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Secretory Duct Structure and Phytochemistry Compounds of Yellow Latex in Mangosteen Fruit

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Yellow latex is the main problem in mangosteen agribusiness, because it is one factor lowering the fruit quality. The structure of yellow latex secretory ducts in the flower and fruit as well as in the root, stem and leaf of mangosteen (*Garcinia mangostana* L.) seedling and the qualitative phytochemistry of yellow latex were studied. The ducts were branched, canal-like type. They were found in the exocarp, mesocarp, endocarp, aril of the fruit, flower, stem, and leaf. In the fruit, the biggest diameter of the secretory ducts was found in the endocarp. There were continuous secretory ducts from fruit stalk to the fruit. Ultrastructural observation showed that the ducts surrounded by specific epithelial cells, which were living cells containing dense cytoplasm with plastid, mitochondria and golgi apparatus organelles. The qualitative test indicated that the yellow latex collected from stem bark, outer part of fruit, young fruit pericarp, mature aril and young aril contained terpenoid, flavonoid and tannin, but not alkaloid, saponin and steroid, except in the young aril containing the steroid.

Key words: secretory ducts, yellow latex, endocarp, aril, epithelial cells

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is one of the main commodities of Indonesian export, known as the Queen of Tropical Fruits. Even though the fruit has been exported, the avaibility of good quality fruit is still inadequate.

The quality of the fruit varies from one production center to an other, because mangosteen plantations are managed traditionally and its production system depends on the nature. One factor lowering the fruit quality is the yellow latex disease. Yaacob and Tindall (1995) suggested that the yellow latex is a physiological disorder which showed symptoms of yellow fruit aril. At present, the yellow latex is the main problem in mangosteen agribusiness, since it does not only damage the appearance and cleanliness of outer part of fruit but also makes the aril bitter. The absence of the yellow latex is one of the criteria of mangosteen fruit to be exported to East Asian countries (Taiwan, Japan, and Korea) and Middle East countries (United Arab Emirates, Saudi Arabia, and Kuwait).

The exact causal agent of yellow latex disease has not been known yet. It is assumed that yellow latex is the natural latex found in mangosteen fruit similar to the latex found in the twig, petiole, leaf, and stem bark of Guttiferae family. The whole parts of the plant will excrete yellow exudate whenever wounded. Gamboge as resin exudate found on various plants from the family of Guttiferae originated from the broken resin canal/duct (Asano *et al*. 1996; Pankasemsuk *et al.* 1996). Any physical damage to the latex vessels could be caused by excessive watering after drought, punctures by sucking insects (capsids), strong wind, rough harvesting, and handling (Verheij 1992). The yellow latex in the mangosteen fruit may be excreted by unknown secretory tissues. Therefore, research concerning tissue or structure producing yellow latex is necessary to be carried out.

The continuity of secretory duct in the fruit and the fruit stalk is also necessary to be examined. The yellow latex found in aril may be excreted from endocarp, therefore, the density, and the size of the yellow latex secretory ducts in the endocarp is necessary to be studied. Research on compounds isolated from mangosteen leaf and pericarp has been conducted (Parveen *et al*. 1991; Ketsa & Atantee 1998). Whereas research on those from the yellow latex originated from mature and young aril, young fruit pericarp, outer part of fruit, and stem bark has never been reported. Therefore, the phytochemistry compounds of the yellow latex on those parts will also be conducted.

MATERIALS AND METHODS

Fruit sampling in the field was carried out on the production center of mangosteen in Cengal, Karacak village (6°60'S and 106°60'E, 490 m above sea level with an annual rainfall appoximately 1,515 mm), Leuwiliang subdistrict, Bogor regency, Indonesia. Ten of 20-years-old productive mangosteen trees were used in this research.

Sampling. Fruit samples were chosen randomly from the trees of mangosteen for further fruit anatomical study. Anatomical study of the fruits were examined in different development stages from buds until mature fruits. Each week

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ten flowers and fruits were taken routinely started one week before anthesis until 105 days after anthesis (DAA). There were 17 times observation namely -7, 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105 DAA.

Histological Study Using Light Microscope. Observation of yellow latex secretory structure of the fruit samples was carried out on pericarp and fruit stalk. As comparison yellow latex secretory ducts in root, stem and leaf of one-month-old mangosteen seedlings were also observed. Microscopic slides of cross and longitudinal sections were prepared by using paraffin method (Johansen 1940). The fruits were fixed in FAA solution (5 ml formaldehyde, 5 ml acetic acid glacial, 90 ml 70% alcohol). Dehydration and infiltration process was conducted by soaking the samples in Johansen series solutions. The samples were sectioned 10 µm thick by using rotary microtome. Then double staining using 2% safranin and 0.5% fast-green was carried out. Characters observed were distribution, density (number/mm^2), and diameter of yellow latex secretory ducts (µm). Observations under light microscope were carried out on five view area with three replication.

Histological Study Using Transmission Electron Microscope (TEM) was Prepared as Follows. The aril and the mesocarp tissues of 28 DAA fruit were fixed in 5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, temperature 4 ^oC for 24 hours, then postfixed in 2% osmium tetroxide in the same buffer 4 ^oC for 2 hours. The tissues were dehydrated in a gradual series of ethyl alcohol and infiltrated by propylene oxide and embedded in Spurr's resin. Ultrathin section (70 nm thick), cut with the ultracut Reichert ultramicrotome using a diamond knife and stained with 2% uranyl acetate and 4% triple lead and then were observed with a JEM 1010 transmission electron microscope at 80 kV.

Determination of Phytochemistry Compounds on the Yellow Latex. The yellow latex samples collected from mature and young aril, young fruit pericarp, outer part of fruit, and stem bark were analyzed qualitatively to check the presence of triterpenoid, flavonoid, tannin, alkaloid, and steroid compounds following the methods used by Harborne (1987). Availability test of triterpenoid and steroid used Lieberman-Burchard reactant (anhydrous acetic acid + concentrated H_2SO_4 + alcohol). Red indicated positive reaction for triterpenoid, green showed positive reaction for steroid. Flavonoid test used Mg powder several drops of concentrated HCl and amyl alcohol. Orange layer of amyl alcohol showed positive reaction of flavonoid. To examine the existence of tannin, 10% Fe Cl_3 was added to the filtrate. Greenish black indicated positive reaction of tannin, while saponin content showed positive reaction if the filtrate was strongly shaken and stable foam appeared. The existence of alkaloid compounds showed positive reaction if there were orange, white, and brown sediments successively after being reacted with Dragendrof, Mayer, and Wagner reactants.

RESULTS

Distribution of the Yellow Latex Secretory Ducts in Mangosteen Fruit. The ducts were found on flower bud (-7 DAA) and anthesis (0 DAA), in their ovaries (Table 1). The ducts were also found in young fruit stages (7-35 DAA), medium fruit stages (42-70 DAA), and mature fruit stages (77-105 DAA). In those stages, the ducts were found on the fruit pericarp either on exocarp, mesocarp or endocarp. The ducts were also seen in the fruit aril (Figure 1). The more mature was the fruit the lower the density of the ducts was on the fruit mesocarp. The less dense the ducts found, the bigger was the diameter of the ducts (Table 1).

Based on the cross section of the fruit pericarp, the structure type of the ducts consisted of a big lumens surrounded by specific epithelial cells (Figure 2). While on the longitudinal section of its pericarp, the ducts were elongated and branched (Figure 3). The yellow latex appeared on the aril when the fruit was 14-weeks-old. This can be seen on the damage of epithelial cells (Figure 4). Therefore, it can be said that the yellow latex seen on the aril originated from the endocarp secretory ducts.

The Yellow Latex Secretory Ducts in the Fruit Stalk. The result of longitudinal section of fruit and stalk indicated that the structure ducts in the fruit stalk was continuous with those of the fruit (Figure 5). The ducts in the fruit stalk were found in the cortex and among the vascular bundles. The diameter of the ducts among the vascular bundles was bigger than that in the cortex, which were 30-162.5 µm and 30-100 µm respectively.

The Yellow Latex Secretory Ducts in the Seedling. The objective of yellow latex secretory duct observation in one month seedling after sowing was to study the continuity of the yellow latex secretory ducts. The ducts were not found in the root. The observation of the ducts in the stem was carried out on various positions from one cm from the soil surface

Table 1. Diameter (µm) and density (number/mm^2) of yellow latex secretory ducts on various stages of mangosteen on ovary of the flower and pericarp of the fruit

Stages	Yellow latex secretory ducts diameter (µm)				Density* (number/mm^2)
	Outer ovary/exocarp	Mesophyl ovary/mesocarp	Inner ovary/endocarp	Aril	
Flower					
Bud	10.0-17.5	25.0-43.5	30.0- 67.5	-	-
Anthesis	12.5-27.5	31.3-68.8	35.0- 75.0	-	57.7-96.3
Fruit					
Young	22.5-50.0	56.3-112.5	50.0-145.0	25.0-100.0	8.3-20.5
Medium	27.5-67.5	62.5-168.8	62.5-190.0	45.0-112.5	6.5-7.6
Mature	30.0-82.5	67.5-175.0	112.5-262.5	45.0-137.5	5.1-6.3

*Yellow latex secretory ducts in mesophyl ovary or mesocarp.

(Figure 6a) until the first leaf. On the position a the ducts were found only in the cortex and were not found in the pith. Whereas on the position b and c (Figure 6) of the stem the yellow latex secretory ducts were found both in the cortex and the pith. In the stem cortex were found around 12-16 initial cells forming the ducts. The initial cells were easily differentiated from the parenchyma cells since the cells were smaller. The diameter of ducts in the stem cortex were approximately 17.5-50.0 µm while in the pith was around 17.5-30.0 µm. The ducts were found on the first leaf of seedling one month after germination. In the leaf the ducts were found in the parenchymal tissues of main vein leaf with around 30.0-37.5 µm in diameter. In the leaf mesophyl the ducts were also found in the intercellular spaces among pallisade and spongy cells, with the diameter of 17.5-37.5 µm and 25.0-37.5 µm, respectively.

Ultrastructure of the Yellow Latex Secretory Ducts in the Fruit. Secretory duct initials had large vacuole, were densely cytoplasmic layer containing a lot of mitochondria,

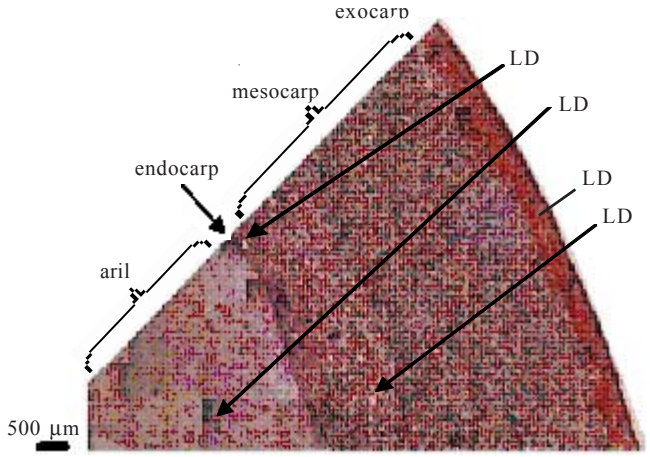

Figure 1. Cross section of mature mangosteen fruit. LD: yellow latex secretory ducts.

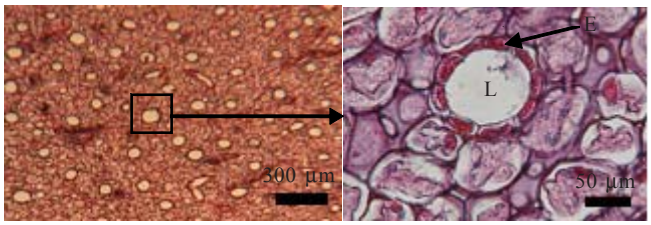

Figure 2. Yellow latex secretory ducts on cross section of mangosteen fruit mesocarp. L: lumen, E: epithelium cell.

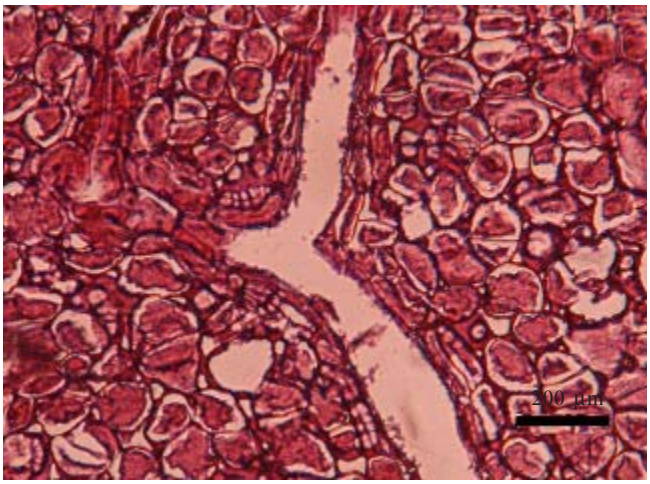

Figure 3. Elongated and branched yellow latex secretory ducts on longitudinal section of mangosteen fruit mesocarp.

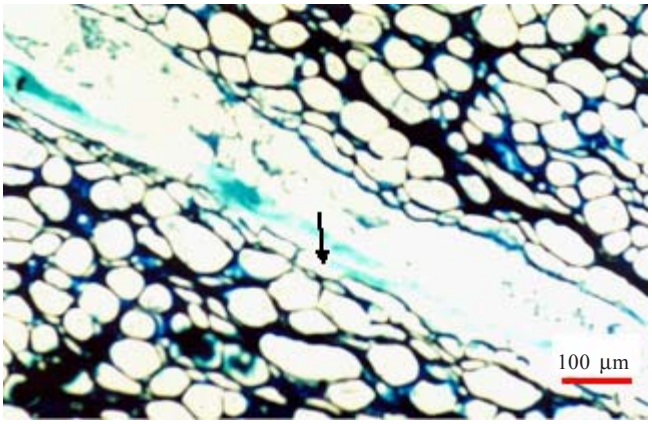

Figure 4. Damaged epithelial cells in yellow latex secretory ducts on longitudinal section.

Figure 5. Longitudinal section of the stalk and the base of mangosteen fruit.

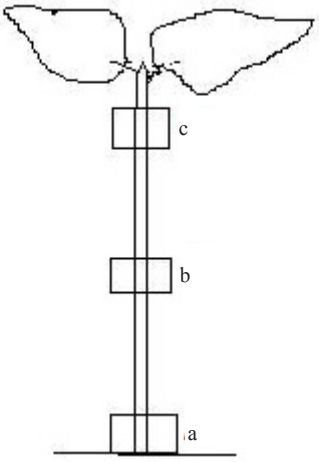

Figure 6. Seedling stem of mangosteen. a: 1 cm, b: 5 cm, c: 9 cm from the ground.

with thicken cell wall (Figure 7a). The ducts were surrounded by specific epithelial cells. These epithelial cells were living cells containing densely cytoplasmic organels with plastid, mitochondria and golgi apparatus organelles (Figure 7b,c). Around the epithelial cells of aril secretory duct were found neighbour cells with latex particles (Figure 7d).

The Yellow Latex Compounds Phytochemistry. The result of qualitative test of phytochemistry compounds of the yellow latex collected from stem bark, outer part of fruit, young fruit pericarp, mature aril, and young aril indicated those samples showed positive reaction for triterpenoid compound, flavonoid and tannin (phenolic) compounds. On the other hand those samples showed negative result for alkaloid, saponin (phenolic), and steroid compounds, except in the young aril the stroid compound showed positive reaction (Table 2).

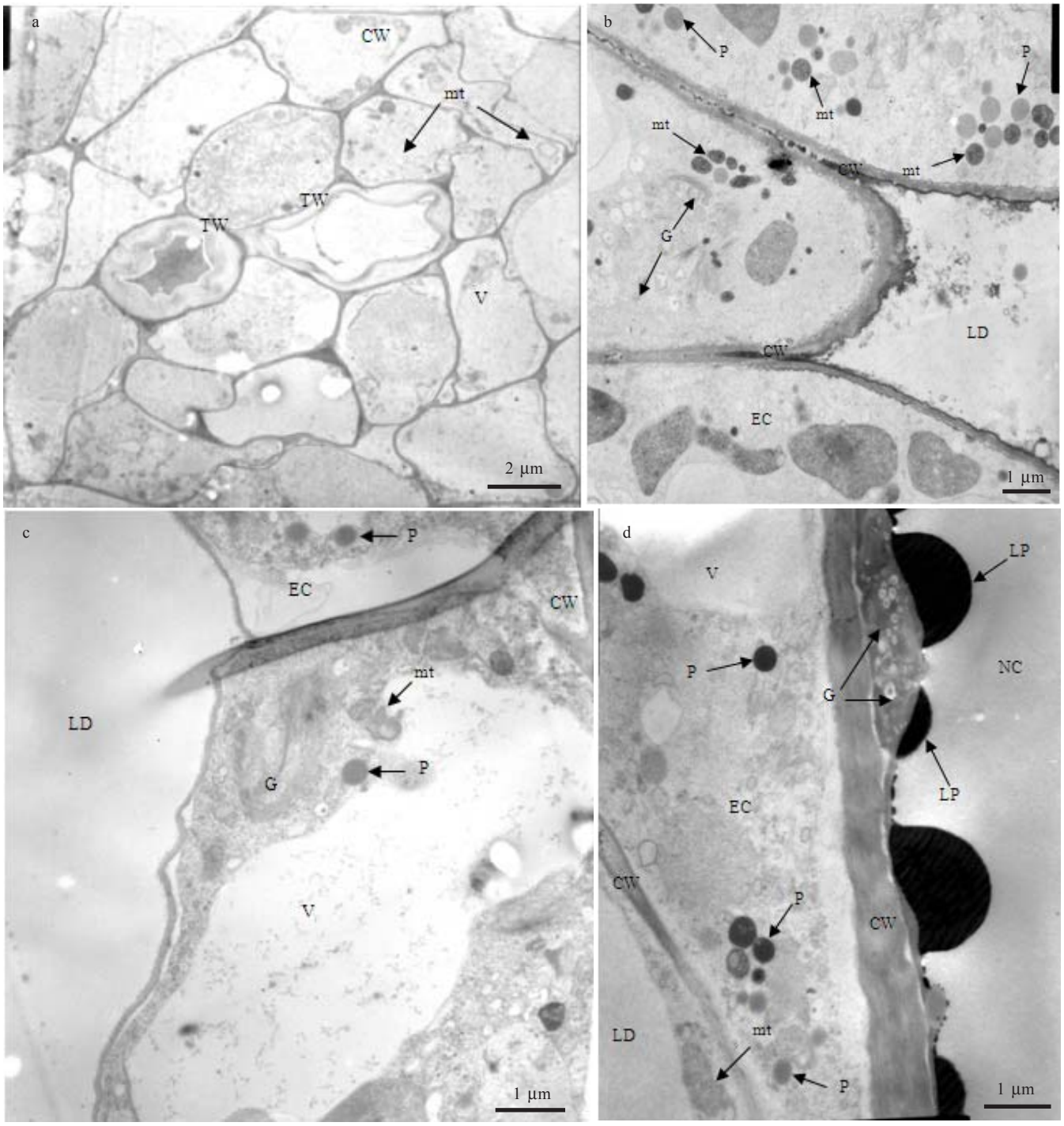

Figure 7. TEM micrographs cross section of yellow latex secretory ducts. a. Secretory duct of aril initial cells, b. Secretory duct of aril epithelial cells, c. Secretory duct of mesocarp epithelial cells, d. Secretory duct of aril neighbour cells, mt: mitochondria, CW: cell wall, V: vacuole, TW: thickened cell wall, EC: epithelium cell, LD: yellow latex duct, P: plastid, G: golgi apparatus, NC: neighbour cell, LP: yellow latex particles.

Table 2. Qualitative test of mangosteen yellow latex phytochemistry compounds

Compound test	Stem bark	Outer part of fruit	Young fruit pericarp	Mature aril	Young aril
Triterpenoid	+	+++	++	++	*
Flavonoid	+	+	++	+	+
Tannin	++	+	+++	+	+
Alkaloid	-	-	-	-	-
Saponin	-	-	-	-	-
Steroid	-	-	-	-	+

+++: high, ++: medium, +: low, -: not detected, *Triterpenoid test: -, terpenoid histochemistry test: +.

The highest concentration of triterpenoid was found in the latex collected from the outer part of fruit, while the flavonoid and tannin collected from the young fruit pericarp latex were the highest.

DISCUSSION

The structure of yellow latex secretory ducts found in the bud and flower ovaries, pericarp and aril of the fruit compared with that found in the fruit stalk, stem and leaves of mangosteen seedling were similar. It consisted of big lumen surrounded by specific epithelial cells. Although mangosteen produces yellow latex the structure of its ducts is not laticifer tissue. Instead, it was branched canal-like type which possibly belongs to schizogenous secretory space (Esau 1974).

Our study showed that the ducts were found in the stadia of bud (-7 DAA) and anthesis (0 DAA). This is in accordance with Rai *et al*. (2006) research that reported on the six days before anthesis stage, aril segments have already begun developed.

The yellow latex seen in the aril and on the outer part of the fruit originated from the damaged epithelial cells of its ducts in the pericarp. Therefore, the yellow latex may not be bacterial exudate. This contradicts to Nurcahyani (2005) stating that *Corynebacterium* spp. Was associated with the yellow latex in mangosteen fruit. The cell wall of the ducts in the endocarp was broken and excreted the yellow latex due to physiological disorder as a result of the fluctuated soil water during the fruiting stage so that there is turgor pressure change (http//www.pustaka-deptan.go.id/inovasi/kl070102.pdf). The damage of the ducts may also be related to the lack of calcium in the cell wall. Callan (1986) stated that calcium deficiency in sweet cherry tended to cause fruit splitting. Yellow latex spots on the outer part of the fruit were caused by mechanical disturbance such as puncture, insect bite, collision, and careless harvesting (Verheij 1992; http//www.pustaka-deptan.go.id/inovasi/kl070102.pdf.).

The yellow latex secretory ducts in mangosteen were similar to those in *Mammillaria heyderi* (Cactaceae) wich have a big lumen surrounded by epithelial cells (Wittler & Mauseth 1984). Systematic study for the types and ontogeny of the ducts in mangosteen is very limited. The duct type on several plants from the same family are not always similar. For example, in *Euphorbia marginata* the laticifer type was non articulated laticifer (Mahlberg 1959; Mahlberg & Sabharwal 1967) while in *Hevea* sp. of the same family, Euphorbiaceae, the laticifer type was articulate (Hao & Wu 2000). Therefore, the laticifer type may not always indicate the taxonomic relationship. The initial cells forming the ducts in mangosteen are 16-26 cells, in *Nerium oleander* and *E. marginata* were 28 and 12 cells respectively (Mahlberg 1961; Mahlberg & Sabharwal 1968), whereas in *Jatropha dioca* 5-7 cells (Cass 1985). The initial cells in *Mammea americana* were found in mesophyl ovary (Mourao & Beltrati 2000), while in mangosteen they were seen in the stem cortex of seedling. According to Behnke and Hermann (1978) articulated laticifers in *Gnetum gnemon* were very commonly found in the cortex and the pith of the stem.

The distribution of non-articulated branched laticifers in the *Euphorbia supina* were found in the bundle-sheath of leaf main vein (Rosowski 1968; Monacelli *et al*. 2005), intercellular spaces of pallisade and sponges cells (Rosowski 1968). It is similar to the distribution of the ducts found in the first leaf of mangosteen seedling.

The ultrastructure of the yellow latex secretory ducts in mangosteen is interesting: the duct initial cell had large vacuole with thicken cell wall. Wheareas the epithelial cells contained dense cytoplasm with plastid, mitochondria and golgi apparatus organelles with a thin cell wall. According to Monacelli *et al*. (2005) the surrounding laticifer cells showed empty vacuoles and plastids rich in starch. The first indication of gum duct initiation was the differentiation of densely cytoplasmic secretory cells in the mesocarp vascular parenchyma (Rachmilevitz & Fahn 1982; Morrison & Polito 1985). Nesller and Mahlberg (1978) reported endoplasmic reticulum, predominantly the rough form with ribosomes attached to its surface, was observed distributed throughout the densely packed cytoplasm of laticifer initials. According to Wittler and Mauseth (1984) mitochondria and lipid bodies were common in young duct cells but ER is rare.

Laticifers of higher plants were known to accumulate various kinds of useful secondary metabolites. The yellow latex of mangosteen contained triterpenoid, flavonoid and tannin compounds, which may protect the plant against herbivores and parasites (Harborne 1987; McGarvey & Croteau 1995). Monacelli *et al*. (2005) reported in *Camtotheca acuminata* Decne (Nyssaceae) that the main compounds accumulated in the latex were flavonoids and tannins. Whereas resin in *Norway spruce* (Pinaceae) did not only contain polyphenollic compound but also terpenoid (Nagy *et al*. 2000; Martin *et al*. 2002). Terpenoids and flavonoids are found in *Salvia candidissima* (Topcu *et al*. 1995) According to Behnke and Herrmann (1978) latex of *G. gnemon* also contained terpenoid likewise triterpenoid, tannin, and flavonoid (phenollic) were also found in the mangosteen yellow latex. Phytochemistry test the latex indicated negative result to alkaloid. It was different from *Papaver somniferum* plant accumulating benzylisoquinoline alkaloid in the multinucleate cytoplasm of specialized laticifers that accompany vascular tissues throughout the plant (Samanani *et al*. 2006). Test for resins, essential oils, and tannins gave different responses in different parts of *Hypericum perforatum* plant (Ciccarelli *et al*. 2001; Soelberg *et al*. 2007). Parveen *et al*. (1991) isolated and characterized triterpenoid

compound from *G. Mangostana* leaves. Furthermore Ketsa and Atantee (1998) reported that mangosteen pericarp contained phenollic and lignin. Other chemical studies in mangosteen have identified, such as xanthones, and benzophenones, which were of interest from a pharmacological point of view (Parveen & Khan, 1988; Chairungsrilerd *et al*. 1996; Gopalakrishnan & Balaganesan 2000; Moongkarndi *et al*. 2004; Nilar *et al*. 2005).

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