Detection of polyisoprenoids in the roots and stems of coastal grasses using a two-dimensional thin layer chromatography

M Basyuni1,2*, R Wati1,2, A N Prabuanisa1, I K T W Kusuma3, Hamiuddin4, Guntur5, and H Sagami5

1 Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatera 20155, Indonesia
2 Mangrove and Bio-Resources Group, Center of Excellence for Natural Resources Based Technology, Universitas Sumatera Utara, Medan North Sumatera 20155, Indonesia
3 Balai PPI dan Karhutla Wilayah Jawa Bali Nusra, Suwung Kauh, Denpasar, Indonesia
4 UPT Taman Hutan Raya Ngurah Rai, Dinas Kehutanan Provinsi Bali, Indonesia
5 Institute of Multidisciplinary Research for Advanced Material, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, 980-8577, Japan

*Email: m.basyuni@usu.ac.id

Abstract. Coastal plants are known to produce secondary metabolites including polyisoprenoid alcohols. Coastal plants have been shown to have phytomedicinal, biological, and pharmacological properties. The present study reports the detection of polyisoprenoids composition from roots and stems of selected coastal medicinal grasses, Cyperus rotundus, Distichlis spicata, and Spinifex littoreus. A two-dimensional thin layer chromatography (2D-TLC) method was used to analyse the content and distribution of polyisoprenoid alcohols (polyprenols/dehydrodolichols and dolichols) in coastal grasses. The presence of polyprenols and dolichols in the roots and stems were identified and grouped into two types. Type-I, showing a preponderance of dolichols over polyprenols, was detected in the roots of C. rotundus and D. spicata. Type-II, displaying the existence of both polyprenols and dolichols, was traced in S. littoreusroots and the stems of C. rotundus, D. spicata, and S. littoreus. The diversity of polyisoprenoids in the root and stem tissues even in the same species, suggesting the chemotaxonomic criterion of polyisoprenoids in coastal grasses.

1. Introduction
Plants that exist in the coastal environment and within mangroves as well are regarded as coastal plants or mangrove associates [1]. Coastal plants are widespread in the intertidal tropical and subtropical areas and have long been eminent a fountain of secondary metabolites including polyisoprenoid alcohols [2]. The selected coastal medicinal grasses, namely Cyperus rotundus, Distichlis spicata, and Spinifex littoreus are common species widespread in Tanjung Benoa and Taman Hutan Raya Ngurah Rai, Bali Province, Indonesia.

A number of studies have been reported for biological and medicinal activities of coastal plants. For example, C. rotundus has been shown to have traditional uses, phytochemical, biological, and
pharmacological potentials [3-4]. Anti-inflammatory and analgesic property of S. littoreus has also been reported [5]. Whereas, D. spicata has been used for food and cover by various species of snails and crabs [6]. Furthermore, polyisoprenoids have been reported to show some pharmacological activity such as anticancer [7], anti-dyslipidaemic [8], anti-influenza and antiviral agents [9]. In addition, dehydrodolichols from Ginkgo biloba leaves has been demonstrated to actively exhibit antibacterial and antifungal activity [10].

Supposed the essential biological and medicinal properties of C. rotundus, D. spicata, and S. littoreus as well as sources of plant polyisoprenoids. However, the profile of polyisoprenoid in C. rotundus, D. spicata, and S. littoreus have not been studied yet. The occurrence and distribution of polyisoprenoid in mangrove and coastal plants have been reported [2, 11-12]. To obtain more understanding the function of polyisoprenoids, it is indispensable to acquire the data of allocation and profile of polyisoprenoid in selected coastal medicinal grasses. Therefore present study aimed to examine the polyisoprenoids (polyprenol and dolichol) profile and composition from selected coastal grasses namely C. rotundus, D. spicata, and S. littoreus to extend our previous works on the search of polyisoprenoids from coastal plants.

2. Materials and Method

2.1 Chemicals
Dolichols (C_{90-95}) and polyrenols (C_{90-100}) standards as earlier reported [2] was used to detect the polyisoprenoids in this study. For at least three independent experiments were performed to determine the family compounds relating to polyrenols or dolichols. Silica gel 60 TLC glass plates and silica RP-18 HPTLC glass plates were obtained from Merck.

2.2 Plant materials
The roots and stems of coastal grasses, C. rotundus, D. spicata, and S. littoreus were collected from Taman Hutan Raya Ngurah Rai, Bali, Indonesia, in May 2017. These coastal florae are commonly growing exposer to sunlight. The mean temperature in the month of the sampling was 30-32°C with an ordinary humidity of 75-77%.

2.3 Polyisoprenoid alcohols extraction
Extraction of polyisoprenoids' protocol was performed as previously described [11-12]. The roots and stems were dried at 65-70°C for 48 hours. The desiccated tissue (5 g each) was powdered and submerged in chloroform/methanol (2/1, v/v) for 2 days. The crude lipid extract of roots and stems was saponified and removed physically with hexane, and the organic solvent was dried up and re-mixed in hexane as earlier reported [2]. The root (100-150 mg) and stem (150-200 mg) extracts were used to apply for each TLC plate.

2.4 Polyisoprenoids were analyzed by two-dimensional thin layer chromatography (2D-TLC)
2D-TLC method contained two steps: a silica gel glass plate (20 x 3 cm) was used for first-dimensional TLC (1D-TLC) for about 45 min with toluene-ethyl acetate (9:1) solvent as earlier reported [11]. The second phase, acetone as the solvent was used to 2D-TLC analysis with for about 40 min. The oblique ladder of polyprenols and dolichols along with the standard are separated and then recognized and visualised with iodine vapour. The enhanced chromatographic was imaged and scanned with a Canon Pixma G2000 series printer. The dehydrodolichols and dolichols family was analyzed by the corresponding to movability on chromatograms with those of certain standards of polyisoprenoids that were practised in the two-plate run. The polyprenols and dolichols contents were assessed using ImageJ version 1.46r [13], with dolichol and dehydrodolichol standards as criterions.
3. **Results and Discussion**

3.1. **Distribution and profile of polyisoprenoids in selected coastal grasses**

The selected coastal grasses, namely *C. rotundus*, *D. spicata*, and *S. littoreus* for long-chain polyisoprenoids was analysed by 2D-TLC [2]. Polyisoprenoids of different chain-lengths were divided into polyprenols and dolichols. Tables 1-2 compile the analytical results of the distribution of the dehydrodolichols and dolichols with the carbon (C)-chain lengths addicted for every species.

TL content of coastal grasses roots and stems ranged 562 to 657 and 569 to 715 mg/g dw, respectively (Table 1). The quantity of PI was highest in *D. spicata* roots (157 mg/g dw) and *S. littoreus* stems (63 mg/g dw). The lowest content of PI was in the roots of *C. rotundus* (13 mg/g dw) and the stems of *D. spicata* (7 mg/g dw) (Table 1).

The comparable results on TL and PI contents of this study were also have been previously supported for North Sumatran coastal leaves [1] and *Nephelium lappaceum* various tissues [14]. The TL and PI concentrations in the coastal and mangrove associates plants presented in this study were relatively higher than those shown from major and minor components of mangrove plants [11-12] and generative and vegetative tissues of oil palm [15].

| Species   | Tissue | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in TL | % of PI |
|-----------|--------|--------------|--------------|-----------|-----------|---------|---------|
| *C. rotundus* | roots | 657.9±18.5 | 13.6 | nd         | 13.6      | 2.1     | nd      | 0.1 |
|           | D. spicata | 562.2±18.3 | 157.1 | nd         | 157.1     | 27.9    | nd      | 27.9 |
|           | S. littoreus | 640.2±12.5 | 14.4 | 6.1        | 8.3       | 2.2     | 0.9     | 1.3 |
| *C. rotundus* | stems | 617.4±15.7 | 32.5 | 13.3       | 19.2      | 5.3     | 2.1     | 3.1 |
|           | D. spicata | 569.7±11.5 | 6.9  | 0.8        | 6.1       | 1.2     | 0.1     | 1.1 |
|           | S. littoreus | 715.9±12.5 | 63.7 | 33.6       | 30.1      | 8.9     | 4.7     | 4.2 |

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

3.2. **Polyisoprenoid analyzed by 2D-TLC**

The spreading of polyprenols and dolichols in the roots were determined and classified into two types. Type-I, which shows a numerousness of dolichols over dehydrodolichols (100%), was found in the roots of *C. rotundus* and *D. spicata* (Figure 1 A-B, Table 2). Type-II, which has the existence of both dehydrodolichols and dolichols, was identified in *S. littoreus* roots (Figure 1 C, Table 2). However, in the stems of *C. rotundus*, *D. spicata*, and *S. littoreus*, the dolichols were not detected as a case in the roots but contained both polyprenols and dolichols as Typed-II (Figure 1 D-F, Table 2).

The abundance of dolichols in the roots of both species of *C. rotundus* and *D. spicata* well agreed with previous results on mangrove plant roots [11-12], coastal plant roots [2], *N. lappaceum* roots [14], rubber roots [16], and soybean roots [17]. It is noteworthy that the existence of both polyprenols and dolichols in the stems of selected coastal grasses indicated the similar pattern of some coastal plants leaves such as *Acanthus ilicifolius* [11], *Casuarina equisetifolia* [2], *Melastoma candidum* [2], *Morinda citrifolia* [2], *Sesuvium portulacastrum* [2]. The existence of both polyprenols and dolichols also have been reported in a few mangrove leaves [11-12], *N. lappaceum* rinds, fruits, and seeds [14], young and old leaves of *Hevea brasiliensis* [16], Ginkgo seeds, embryo, young and old leaves [16], and pine (*Pinus sylvestris*) [16]. The presence both polyprenols and dolichols are typical of oil palm *pisifera* and *tenera*-type fruit [15].

---

Table 1. Distribution of polyisoprenoids in coastal grasses roots and stems

| Species   | Tissue | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in TL | % of PI |
|-----------|--------|--------------|--------------|-----------|-----------|---------|---------|
| *C. rotundus* | roots | 657.9±18.5 | 13.6 | nd         | 13.6      | 2.1     | nd      | 0.1 |
|           | D. spicata | 562.2±18.3 | 157.1 | nd         | 157.1     | 27.9    | nd      | 27.9 |
|           | S. littoreus | 640.2±12.5 | 14.4 | 6.1        | 8.3       | 2.2     | 0.9     | 1.3 |
| *C. rotundus* | stems | 617.4±15.7 | 32.5 | 13.3       | 19.2      | 5.3     | 2.1     | 3.1 |
|           | D. spicata | 569.7±11.5 | 6.9  | 0.8        | 6.1       | 1.2     | 0.1     | 1.1 |
|           | S. littoreus | 715.9±12.5 | 63.7 | 33.6       | 30.1      | 8.9     | 4.7     | 4.2 |

nd= not detected, TL = Total lipids, PI = Polyisoprenoids, Pol = Polyprenols, Dol = Dolichols, dw = dry weight. Data are stated as mean ± SD (n= 3).
The alteration of polyisoprenoids including in polyprenols, dolichols, and secretor and non-secretor species in coastal grasses roots and stems has been demonstrated in triterpenoids as well. The content of polyisoprenoid also altered upon abiotic stress as well as with senescence has been shown [11-12, 19]. The similar function has been demonstrated in triterpenoids as well.
a defence mechanism to external stress such as salinity [20]. In this context, polyisoprenoids are known to have some pharmacological and biological activities [7-9].

4. Conclusions
This study confirmed the presence of polyisoprenoids profile in the roots and stems of selected coastal grasses: dolichols dominated in the roots and the incidence of both polyprenols and dolichols in the stems. The significant findings showing the diversity of polyisoprenoids in the root and stem tissues even in the same species, suggesting the chemotaxonomic marker of polyisoprenoids in coastal grasses for the potential use of phytomedicinal, biological, and pharmacological activities.

Acknowledgement
This work was funded 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.

References
[1] Baba S, Chan H T and Aksornkoae S 2013 Useful Products from Mangrove and other Coastal Plants ISME Mangrove Educational Book Series No. 3. International Society for Mangrove Ecosystems (ISME), Okinawa, Japan, and International Tropical Timber Organization (ITTO) (Japan: Yokohama) 2
[2] Basyuni M, Wati R, Sagami H, Sumardi, Baba S and Oku H 2018 Biodiversitas 19 1
[3] Peerzada A M, Ali H H, Naeem M, Latif M, Bukhari A H and Tanveer A 2015 Ethnopharmacol. 174 540
[4] Kumar K H, Razack S, Nallamuthu I and Khanum F 2014 Ind. Crops and Prod. 52: 815
[5] Lonard R I, Judd F W and Stalter R 2013 J Coastal Res. 29 105
[6] Yogamoothidi A and Priya E S 2006 J. Environ. Biol. 27 271
[7] Kuznecovs S, Jelina K and Kuznecovs I 2007 The Breast 16 515
[8] Singh G, Gupta P, Rawat P, Puri A, Bhatia G and Maurya R 2007 Phytomedicine 14 792
[9] Safatov A S, Boldyrev A N, Bulychev L E, Buryak G A, Kukina T P, Poryvaev V D, P'Yankov O V, Raldugin V A, Ryzhikov A B, Sergeev AN, Shishkina L N, Tolstikov G A and Zhukov V A 2005 J. Aerosol Med. 18 55
[10] Tao R, Wang C Z and Kong Z W 2013 Molecules 18 2166
[11] Basyuni M, Sagami H, Baba S, Iwasaki H and Oku H 2016 Dendrobiology 75 167
[12] Basyuni M, Sagami H, Baba S and Oku H 2017 Dendrobiology 78 18
[13] Schneider, Rasband W S and Eliceiri K W 2012 Nature Methods 9 671
[14] Basyuni M and Wati R 2017 Earth Environ. Sci 101 012001
[15] Arifiyanto D, Basyuni M, Sumardi, Putri L A P, Siregar E S, Risnasari I and Syahputra I 2017 Biodiversitas 18 1487
[16] Tateyama S, Wititsuwannakul R, Wititsuwannakul D, Sagami H and Ogura K 1999 Phytochemistry 51 11
[17] Kurisaki A, Sagami H and Ogura K 1997 Phytochemistry 44 45
[18] Basyuni M, Sagami H, Baba S, Putri L A P, Wati R and Oku H 2017 HAYATI J. Biosci. 24 206
[19] Swiezewska E and Danikiewicz W 2005 Prog. Lipid Res. 44 235
[20] Basyuni M, Baba S, Kinjo Y, Putri L A P, Hakim L and Oku H 2012 J. Plant Physiol. 169 1903