Analysis of polyisoprenoids in the leaves and roots of *Aegiceras floridum* and *Lumnitzera littorea*

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**Abstract.** *A. floridum* and *L. littorea* are the members of significant mangroves which are abundant in Indonesia that has been reported to have biological properties. The pattern and existence of polyisoprenoid alcohols (polyprenols/dehydrodolichol and dolichols) in the leaves and roots of *A. floridum* and *L. littorea* were investigated using a convenient two-plate thin layer chromatography method. The polyprenols and dolichols distribution was determined and classified into two groups. Group-I, having a dominating of dolichols over dehydrodolichols, was found in the roots of *L. littorea* with one dolichol family (C90-C95). Type-II, showing the existence of both dehydrodolichols and dolichols, was observed in the leaves *A. floridum* (with polyprenol and dolichol chain length C60-C80 and C90-C95, respectively). Furthermore detected in leaves of *L. littorea*, polyprenol and dolichol occurred longer chain length (C60-C140 and C70-C140, respectively) and roots of *A. floridum* with a chain length of dolichol (C75-C100) few longer that polyprenol (C85-C95). The composition of polyisoprenoids in leaves and roots tissues of *A. floridum* and *L. littorea* is the first description to extend our previous finding on the occurrence polyisoprenoids in mangrove plants.

1. **Introduction**

Mangroves are woody plant communities that grow in the intertidal of the coastal zone in tropical and subtropical climates. Indonesia has the most extensive area of mangroves, comprising 22.6% of the world's mangroves [1]. Mangroves forests in Bali mainly distributed in Tanjung Benoa and Ngurah Rai Grand Forest Park, Nusa Lembongan, and Bali Barat National Park [2]. *Aegiceras floridum* has been reported to be found only in Indonesia [3] whereas *Lumnitzera littorea* distributed throughout of East coast of Africa to Southeast Asia, Australia and Polynesia [4]. Mangrove plants together with coastal plants are known to produce secondary metabolites including polyisoprenoid alcohols [5-7].

The polyisoprenoids distribution has been shown in different plant organs either in leaves and roots [5-7] flower, fruits, and seeds [8-10]. These reports disclosed the ubiquitous pattern of polyisoprenoid
in the plant family. The occurrence and distribution of polyisoprenoid in mangrove and coastal plants have been reported [5-7]. To obtain more insight into the function and significant polyisoprenoid in mangroves, detail information on the occurrence of mangroves are entirely required. Therefore present study aimed to analyze the polyisoprenoid (polyisoprenol and dolichol) profile and composition from selected mangrove significant leaves and roots namely A. floridum and L. littorea to extend our previous results.

2. Materials and Method

2.1. Plant materials

Fresh leaves and roots of Aegiceras floridum and Lumnitzera littorea were collected from Taman Hutan Raya Ngurah Rai, Bali, Indonesia, in May 2017. A. floridum and L. littorea are grown under direct sunlight and natural conditions. The mean temperature during the sampling was 29-31 ºC with an ordinary humidity of 74-76%. All of the plant materials were provided at freezer until utilised.

2.2. Chemicals

In this study, a mixture of dolichols (C_{90}-C_{95}) and polyisoprenols (C_{90}-C_{100}) standard as earlier reported [5] was used to detect the polyisoprenoids. The determination of the compounds relating to dehydrodolichols or dolichols was made at triplicate independent investigations. Silica gel 60 TLC glass plates and silica RP-18 HPTLC glass plates were obtained from Merck. All of the other chemicals and solvents were of the analytical level.

2.3. Protocol of polyisoprenoid alcohols

A protocol for the separation of polyisoprenols from dolichols as preceding reported [6]. Concisely, the dried leaves and roots (5 g each) were powdered and involved in chloroform/methanol (2/1, v/v) for two days. The crude lipid extract of leaves and roots was saponified, and the un-saponifiable lipids of each organ sample drew out with hexane, and this basic solution was dried-up and re-mixed in hexane as previously described [6].

2.4 Search by two-plate thin layer chromatography (2 plate-TLC)

Search for polyisoprenoids was done through two steps as earlier shown [6-7]. Firstly, a silica gel glass plate (20 × 3 cm) developed in toluene-ethyl acetate (9:1) for about 50 min. The silica gel face which polyisoprenols and dolichols represented was formed transverse to the first plate to move dehydrodolichol or dolichol to the focusing area of the reversed-phase plate.

Secondly, acetone solvent was used for approximately 30 min for reversed-phase silica gel TLC. The position spots of polyisoprenols and dolichols are separated and then determined and imaged with iodine vapour. Canon E-400 series printer was used to scan the developed chromatographic images. The polyisoprenols and dolichols family was identified by the comparing mobility on chromatograms with standards samples that were run in the reverse-phase. The polyisoprenols and dolichols contents were measured using ImageJ version 1.46r [11], with dolichol and polyisoprenol standards as markers.

3. Results and Discussion

3.1. Pattern of polyisoprenoid

Table 1 illustrates the total lipid (TL) concentration of leaves tissue of A. floridum and L. littorea ranged from 549.0 to 577.7 mg/gdw with the minimum and maximum content in the leaves of L. littorea and A. floridum, correspondingly. The TL content of roots tissue aligned from 487.0 to 696.9 mg/g dw with the undermost and topmost materials in the leaves of L. littorea and A. floridum, subsequently. The minimal value of polyisoprenoids was in the leaves and roots of A. floridum, and the highest was in the leaves and roots of L. littorea (17.2 - 10.6 and 93.5 - 13.9 mg/gdw, respectively) (Table 1).
The present data suggests that profile of polyisoprenoid in the vegetative organ of *A. floridum* and *L. littorea* rely upon each organ. In the leaves, polyrenols were plentifully observed, this data help up the preceding study on the preeminence of polyrenols over dolichols in the plant leaves [5-7, 12-13], exemplified by minor component of mangroves: *Excoecaria agallocha*, *Heritiera littoralis*, and mangrove associates of *Hibiscus tiliaceus* [5]. Ficaprenol types of polyrenols have been typified by the younger to older *Hevea brasiliensis* leaves [13], *Glycine max* leaves [14], young leaves of Spinach [15], and *Lauraceae*, *Tiliaceae*, and *Magnoliaceae* tribe [16].

### 3.2. Polyisoprenoid detection by two-plate thin layer chromatography

The pattern types of dehydrodolichols and dolichols in the leaves and roots of *A. floridum* and *L. littorea* were classified into two categories (I and II), as previously described [7-8]. In leaves organs, category-II only was detected, and in the roots tissues category I and category II were observed. In group-I, the majority of dolichols over polyrenols was found in roots of *L. littorea*.

Dolichol has been established as leading polyisoprenoids in the plant roots, for instance, young *H. brasiliensis* roots [13], in the roots of several coastal plants such as *Acacia auriculiformis*, *Barringtonia asiatica*, *Casuarina equisatfolia*, *Calophyllum inophyllum*, *Hibiscus tiliaceus*, *Pandanus odoratissima*, *Pongamia pinnata*, *Ricinus communis*, *Schypilhara hydropflaceae*, *Stachytar jamacensis*, *Sesivium portulacastrum*, and *Terminalia catappa* [7], *G. max* roots [14], and the most of mangrove forests [5-6]. A particular component of polyisoprenoids is their incidence in leaf tissues as anassortment of correspondent, more complex dehydrodolichols (as in shorter, medium and longer prenols). However, several studies have been shown [5-7] and in the present study (*L. littorea* leaves), dolichols also served as longer-chains-length in numbers of mangrove plant leaves. For instance, found in *Avicennia marina* (C_{65–130}), *L. racemosa* (C_{60–140}), *Phempsis acidula* (C_{50–140}), *Sonneratia alba* (C_{65–130}), *Acanthus ilicifolius* (C_{60–125}), *Aegicerascorniculatum* (C_{60–140}), and *S. caseolaris* (C_{50–120}) (5-7).

| Species       | Tissue | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in TL | % of PI | Type |
|---------------|--------|--------------|--------------|------------|------------|---------|---------|------|
| *A. floridum* | Leaves | 549.0±15.2   | 17.2         | 10.1       | 7.1        | 3.1     | 1.8     | 1.3  | 58.7 | 41.3 | II   |
| *L. littorea* | Leaves | 577.7±28.9   | 93.5         | 71.9       | 21.4       | 16.2    | 12.4    | 3.7  | 76.9 | 22.9 | II   |
| *A. floridum* | Roots  | 696.9±14.9   | 10.6         | 5.5        | 5.1        | 1.5     | 0.8     | 0.7  | 51.9 | 48.1 | II   |
| *L. littorea* | Roots  | 487.0±21.0   | 13.9         | nd         | 13.9       | 2.8     | nd      | 2.8  | nd   | 100  | I    |

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

This finding has the similar result with a case in mangroves roots of Okinawan mangrove [5]. *L. littorea* leaves, a observe quantity of dehydrodolichols with carbon chain-lengths identical to those of dolichols. On the other hand, dehydrodolichols found much longer than dolichols (>C100 and more) in chain-length were too, as displayed in Figure 1 and Table 2. Similarly, group-II have been described in seeds of *H. brasiliensis*, *Ginkgo biloba*, and *Pinus sylvestris* [13]. The presence of both dehydrodolichols and dolichols was also detectable in *Coluria geoides* seeds [17]. By contrary to this result, a number of gymnosperms species comprised only dehydrodolichols but not dolichols found [18], however, in case of soybeans seeds included only dolichols [14]. These observations are slightly opposite to the leaves and roots of mangrove forests, where the primary polyisoprenoid alcohols are dolichols rather than polyprenols. Dolichols were found in all tissues of North Sumatran mangroves [6] as well as in coastal plants [7].
Figure 1. Two-plate TLC chromatograms of polyisoprenoid from *A. floridium* leaves (1), *L. littorea* leaves (2), *A. floridium* roots (3), and *L. littorea* roots (4).

Table 2. Chain lengths of polyprenol and dolichol of leaves and roots of *A. floridium* and *L. littorea*

| Species    | Tissue | Polyprenol                  | Dolichol                  |
|------------|--------|-----------------------------|---------------------------|
| *A. floridium* | Leaves | 60 65 70 75 80              | 90 95                     |
| *L. littorea* | Leaves | 60 65 70 75 80 85 90 95 100 105 110 | 70 75 80 85 90 95 100 105 110 |
|             |        | 115 120 125 130 135 140 and more | 115 120 125 130 135 140 and more |
| *A. floridium* | Roots  | 85 90 95                    | 75 80 85 90 95 100       |
| *L. littorea* | Roots  | 90 95                       |                           |

Furthermore, the physiological function of polyisoprenoids remains unclear. It has been reported that salinity alters the polyisoprenoid contents in four salt-secretor and non-salt-secretor mangroves [19]. The changing of polyisoprenoids including in polyprenols, dolichols, and bombiprenone as well has been shown [19].

4. Conclusions
The current work clarified the profile and composition of polyisoprenoids in *A. floridum* and
L. littorea. The pattern of polyisoprenoids in leaves and roots tissues of A. floridum and L. littorea is the first report to extend our previous finding on the occurrence polyisoprenoids in mangrove forests.

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