₁₁₀) [14].

Table 1. Pattern of polyisoprenoids in selected coastal plants fruits and flowers.

Species	Tissue	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/ g)	Dol (mg/ g)	% in TL			% in PI		Type
						PI	Pol	Dol	Pol	Dol	
<i>A. paeoniifolius</i>	flower	640.1±18.3	45.0	18.6	26.4	7.0	2.1	4.1	41.3	58.7	II
<i>G. speciosa</i>	flower	654.0±12.4	121.1	67.8	53.3	18.5	10.3	8.1	56.0	44	II
<i>J. curcas</i>	flower	582.6±10.9	46.9	36.5	10.4	8.0	6.3	1.8	77.8	22.2	II
<i>A. paeoniifolius</i>	fruit	880.9±13.1	11.9	6.0	5.9	1.3	0.7	0.7	50.4	49.6	II
<i>G. speciosa</i>	fruit	533.1±14.5	8.9	2.9	6.0	1.7	0.5	1.1	32.6	67.4	II
<i>J. curcas</i>	fruit	888.0±16.4	8.3	3.7	4.6	0.9	0.4	0.5	44.6	55.4	II

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

Fig 1. 2D-TLC chromatograms hexane extracts of polyisoprenoids from *A. paeoniifolius* fruits (1), *G. speciosa* fruits (2), *J. curcas* fruits (3), *A. paeoniifolius* flowers (4), *G. speciosa* flowers (5), and *J. Curcas* flowers (6).

A similar pattern was shown in *F. magellanica* flowers where polyprenols with chain length (C_{65} - C_{130}) was described [14]. By contrast to this observation, in *N. lappaceum* flowers, the predominance of dolichol was observed [11]. These reports indicated difference

polyisoprenoid pattern is modulated in plant generative organs, which may be influenced by tropical or sub-tropical climates [15]. Further investigation on other mangrove or coastal plant flowers and fruits may give to answer this interesting and important matter.

Table 2. Carbon-chain lengths of polyprenol and dolichol of selected coastal plants.

Species	Tissue	(C43)	Polyprenol	Dolichol
<i>A. paeoniifolius</i>	Fruit	o	80 85 90 95 100	80 85 90 95 100
<i>G. speciosa</i>	Fruit		45 50	70 75
<i>J. curcas</i>	Fruit		65 70 75	65 70 75 80 85 90 95 100 105 110 115
<i>A. paeoniifolius</i>	Flower	o	75 80 85 90 95 100	75 80 85 90 95 100
<i>G. speciosa</i>	Flower		60 65 70 75 80	60 65 70
<i>J. curcas</i>	Flower		55 60 65 70 75 80 85 90 95 100 105 110 115 120 125	65 70 75 80 85 90 95 100 105 110 115 120 125

Furthermore, the physiological function of polyisoprenoids remains unclear. Recently it has been described that salinity changes the polyisoprenoid contents in four salt-secreter and non-salt-secreter mangroves [6]. The alteration of polyisoprenoids including in polyprenols and dolichols, as well. The content of polyisoprenoid also altered upon abiotic stress as well as with senescence has been shown [5, 13]. In this context, polyisoprenoids are known to adapt to external stresses.

4 Conclusion

This study confirmed the presence of polyisoprenoids profile in the fruits and flowers of selected coastal grasses: the occurrence of both polyprenols and dolichols in all samples. The significant findings are showing the diversity of polyisoprenoids in the flower and fruit tissues even in the same species, suggesting the chemotaxonomic marker of polyisoprenoids in coastal plants for the potential use of phytopharmacological, medicinal, and biological properties.

This study was supported by an International Research Collaboration and Scientific Publication Grant 2018 and an Excellent Research of Higher Education Grant 2017 (No. 003/SP2H/LT/DRPM/IV/2017 to MB) from the Directorate for Research and Community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia.

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