

Response of polyisoprenoid concentration and profile in three groups of mangrove seedlings of coping with long-term salinity

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Abstract. Basyuni M, Sagami H, Baba S, Oku H. 2019. Response of polyisoprenoid concentration and profile in three groups of mangrove seedlings of coping with long-term salinity. *Biodiversitas* 20: 320-326. The response of polyisoprenoid (polyprenol and dolichol) concentration and distribution was investigated with three groups mechanism of coping with salinity: a secreting mangrove species of *Avicennia officinalis*, a non-secreting species (excluder) of *Bruguiera cylindrica*, and a salt-accumulating species of *Xylocarpus granatum*. The seedlings of three mangroves were grown under 0 and 3% salinity concentration for five months. Polyisoprenoids in the lipid extracts were examined by two-dimensional thin layer chromatography (2D-TLC). The pattern of the polyprenols and dolichols in the leaves and roots were categorized as two types (I and II). In category I, dolichols dominated over polyprenols, however, in category II, the existence of both polyprenols and dolichols was found. In the leaves, type-I was observed in *A. officinalis* under 0 and 3% salinity. On the other hand, type-II was found in *B. cylindrica* and *X. granatum* under 0 and 3% salt concentrations. A similar pattern was found in the roots, *A. officinalis* (type-I), and that in *B. cylindrica* and *X. granatum* was type-II. This finding depicted that the seedlings of *A. officinalis*, *B. cylindrica*, and *X. granatum* leaves and roots imply no change in the distribution type: the categories were distributed as type I or II under 0% salt concentrations, as well as type-I or II under 3% salt concentrations. This study implied that polyisoprenoids may play a protective function against salinity in the mangrove leaves and roots of three groups scheme (secreting, excluding, and accumulating) of salt management.

Keywords: Dolichols, mangrove, polyprenols, protective role, secreting and non-secreting species

INTRODUCTION

Mangrove forests are widespread in the intertidal area of tropical and subtropical areas and are denoted physiologically by their ability to grow under varying salinity concentration, differing from freshwater to seawater conditions (Tomlinson 1986; Basyuni et al. 2017a). Mangrove plants are categorized into three groups according to their mechanisms of coping with salinity, i.e., salt-secreting, non-salt-secreting or excluder, and salt accumulating (Tomlinson 1986; Hogarth 1990; Basyuni et al. 2017a). The salt secreting species includes *Avicennia officinalis* (Acanthaceae), which has salt glands or salt hairs to eliminate excessive salt. The non-secreting species (salt exclusion) is exemplified by *Bruguiera cylindrica* (Rhizophoraceae), which has no salt glands but an ultrafiltration scheme. The salt accumulators species such as *Xylocarpus granatum* can cope with high salinity levels in their cells. *A. officinalis*, *B. cylindrica*, and *X. granatum* are common mangrove species on Lubuk Kertang forest, Langkat, North Sumatera, Indonesia and are considered to be the type of every group in concern of salinity characteristics. The three species are therefore ideal exemplary for studying physiological mechanisms of salinity tolerance at biochemical level.

Although mangroves are well known to produce secondary metabolites along with polyisoprenoid alcohols, the physiological function of these compounds remains poorly understood (Basyuni et al. 2016, 2017a, 2018a). Polyisoprenoid is a secondary metabolite compound with a linear polymer unit of five carbon chains found in almost all living cells (Swierzewski and Danikiewicz 2005; Basyuni et al. 2016, 2017b, 2018a; Sagami et al. 2018). Our previous studies have shown that salinity enhanced the triterpenoid content and triterpenoid synthase genes in mangrove seedlings (Basyuni et al. 2009, 2012a,b). By contrast, salinity decreased the mangrove seedling growth (Basyuni et al. 2009, 2012a,b, 2014; Hoppe-Speer et al. 2011; Ye et al. 2005). These studies suggested the complexity and diversity level of mangrove species. It is essential, therefore, to investigate the salt tolerant mechanism of each species of mangroves. At present, we are the first group to show that the salinity changes polyisoprenoid concentration in mangroves seedlings (Basyuni et al. 2017a). The present study extends our previous study and describes the response of polyisoprenoid (polyprenol and dolichol) content and distribution in three groups of salinity coping salinity, i.e., a secreting mangrove species of *Avicennia officinalis*, a non-secreting species of *Bruguiera cylindrica*, and a salt-

accumulating of *Xylocarpus granatum*.

MATERIALS AND METHODS

Plant materials

Mature and healthy propagules (seeds) of salt-secreting species, *Avicennia officinalis* L. (Acanthaceae), a non-secreting (excluder) species, *Bruguiera cylindrica* Blume (Rhizophoraceae), and a salt-accumulating species, *Xylocarpus granatum* Koen (Meliaceae) were collected in Lubuk Kertang forest, Langkat, North Sumatra, Indonesia and planted in bottle pots under 0 and 3% salinity level in a glass house. Mature seeds of *A. officinalis* had a greenish yellow pericarp with dark green to russet endosperm, 2.5–3.0 cm long and 2.0–2.5 cm wide. The mature propagules of *B. cylindrica* was slightly curved, cylindrical fruit hypocotyls with green to purplish green color, 0.5–1.0 cm in diameter, and 10–15 cm in length; mature *X. granatum* seeds were yellowish brown with a durable surface, greenish yellow cotyledons and were 15–20 cm in diameter. The *X. granatum* fruit is heavy (1–2 kg) like spherical melon, contained 6–16 seeds.

Salt tolerance experiments

The propagated seedlings grew for five months with exposure to normal temperature and sunlight in a controlled glass house. The mean temperature was 24.1 °C. A seawater solution was prescribed by liquefying commercial salt powder (Marine Salt, Singapore) to make 0% and 3% (equal to seawater salinity level) salt concentrations following the manufacturer's protocol. Salinity in this term was denoted as the mass of salt powder/weight of the solution (Basyuni et al. 2012b). The salinity level in each pot treatment was verified weekly during the experiments by an S/Mill-E salinity refractometer (Atago Co., Ltd, Tokyo, Japan) and was regulated by adding tap water or pure water to the control treatment (0%) or pure water to the salt treatments to recompense water lost due to evapotranspiration. Three plants in a detached pot, i.e., five bottle pots per species per salinity treatment, were grown for five months. After five months of cultivation, the three species of mangrove seedlings were collected and splashed, and then the leaves and roots were stored at -20 °C for further analysis.

Chemicals

A mixture of dolichol (C₉₅–C₁₁₀) standard compounds was isolated from horse testicles together with a combination of polyprenol (C₉₀–C₁₀₀) as previously reported (Basyuni et al. 2017a). These mixtures were used to classify the polyisoprenoids that were traced in this work. A mixture of dolichol (C₉₅–C₁₁₀) standard derived from skipjack tuna livers (Ishiguro et al. 2014) was also used in this study. The description of the family parallel to polyprenols or dolichols was carried out in at least three independent repetitions. Silica gel 60 TLC plates and reversed-phase silica RP-18 HPTLC plates were obtained from Merck. All of the other chemicals and solvents were of reagent grade (Merck).

Procedures

Isolation of polyisoprenoid alcohols

The protocol for the separation of polyisoprenoids was done as foregoing reported (Sagami et al. 1992; Basyuni et al. 2016). The leaves and roots of the three mangrove species were kept on an oven at 60 °C for two days. The dried tissue (2 g each) was crushed into a fine powder and submerged in chloroform/methanol (2/1, v/v) solvent for two days. The lipid extract of the leaves and roots was saponified at 65 °C for 24 h in 50% ethanol containing 2 M KOH. The unsaponifiable lipids of each tissue sample were extracted with hexane, and the organic solvent was evaporated and re-dissolved in hexane. The leaf (≈100 µg) and root (≈200 µg) extracts were applied to each TLC plate.

Analysis by two-dimensional thin layer chromatography (2D-TLC)

First-dimensional TLC was preformed for 45 min on a silica gel glass plate (20 cm × 3 cm) with a solvent system of toluene-ethyl acetate (9: 1) as previously described (Basyuni et al. 2016; 2017a). The second-dimension reversed-phase C-18 silica gel TLC was done with acetone as the solvent for about 45 min. The position of the spaced polyisoprenoid alcohols being developed by 2D-TLC was determined and envisaged with iodine vapor (Basyuni et al. 2017b). To identify whether the family corresponds to dolichols or polyprenols, a dolichol or polyprenol standard was applied to the sample line of the first-dimensional TLC. In the case that one family was observed on 2D-TLC, the standard was also employed to the sample and generated with a solvent system as previously reported (Basyuni et al. 2016). The improved chromatographic images were acquired and digitally scanned with a Canon G-2000 series printer.

The polyisoprenoid family was confirmed by the correlation of mobility on TLC with that of authentic standards of dolichol or polyprenol used in the second-dimensional phase. The polyprenols and dolichols found on RP-18 HPTLC plates were measured using ImageJ ver. 1.46r (Schneider et al. 2012) with dolichol and polyprenol standards as references.

Statistical analysis

The data were statistically evaluated by Dunnett's to test between the two treatments: 3% salt concentration against 0% salt concentration. The significant value of $P < 0.05$ was selected as the threshold of statistical significance. The analysis was carried out using the SAS 9.1 statistical software program (SAS Institute Inc., Cary, NC, USA)

RESULTS AND DISCUSSION

The effect of salinity concentration (0 and 3%) on the existence and content of polyisoprenoids in three mangrove groups of salt management, namely salt-secreting (*A. officinalis*), excluding (*B. cylindrica*), and accumulating (*X. granatum*) were analyzed by 2D-TLC (Basyuni et al.

2017a, 2018a). Alteration of polyisoprenoids into polyprenols and dolichols differing in chain length was detected. The fundamental types of the polyprenols and dolichols distribution in the leaves and roots were grouped into two types (I and II) as previously reported (Basyuni et al. 2016, 2017a). In type I of polyprenols and dolichols distribution, the dolichols is more dominant while in type II, both were observed in leaves and roots and no dominance did occur. Type III which shows the predominance of polyprenols over dolichols was not observed in this study.

Table 1 and 2 show the polyisoprenoid and distribution in the leaves and roots of three species under 0 and 3% salinity. As depicted in Table 1, the highest total lipid content in leaves tissues was in found *X. granatum* under 0 and 3% salinity (130.0 mg/g dw, respectively), and the lowest was found in *B. cylindrica* under 0 and 3% salinity (46.7 and 76.7 mg/g dw, respectively). Table 2 shows that the highest total lipid in roots tissues were found in *A. officinalis* under 0% salinity (83.3 mg/g dw) and *B. cylindrica* under 3% salinity (120.0 mg/g dw), and the lowest was in *X. granatum* and *A. officinalis* (40.0 and 26.7 mg/g dw, respectively under 0 and 3% salinity). *A. officinalis* leaves under 0%, and 3% were found as type II, by contrast, *B. cylindrica* classified as type I, and *X. granatum* observed as type II. These findings implied changes in two salinity level; polyisoprenoid, polyprenol, and dolichol contents were increasing or decreasing along with the enhanced salt concentration.

Table 2 shows that polyisoprenoids distribution in roots of *A. officinalis* was categorized as type I while that in *B. cylindrica* and *X. granatum* roots was classified as type II. The physiological role of polyisoprenoid in leaves and roots of mangrove differed between the two salinity levels. The contrasting responses in the investigations of isoprenoid, polyisoprenoid and osmotic stress have been reported in mangrove and halophyte plants. A previous study by Basyuni et al. (2009) shows that the mRNA levels of triterpenoid synthase genes and triterpenoid content increase in the root tissues and decrease in the leaves tissue of mangrove species *Kandelia candel* and *Bruguiera gymnorhiza* as the salinity level increased. A similar report was shown by Basyuni et al. (2017a) in the polyisoprenoid content of leaves and roots of mangrove seedling. Furthermore, this result was also supported by previous study; in this circumstance, Silveira et al. (2009) reported a distinct osmotic adjustment of a halophyte plant *Atriplex nummularia* in response to salinity. The *A. nummularia* leaves showed a strong osmotic adjustment, however, it was less significant in the roots. In the present study, only *X. granatum*, an accumulating-salt species, displayed a consistent polyisoprenoid type between leaves and roots, type II, respectively. *A. officinalis* and *B. cylindrica*, on the other hand, in term of polyisoprenoid type they produced as type I or type II. This explanation may be coherent with the divergent and complex adaptation mechanism of halophyte plants including mangroves to the salinity level (Slama et al. 2015).

Table 1. Effect of salt concentration on the occurrence and distribution of polyisoprenoids in leaves of three mangrove species

Species	Salinity (%)	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% total lipid			% polyisoprenoid		Type
						PI	Pol	Dol	Pol	Dol	
<i>A. officinalis</i>	0	96.7±41.6	92.7±29.8	20.2±3.4	72.5±5.1	95.8	20.8	75.0	21.8	78.2	II
<i>B. cylindrica</i>	0	46.7±20.8	32.3±12.8	nd	32.3±12.8	69.1	nd	69.1	nd	100	I
<i>X. granatum</i>	0	130.0±40.0	14.5±7.9	6.9±3.1	7.6±4.9	11.1	5.3	5.8	47.5	52.5	II
<i>A. officinalis</i>	3	106.7±23.1	47.2±13.2a	11.8±7.5a	35.4±13.0a	44.2	11.0	33.1	25	75	II
<i>B. cylindrica</i>	3	76.7±20.8a	61.0±5.1a	nd	61.0±5.1a	79.5	nd	79.5	nd	100	I
<i>X. granatum</i>	3	130.0±10.0	38.5±19.6a	15.3±4.3a	23.2±4.8a	29.6	11.9	3.5	40.3	59.7	II

Note: nd= not detected, TL = Total lipids, PI = Polyisoprenoids, Pol = Polyprenols, Dol = Dolichols, dw = dry weight. Data are stated as mean ± SD (n= 3) at least three independent experiments. "Significantly different from 0% at $P < 0.05$ using Dunnet's test. Total lipids are expressed as a fraction of a crude lipid estimated gravimetrically.

Table 2. Effect of salt concentration on the occurrence and distribution of polyisoprenoids in roots of three mangrove species

Species	Salinity (%)	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% total lipid			% polyisoprenoid		Type
						PI	Pol	Dol	Pol	Dol	
<i>A. officinalis</i>	0	83.3±30.6	33.6±8.8	nd	33.6±8.8	40.3	nd	40.3	nd	100	I
<i>B. cylindrica</i>	0	46.7±23.1	41.2±10.4	16.6±4.9	24.6±5.5	88.2	35.5	52.6	40.4	59.7	II
<i>X. granatum</i>	0	40.0±20.0	11.6±2.87	3.2±1.6	8.4±1.3	29.1	7.9	21.2	27.2	72.8	II
<i>A. officinalis</i>	3	26.7±5.8a	21.4±3.5	nd	21.4±3.0	80.1	nd	80.1	nd	100	I
<i>B. cylindrica</i>	3	120.0±26.5a	96.5±23.6a	60.3±8.1a	36.2±11.6	80.4	50.2	30.1	62.5	37.5	II
<i>X. granatum</i>	3	73.3±11.5a	37.4±14.4a	32.8±6.1a	4.6±4.2a	51.1	44.8	6.2	87.7	12.2	II

Note: nd= not detected, TL = Total lipids, PI = Polyisoprenoids, Pol = Polyprenols, Dol = Dolichols, dw = dry weight. Data are stated as mean ± SD (n= 3) at least three independent experiment. "Significantly different from 0% at $P < 0.05$ using Dunnet's test

Table 3. Carbon-chain lengths of polyprenol and dolichol occurring in three mangrove seedlings

Species	Tissue	Salinity (%)	Polyprenol*	Dolichol*
<i>A. officinalis</i>	Leaves	0	55 60	65 70 75 80 85 90 95 100 105 110 115 120 125 130
<i>B. cylindrica</i>	Leaves	0	nd	90 95
<i>X. granatum</i>	Leaves	0	60 65	80 85 90
<i>A. officinalis</i>	Leaves	3	45 50	65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140
<i>B. cylindrica</i>	Leaves	3	nd	80 85 90
<i>X. granatum</i>	Leaves	3	60 65	80 85 90
<i>A. officinalis</i>	Roots	0	nd	80 85 90 95 100 105
<i>B. cylindrica</i>	Roots	0	nd	80 85 90
<i>X. granatum</i>	Roots	0	75 80 85 90 95 100	75 80 85 90 95 100 105
<i>A. officinalis</i>	Roots	3	nd	75 80 85 90 95
<i>B. cylindrica</i>	Roots	3	nd	80 85 90 95
<i>X. granatum</i>	Roots	3	75 80 85 90 95	75 80 85 90 95 100

Note: *The numbers refer to the carbon-chain length of polyisoprenoid alcohols. nd= not detected

Table 3 summarizes the carbon-chain length in the leaf and root tissues of three mangrove species under 0% and 3% salinity. There was no significant change in polyisoprenoid composition in leaf tissues under 0 and 3% salinity level. Polyprenol with small different chain lengths as a shorter chain length of polyprenol was observed in *A. officinalis* under 0% salinity (C₅₅-C₆₀) and 3% salinity (C₄₅-C₅₀). On the other hand, carbon chain length of dolichol in *B. cylindrica* under 0% and 3% salinity level, respectively, C₉₀-C₉₅ and C₈₀-C₉₀. Salt stress increased the dolichols composition in *A. officinalis* leaves. Dolichol with a longer chain length was detected under 3% salt concentration (Table 3). A similar case was found in roots tissues, where the content and the chain length of dolichols of *A. officinalis* decreased with salt concentration (C₈₀-C₁₀₅) under 0% salinity, and C₇₅-C₉₅ under 3% salinity), but the polyprenol content and carbon chain length did increase in *B. cylindrica* roots (from C₈₀-C₉₀ to C₈₀-C₉₅). Polyprenol was only found in *X. granatum* roots with insignificant change of carbon chain lengths from C₇₅-C₁₀₀ to C₇₅-C₉₅.

Figure 1-3 depict the two-dimensional TLC chromatograms of polyisoprenoid from leaves and roots of *A. officinalis*, *B. cylindrica*, and *X. granatum*, under 0 and 3% salinity. Longer chain length of dolichol was found in *A. officinalis* under 0, and 3% salt concentration (C₆₅-C₁₃₀ and C₆₅-C₁₄₀, respectively). Ficaprenol, a shorter polyprenol with carbon chain length under 0% and 3% salinity of C₅₅-C₆₀ and C₄₅-C₅₀, respectively, was detected in leaves but not detected in root tissues. In *B. cylindrica*, C₈₀-C₉₅ dolichols were identified but polyprenols were not detected (Figure 2). In leaves of *X. Granatum*, both ficaprenols (C₆₀-C₆₅) and longer-chain dolichols (C₈₀-C₉₅) were detected in both salt concentrations (Figure 3) while in the root tissue of those species, C₇₅-C₁₀₅ dolichol was detected in 0% salinity level and C₇₅-C₁₀₀ dolichol was detected in 3% salinity level. Additionally, in roots of *X. granatum*, longer polyprenols ((C₇₅-C₉₅ and C₇₅-C₁₀₀) were also found in, respectively, 0 and 3% salinity level.

Shorter polyprenol (ficaprenols) was only detected in a secretor species of *A. officinalis* leaves, which supported our previous findings on the accumulated ficaprenols on

non-secretor species of *B. gymnorhiza*, and *K. candel* leaves under 3%. The diverse salinity coping mechanism resulted in different chain lengths of polyisoprenoids from shorter to longer chain lengths of polyisoprenoid (Basyuni et al. 2016, 2017a). Quantities of ficaprenol have been shown in the old leaves of *K. obovata* (Basyuni et al. 2016) which stores salt in its yellow leaves. The increase ficaprenols is supported by previous results (Baczewska et al. 2014) concerning the accumulation of short-chain polyprenols (C₄₅-C₆₀) of *Tilia* ‘Euchlora’ leaves under the increased soil salinity levels. Polyprenols with shorter carbon chain length have been identified in the Euphorbiaceae tribe (Swiezewska et al. 1994) and other families such as Lauraceae, Tiliaceae, and Magnoliaceae (Roslinska et al. 2002), soybean leaves (Kurisaki et al. 1997); in young and old rubber leaves (Tateyama et al. 1999); in spinach leaves (Sakaihara et al. 2000); and in the leaves of number of mangrove species (Basyuni et al. 2016, 2017b). The present study suggested the importance of investigation on the salt tolerance mechanism of different species or genera of mangrove plants.

The physiological role of polyisoprenoids remains unclear. Polyisoprenoids, analogous to earlier studies on triterpenes (Basyuni et al. 2009, 2012b), might control salinity stress with reference to the triterpene synthase gene as well as the physicochemical features of cell membranes by enhancing the permeability and adaptation to external stress (Inafuku et al. 2018; Basyuni and Wati 2017c). Dolichol contents were expanded in the roots of *Coluria geoides* and *Cucumis sativus* under severe abiotic stress conditions, including salinity, heavy metal, and low temperatures (Skorupinska et al. 2009). Likewise, dolichol content enhances the drought tolerance in *A. thaliana* (Zhang et al. 2008). These latter studies were parallel with our current findings in term of the accumulation of dolichols in three mangrove seedlings.

The dominance of dolichols over polyprenols and vice versa in some plant tissues in agreement with predominance dolichols over polyprenols in this study and other reports is still poorly understood. It has been shown that the polyisoprenoid distribution and dominance might

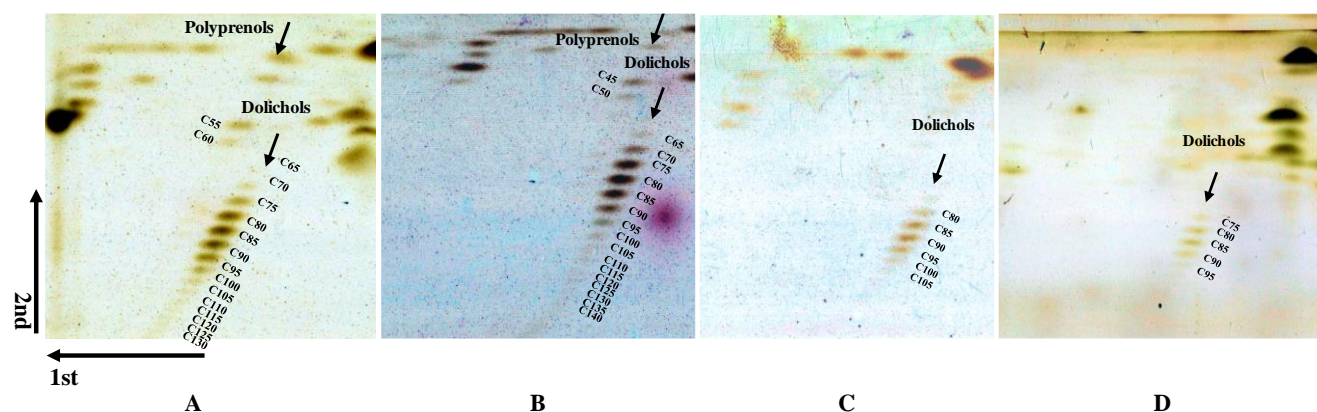


Figure 1. Two-plate TLC chromatograms of polyisoprenoids from *A. officinalis*: in leaves under 0% (A) 3% (B) salinity level, and in roots under 0% (C) and in 3% (D) salinity level. Data are from three independent experiments. The number shows the carbon chain length of polyisoprenoid alcohols. Arrow indicates the direction of TLC phase.

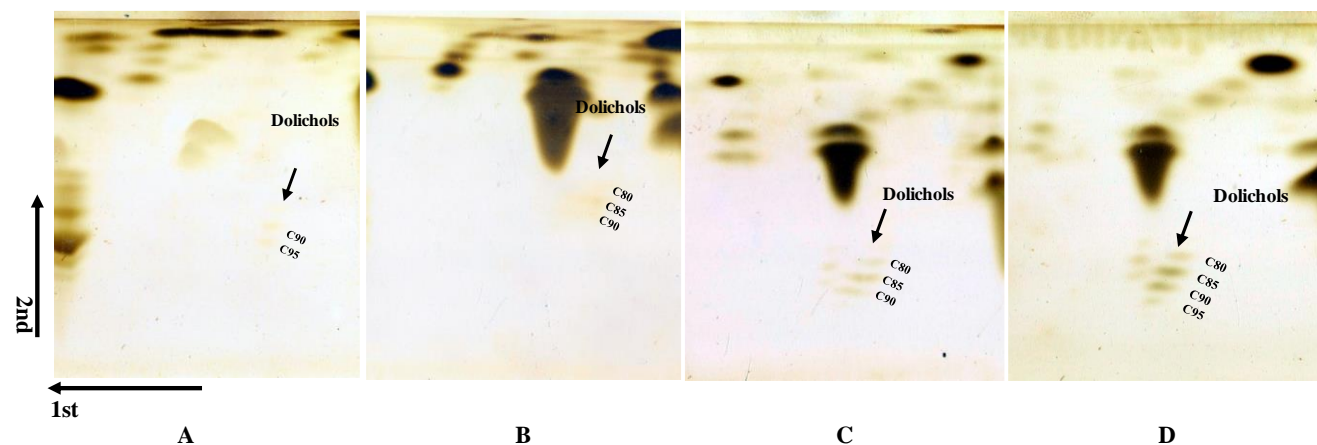


Figure 2. Two-plate TLC chromatograms of polyisoprenoids from *B. cylindrica*: leaves under 0% (A) and 3% (B) salinity levels, and in roots under 0% (C) and 3% (D) salinity level. Data are from three independent experiments. The number shows the carbon chain length of polyisoprenoid alcohols. Arrow indicates the direction of TLC phase.

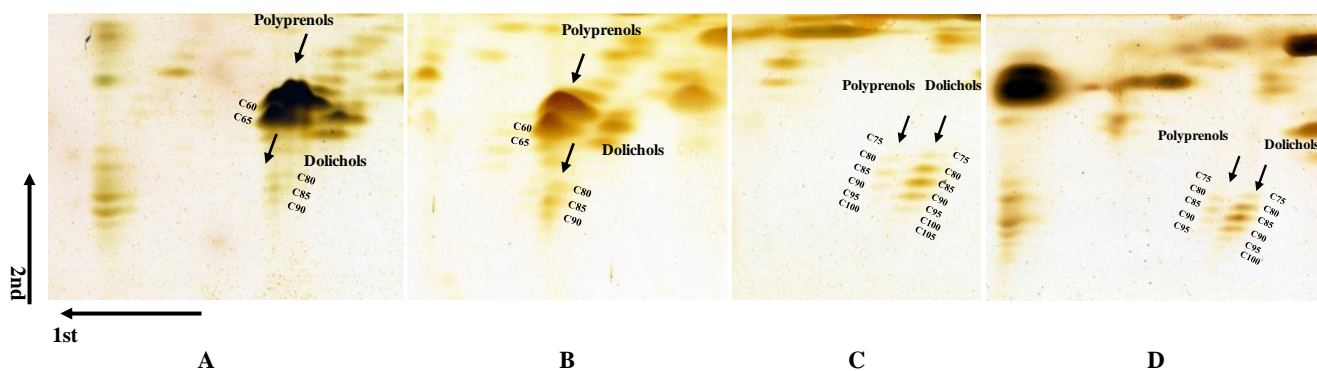


Figure 3. Two-plate TLC chromatograms of polyisoprenoids from *X. granatum*: in leaves under 0% (A) and 3% salinity (B), and in roots under 0% (C) and 3% (D) salinity level. Data are from three independent experiments. The number shows the carbon chain length of polyisoprenoid alcohols. Arrow indicates the direction of TLC phase

have resulted from the irregular stability of specific compounds of biosynthetic end-products (Skorupinska et al. 2007). Additionally, the predominance of dolichols over polyprenols in mangrove leaves and roots may be due to the existence of active enzymes of polyprenol reductase (Basyuni et al. 2018b, c, d) as a result of tropical or sub-tropical conditions such as climatic or environmental conditions. Recently, the predicted polyprenol reductase enzyme and its bioinformatics data had been reported from *Kandelia obovata* (Basyuni et al. 2018b,c).

The present study findings, therefore, well agreed with the view that there may be partly three different biosynthetic pathways responsible for the formation of shorter-chain polyprenols (ficaprenols-type), longer-chain polyprenols, and shorter and longer dolichols. These results suggested that the biosynthetic pathway from polyprenols to dolichols might be modulated by polyprenol reductase with firm precursor distinction to chain-length in mangrove plants. Recently, the existence of three fragments predicted polyprenol reductase genes had been shown in mangrove species of *K. obovata* (Basyuni et al. 2018b,c).

Moreover, a new polyprenol reductase (KoPPRD1) from *K. obovata* was found to be associated with other polyprenol reductases in the phylogenetic of plant kingdom (Basyuni et al. 2018d). These recent studies including this study, have confirmed the widespread polyisoprenoids in mangrove, and coastal plants were not polyprenols but dolichols. The polyprenol reductase which altered polyprenols into dolichols was elaborated in mangrove and coastal plants activities (Basyuni et al. 2016, 2017b, 2018a).

In conclusion, the present study results revealed a similar pattern and distribution of polyisoprenoid among mangrove groups of salinity coping mechanism (secreting, excluding, and accumulating). There was no substantial change in polyisoprenoid distribution types between leaves and roots of *A. officinalis*, *B. cylindrica*, and *X. granatum* seedlings: the groups were categorized as type I or II under 0% or 3% salt concentrations. The present study results also indicated that polyisoprenoids might play a protective function against salt concentration in the mangrove leaves and roots of three groups of salinity management.

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