Probably occurrence of epoxy polyprenol and epoxy dolichol in Sonneratia alba and S. caseolaris old leaves of mangrove plants

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Abstract. Mangrove plants are known to produce secondary metabolites including isoprenoid and long chain polyisoprenoid. Sonneratia alba and S. caseolaris, are true mangroves that contain alkane (C₂₅-C₃₃), triterpenoid, phytosterol, dolichol, polyprenol, and polypropenyl acetone. Here we reported new compounds of probably epoxy polyprenol and epoxy dolichol from S. alba and S. caseolaris old leaves using two-dimensional thin layer chromatography (2D-TLC). In the S. alba old leaves, having the occurrence of polyprenol, dolichol, epoxy polyprenol, and epoxy dolichol with a dominating pattern of polyprenol. A similar result obtained in the S. caseolaris old leaves, displaying the presence of polyprenol, dolichol, epoxy polyprenol, and epoxy dolichol with a dominating carbon chain length of dolichol. By contrast, no epoxy polyprenol or epoxy dolichol were detected in S. alba and S. caseolaris leaves. The present study indicated a new type of polyisoprenoid distribution and further investigation is needed to clarify the chemical structure of this new compound.

1. Introduction
Mangrove plants well produce secondary metabolites such as isoprenoid and long chain polyisoprenoid [1]. Sonneratia alba and S. caseolaris, which are a family member of true mangroves, have been reported to contain alkane (C₂₅-C₃₃), triterpenoid, phytosterol, shorter and longer polyprenols, and longer-chain dolichol [1-4]. Recently a new compound of polyprenol derivatives, polypropenyl acetone has been detected in S. caseolaris leaves [4]. Biological and pharmaceutical activities of Sonneratia have been described, for example, S. alba and S. caseolaris leaf extracts have been used as traditional folk medicinal in Thailand and showed a promising antioxidant activity[5]. Polyisoprenoid alcohols are regarded as polyprenol or dehydrodolichol and dolichol with reference to the isoprene structure [3-4]. A number of studies have been reported that plant polyisoprenoids involved in pharmacological activities [6-8]. Polyprenols have been indicated to prevent toxic liver and to maintain disturbed hepatic role [6]. Polyisoprenoid extract from selected mangrove leaves...
showed potential for the antimicrobial drug [7]. Furthermore, dolichol content from a mangrove plant, Nypafruticans leaves promises as a chemopreventive agent in colon cancer [8]. Despite the importance of polyisoprenoids from mangrove plants as potential sources of medicines have been described, search for new compounds are required. Our previous studies showed that polyisoprenoid content changed with age tissues [3-4]. Therefore, the present study aimed to describe new polyisoprenoid derivative compounds of probably epoxy polypreneol and epoxy dolichol from S. alba and S. caseolarisold leaves to extend our previous works on exploration for polyisoprenoids from mangrove plants.

2. Materials and methods

2.1. Plant materials
Old leaves of S. alba J. Sm. and S. caseolaris (L.) Engl. (Sonneratiaceae) were collected from Lubuk Kertang forest, Langkat, North Sumatra, Indonesia in August 2016. The sampling condition was depicted by mean temperature was 32-34 °C with a mean value of humidity of 75-78%. All collected samples were kept in the freezer (-20 °C) until used.

2.2. Separation of polyisoprenoidal alcohols
The separation of dolichols from dehydrodolichols or polypreneols used an existing protocol [5]. Briefly, old leaves from both mangrove plants were oven-dried at 65-70°C for 48 h. The dried-up tissue (5 g each) was homogenized into a fine grain and submerged in chloroform/methanol (2/1, v/v) solvent for two days. The crude lipid extract of leaves was saponified and then dissolved with hexane, and this organic solvent was evaporated and treated with hexane [3].

2.3. Investigation of epoxy polypreneol and dolichol using two-dimensional thin layer chromatography (2D-TLC)
Separation of polypreneol from dolichol was investigated by 2D-TLC [5-6]. This procedure involved the development of two kind plates; firstly, a silica gel plate was done with toluene-ethyl acetate (9:1) solvent system of as earlier reported [4]. The TLC plate was then developed vertically to transfer polypreneol or dolichol to the central area of the reversed-phase plate. The secondly, the reversed-phase plate was carried out with acetone for about 60 min. The spots of dehydrodolichols, dolichols, epoxy polypreneols, epoxy dolichols, and standard solutions are distinguished and then characterized and imaged with iodine vapor. The enhanced chromatographic was scanned using a Canon E-470 printer. The association of movement detected the polyisoprenoid on a plate with that of standards of dolichol or dehydrodolichol that were used in reversed-phase run as previously described.

2.4. Purification of epoxy polypreneol and epoxy dolichol compounds from S. caseolaris leaves
Dried leaves of S. alba (5 g) S. caseolaris (5.8 g) were cut into small pieces and placed in chloroform/methanol solvent for 48 h and saponified after adding 4 mL of a mixture containing KOH (0.45 g), ethanol (2 mL), and H2O (2 mL) at 65 °C for 24 h [4]. Non-saponifiable lipids were extracted with hexane, and the hexane extract (1.7 and 2.0 mg, respectively) was applied to a silica-gel 60 column (2.0 x 14 cm) that was equilibrated with toluene as previously reported [4]. All fractions of both species of 5 mL each were collected. Fraction 26 of S. alba and fraction 24 of S. caseolaris were identified as pure-dominating epoxy polypreneol and epoxy dolichol, respectively. Fractions 26 and 24, were pooled and evaporated to dryness to yield 1 mg and 1.2 mg, respectively and used for further structure investigations.

3. Results and Discussion
The exploration for polyisoprenoids compound from S. alba and S. caseolaris old leaves were performed using 2D-TLC as previously reported [9]. Figure 1 shows the occurrence of four family
compounds in the leaves of both species. In the *S. alba* old leaves (Figure 1A), having the existence of polyprenol, dolichol, epoxy polyprenol, and epoxy dolichol with a dominating pattern of polyprenol. It is noteworthy that predominance polyprenols over dolichols had analogous distribution in epoxy. The compound, epoxy polyprenols dominated over epoxy dolichol. A similar result obtained in the *S. caseolaris* old leaves (Figure 1B), displaying the presence of polyprenol, dolichol, epoxy polyprenol, and epoxy dolichol with a dominating carbon chain length of dolichol. Likewise, epoxy dolichol also dominated over epoxy polyprenol in *S. caseolaris* old leaves. The occurrence of epoxy dolichol only has been reported from skipjack tuna liver [10]. Recently it has been suggested that the presence of epoxy polyprenol, as well as epoxy dolichol in fish, may also be present in plants [11].

![Figure 1](image1.png)

**Figure 1.** 2D-TLC chromatograms of *S. alba* old leaves (A) and *S. caseolaris* old leaves (B)

![Figure 2](image2.png)

**Figure 2.** 2D-TLC chromatograms of *S. alba* (A) [3] and *S. caseolaris* old leaves (B) [4]

By contrast to this epoxypolyprenol nor epoxy dolichol, was detected in *S. alba* and *S. caseolaris* leaves (Figure 2). The presence of both polyprenols and dolichols was identified in *S. alba* leaves [3] with dominating of polyprenols, as a similar case with *S. alba* old leaves. In the *S. caseolaris* leaves found dominating dolichol, polyprenol, bombiprenone, and a new compound of polyprenyl acetone (Figure 2B). The polyprenyl acetone also was occurred in *Allium tuberosum*, *Boehmerianivea var. nipponnivea*, *Laurusnobilis*, and *Euphorbia supina* [11]. Polyprenyl acetone was
characterized in some mangroves such as Xylocarpus granatum and Aegiceras corniculatum [4]. The difference pattern and the composition of polyisoprenoid in fresh or old leaves might result from the age differences of the leaves or environmental condition [3-4].

![Figure 3](image1.png)

**Figure 3.** TLC chromatograms of *S. alba* and *S. caseolaris* old leaves fractions

![Figure 4](image2.png)

**Figure 4.** 2D-TLC chromatograms of purified fractions of *S. alba* (#26, A) and *S. caseolaris* (#24, B).

The profile of polyprenol, dolichols, epoxy polyprenol, and epoxy dolichol could be a new type (type VI) of polyisoprenoid pattern to extend previous findings. The polyisoprenoid distribution as previously described [3-4, 9] were categorized into five types (I, II, III, IV, and V). In type-I, the majority of dolichol over polyprenols (100%), type-II, the presence of both polyprenols and dolichols, type-III,
having a predominance of dolichol over polyprenol, type-IV, the occurrence of both polyprenyl acetone and dolichol, type –V, displaying polyprenol acetone, polyprenol, and dolichol. This present study adds a new kind of polyisoprenoid, type-VI, having polyprenol, dolichol, epoxy polyprenol, and epoxy dolichol [9, 12].

Figure 3 depicts TLC chromatograms of *S. alba* and *S. caseolaris* old leaves fractions. From this fraction, fraction number 26 was picked up from *S. alba*(Figure 4A), and fraction number 24 was taken from *S. caseolaris*. Figure 4 confirmed the pure epoxies both polyprenols and dolichols. These fractions showed epoxy polyprenol and epoxy dolichol, however, to confirm this finding, further investigation is needed to clarify the chemical structure such nuclear magnetic resonance (NMR) analysis and electrospray ionization (ESI) [10]. These findings suggested that occurrence epoxy polyprenol or epoxy dolichol in the old leaves of *S. caseolaris* may be due to the catalysis process from polyprenol and dolichol to be epoxy polyprenol and epoxy dolichol. This result was supported by [11, 13-15] that epoxy is more polar than alcohol and the presence of epoxy is due to isoprene residue. More recently, it has been reported that salinity changes the polyisoprenoid contents in salt-secretor and non-salt-secretor mangrove plants [16]. The changes of polyisoprenoids in salt-stressed mangrove including was in polyprenols and dolichols. Mangrove plants may be a prospective source of natural medicinal compounds, which may open up promising mangrove utilization.

4. Conclusion

The present study confirmed the profile and composition of polyisoprenoids from old leaves of *S. alba* and *S. caseolaris* which differed with those reported from *S. alba* and *S. caseolaris* leaves. The present study also indicated a new type of polyisoprenoid distribution, epoxy polyprenol, and epoxy dolichol and further research is needed to clarify the chemical structure of this new compound.

5. References

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