Distribution and occurrence of polyisoprenoids in rambutan 
(Nephellium lappaceum)

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Abstract. Rambutan (Nephellium lappaceum L., Sapindaceae) is a tropical plant having some biological activities. All parts of N. lappaceum either edible or non-edible part have shown some components beneficial to human health. Plant polyisoprenoids have been reported to have biological and pharmaceutical activities. Using a two-dimensional thin layer chromatography (2D-TLC), we analyzed composition and occurrence of polyisoprenoid alcohols (polyprenols and dolichols) in N. lappaceum consisting of leaves, roots, fruits, flowers, seeds, and rinds. The distribution of polyprenols and dolichols were detected and categorized into three types. Type-I, displaying a predominance of dolichols over polyprenols, was observed in the roots and flowers. Type-II, having the presence of both polyprenols and dolichols, was found in the fruits and seeds. Type-III, having the predominance of polyprenols over dolichols, was observed in the leaves. The diversity of polyisoprenoids in various tissues of N. lappaceum is the first report, suggested the chemotaxonomic importance of polyisoprenoids in this tropical fruit plant.

1. Introduction
Rambutan (N. lappaceum L.) is a native tropical fruit plant to Malaysia and Indonesia and grown throughout Southeast Asia, Australia, South America, and Africa [1]. The N. lappaceum trees distributed in the lower or middle story in different kinds of primary and secondary forests [2]. In Indonesia, there are several cultivars of rambutan with different characteristics, namely Rapiah, Narmada, Sinyonya, Binjai, Garuda, Kapulasan, Lebak bulus, Tangkue Lebak, Bahrang, and Sibongkok. Rambutan fruit is ovoid with red or yellow pericarp. It is covered with soft spines and varied in color from green, yellow, and red [1].

Rambutan has several biological activities derived from various tissues of rambutan either edible or non-edible part have been reported to contain some components beneficial to human health [3]. For example, fruits have astringent, stomachic, and anthelmintic; the leaves are used in poultices for headaches. Furthermore, rambutan fruit peels/rinds had as an antihyperglycemic activity, antidiabetic, anti-hypercholesterolemia activities, anti-inflammation, anticancer, antioxidant, and antibacterial [3-5].

Several studies have reported that plant polyisoprenoids play a significant role in controlling cholesterol level [6]. Polyprenols from Ginkgo biloba leaves to have antibacterial and antifungal activity [7]. Given the critical biological and pharmaceutical activities of rambutan as well as plant polyisoprenoids. Nonetheless, the distribution and occurrence of polyisoprenoid in N. lappaceum have
not been studied yet. To get more insight into the biological activities of polyisoprenoids, it is important to obtain the information of distribution and occurrence of polyisoprenoid in tropical fruit plants especially rambutan. Here we report the distribution and occurrence of polysioprenoids from edible and non-edible parts of *N. lappaceum* for the first time.

2. Materials and Methods

2.1 Chemicals

A mixture of dolichols (C_{90}-C_{95}) and polyprenols (C_{90}-C_{100}) standard compounds as previously described [8] was used to identify the polyisoprenoids in this study. The identification of the family corresponding to polyprenols or dolichols was performed in at least three experiments. Silica gel 60 TLC plates and reversed-phase silica RP-18 HPTLC plates were purchased from Merck (Darmstadt, Germany). All of the other chemicals and solvents were of reagent grade.

2.2 Plant materials

The leaves, roots, fruits, flowers, seeds, and rinds of rambutan (*N. lappaceum*) were collected from Binjai, North Sumatra, Indonesia, in September 2016. Rambutan is naturally growing in direct sunlight. The average temperature in the month of the collection was 29 °C with an average humidity of 74%. All of the fresh samples were kept at -20 °C until used.

2.3 Isolation of polyisoprenoid alcohols

A procedure for the isolation of polyisoprenoids as previously described [9]. The leaves, roots, fruits, seeds, flowers, and rinds were dried at 70°C for two days. The dried tissue (5 g each) was crushed into fine powder and immersed in 30 ml of chloroform/methanol (2/1, v/v) solvent for 48 h. The lipid extract of leaves, roots, fruits, flowers, seeds, and rinds was saponified at 65°C for 24 h in 50% ethanol containing 2 M KOH. The non-saponifiable lipids of each tissue sample extracted with hexane and the organic solvent were evaporated and redissolved in hexane.

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

First-dimension TLC was carried out for about 45 min on a silica gel glass plate (20 × 3 cm) with a solvent system of toluene-ethyl acetate (9:1) as previously described [9]. The longitudinal edge of the first dimension TLC plate was 1 cm in width, and the concentration zone of a reversed-phase C-18 TLC was clamped using two magnetic bars (4.0 × 1.1 × 0.8 cm) facing each gel phase. The bound TLC plate was then developed vertically to the first-dimension to transfer polyprenol or dolichol to the concentration zone of the reversed-phase TLC plate.

The second-dimension reversed-phase C-18 silica gel TLC was performed with acetone as the solvent for approximately 30 min. The spots of plants extract mixtures, and standard mixtures are separated and being developed by 2D-TLC was then identified and visualized with iodine vapor. The improved chromatographic images were obtained and digitally scanned with a Canon E-400 series printer. The polyisoprenoid family was determined by the comparison of mobility on TLC with that of authentic standards of dolichol or polyprenol that were applied in the second-dimension development. The polyprenols and dolichols that were detected on RP-18 HPTLC plates were quantified using ImageJ version 1.46r [10], with dolichol and polyprenol standards as references.

3. Results and Discussion

The results discussed in two subsections; they are the occurrence of polyisoprenoid and analysis polyisoprenoid by two-dimensional thin layer chromatography (2D-TLC).

3.1. Occurrence of polyisoprenoid

Table 1 shows the total lipid content of vegetative organs of *N. lappaceum* ranged from 509.5 to 786.5 mg g⁻¹ dry weight with the lowest and highest pressures in the rinds and leaves of *N. lappaceum*,
respectively. The total lipid content of generative organs ranged from 696.7 to 987.8 mg g\(^{-1}\) dry weight with the lowest and highest contents in the fruits and flowers of *N. lappaceum*, respectively. The number of polyisoprenoids was least in the leaves (42.3 mg g\(^{-1}\) dry weight), and the highest was in the seeds (110.2 mg g\(^{-1}\) dry weight). The lowest content of polyisoprenoids was in the rinds (509.7 mg g\(^{-1}\) dry weight) and the fruits (696.7 mg g\(^{-1}\) dry weight) (Table 1). Tables 1 and 2 summarize the occurrence and distribution polyenols and dolichols with the carbon-chain lengths given for each family.

Present results indicate that distribution of polyisoprenoid in rambutan varies depend on each tissue. In *N. lappaceum* leaves, polyenols were abundantly detected, this finding supported the previous report on the dominance of polyenols over dolichols in plant leaves [11-13], minor mangrove species, such as *Excoecaria agallocha*, *Heritiera littoralis*, and *Hibiscus tiliaceus* [8]. Shorter chain of polyenols has been characterized by the young and old rubber leaves [13], soybean leaves [14], young spinach leaves [15], and in the families of Lauraceae, Tiliaceae, and Magnoliaceae [16]. The occurrence of ficaprenols in this study and other plants above-mentioned suggested the localization of ficaprenols in the chloroplast [14-15]. The presence of phytol may be biosynthesized ficaprenols in this organelle [15].

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

The structural groups of polyenols and dolichols in the *N. lappaceum* tissues were classified as previously described [8-9] into three types (I, II, and III). In the tissues of roots, rinds, and leaves, type I, II and III was detected, respectively and on the other hand, in flowers, fruits, and seeds only type I and type II were found. In type-I, the predominance of dolichols over polyenols (nine-fold) was observed in root and flowers of *N. lappaceum* (Figure 1A and B).

Dolichol has known as dominating polyisoprenoids in the roots of plants, for example, young rubber roots [13], soybean roots [14], and the majority of mangrove plants [8-9]. A typical feature of polyisoprenoids is their occurrence in leaf tissues as a mixture of homologous, more complicated polyenols (such as ficaprenols; medium and longer prenols). By contrast, dolichols in the root family are enough "small" (6-8 dolichols) when accumulated in this tissue [17]. However, as has been reported recently [8-9] and in *N. lappaceum* seeds of the present study, dolichols also occurred as longer-chains in several mangrove leaves. For example, *Avicennia marina* (C\(_{65-130}\)), *Lumnitzera racemosa* (C\(_{60-140}\)), *Phempis acidula* (C\(_{50-140}\)), *Sonneratia alba* (C\(_{65-130}\)), *Acanthus ilicifolius* (C\(_{60-125}\)), *Aegiceras corniculatum* (C\(_{60-140}\)), and *S. caseolaris* (C\(_{50-120}\)).

### Table 1. Occurrence and distribution of polyisoprenoids in *N. lappaceum*

| Species         | Tissue   | TL (mg/g dw) | PI (mg/g dw) | Pol (mg/g) | Dol (mg/g) | % in total lipid | % in polyisoprenoid Type |
|-----------------|----------|--------------|--------------|------------|------------|-----------------|----------------------------|
| *N. lappaceum* leaves | 786.5    | 42.3         | 42.3         | nd         | 5.4        | 5.4             | nd                         |
| *N. lappaceum* roots   | 761.5    | 25.3         | nd           | 25.3       | 3.3        | 3.3             | nd                         |
| *N. lappaceum* rinds   | 509.7    | 22.0         | 9.1          | 12.9       | 4.3        | 1.8             | 2.5                        |
| *N. lappaceum* fruits   | 696.7    | 65.4         | 42.0         | 23.4       | 9.4        | 6.0             | 3.4                        |
| *N. lappaceum* flowers | 987.8    | 8.2          | nd           | 8.2        | 0.8        | 0.8             | nd                         |
| *N. lappaceum* seeds   | 808.2    | 11.0         | 45.9         | 64.2       | 13.6       | 7.9             | 41.7                       |

nd= not detected, TL = Total lipids, PI = Polyisoprenoids, Pol = Polyenols, Dol = Dolichols. Data are expressed as mean of triplicate analyses.

In seeds, a trace amount of polyenols with chain-lengths similar to those of dolichols was detected (Figure 1D). In type-II, the presence of both polyenols and dolichols was observed in rinds, fruits, and seeds of *N. lappaceum* (Table 2, Figure 1). In the fruits and rinds polyenols detected (ficaprenols and longer polyenols) with a chain-length similar to that of dolichols and also polyenols much longer than dolichols (>C\(_{10}\) and more) in chain-length were also detected, as shown
in Figure 1C and 1E. Similar results of type-II have reported in seeds of rubber plant, ginkgo, and pine [13]. The occurrence of both polyprenols and dolichols was also detected in *Coluria geoides* seeds [17]. In contrast to these observations, several species of gymnosperms contained only polyprenols no dolichols identified [18], however, soybean seeds contained only dolichols [14].

In a case of *N. lappaceum* flowers, the occurrence dolichols only were found. This result did not agree with previous reports on occurrence polyprenols only in rubber flowers [13], *Philesia magellanica* and *Fuchsia magellanica* flowers [19]. It is yet known, the accumulated dolichols detected only in *N. lappaceum* flowers and roots.

![Figure 1](image-url)

**Figure 1.** 2D-TLC chromatograms of polyisoprenoid extract from *N. lappaceum* roots (A), flowers (B), fruits (C), seeds (D), rinds (E), and leaves (F).

| Table 2. Carbon-chain lengths of polyprenol and dolichol of Rambutan |
|--------------------|-----------------|-----------------|-----------------|
| Species            | Tissue          | Polyprenol      | Dolichol        |
| N. lappaceum       | leaves          | 60 65           |                 |
| N. lappaceum       | roots           | 70 75 80 85     |                 |
| N. lappaceum       | rinds           | 60 65 70 75 80 85 | 70 75 80 85   |
| N. lappaceum       | fruits          | 65 70 75 80 85 90 95 100 | 75 80 85 90 95 100 |
| N. lappaceum       | flowers         | 85 90 95 100    |                 |
| N. lappaceum       | seeds           | 45 50 55 60 65 70 75 80 85 90 95 100 | 65 70 75 80 85 90 95 100 |
|                    |                 | 110 115 120 125 130 135 140 and more | 105 110 115 120 125 130 135 140 and more |

For type-III, the occurrence of polyprenols over dolichols (more than nine-fold) was observed in leaves of *N. lappaceum* as shown in Figure 1D, in these species contained shorter-chain polyprenols only, ficaprenol-type (C_{60}-C_{65}), and that dolichols and longer polyprenols are in the detectable level.

The predominance dolichol over polyprenol in some plant tissue, on the other hand, polyprenols dominated on polyprenols in this study and other reports remain unclear. It has been suggested that it
might be the result of changing the balance of specific compounds as biosynthesis end-products [12]. Furthermore, the predominance dolichols over polyprenols in mangrove leaves may be the presence active enzyme of polyprenol reductase [8,9], as consequence of tropical or sub tropical conditions. In this context, *N. lappaceum* is a tropical plant may have a similar pattern as mangrove plants.

4. Conclusion

The diversity of polyisoprenoids in various tissues of *N. lappaceum* is the first report, suggested the chemotaxonomic importance of polyisoprenoids in this tropical fruit plant. Present findings indicate that distribution of polyisoprenoids in rambutan varies depend on each tissue.

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