

**Chemical constituents of leaves of *Rhizophora x lamarckii*, *R. apiculata* and *R. stylosa*****Background**

*Rhizophora x lamarckii* is the sterile hybrid of *R. apiculata* and *R. stylosa* (Tomlinson, 1986; Chan, 1996). In the Indo-West Pacific region, the hybrid is widespread and shares many characters of its parents (Duke, 2006). Morphological features which distinguish the hybrid from its parents are shown in Table 1.

This study is a follow-up from Chan (1996) who reported the co-existence of *R. x lamarckii* (Fig. 1) and its parents on Pulau Burung (2°32'N, 101°47'E), an islet at Port Dickson, Negeri Sembilan, Malaysia. For the first time, chemical constituents of leaves of the hybrid have been compared with those of its parents.

**Methodology**

Two healthy mature leaves from three trees of *R. x lamarckii*, *R. apiculata* and *R. stylosa* on Pulau Burung were collected, kept in sealed plastic bags and brought to the laboratory for analysis.

Leaves were extracted with 50% MeOH and analysed using an Agilent Technologies 1200 Series reverse-phase HPLC with a Thermo Scientific BDS Hypersil Phenyl Column (4.6 x 100 mm).



Fig. 1. Young inflorescences of *Rhizophora x lamarckii*

A 15-min linear gradient from 5% to 100% MeOH was used to elute the samples at 1 ml/min. Mobile phases were acidified with 0.1% trifluoroacetic acid for better resolution. Elution was monitored at 280 nm.

Chromatograms of leaves of the hybrid and its parents were compared in terms of similarities and/or differences in chemical constituents and peak areas.

Table 1. Morphological features of *Rhizophora x lamarckii*, *R. apiculata* and *R. stylosa* (Chan, 1996; Duke, 2006)

Plant part	<i>R. x lamarckii</i>	<i>R. apiculata</i>	<i>R. stylosa</i>
Bark	Gray with fissures	Dark gray with shallow fissures	Reddish brown with no fissures
Leaf	Stalk 2-3 cm, midrib light green at under surface, blade obovate-elliptic, apex with clear mucronate spike, dense black dots at under surface	Stalk 1.5-3 cm, midrib with pinkish tinge at under surface, blade elliptic oblong to sub-lanceolate, apex with extended tip, fine black dots at under surface	Stalk 2.5-3.5 cm, midrib pale green at under surface, blade broadly elliptic, apex with prominent mucronate spike, prominent black dots at under surface
Inflorescence	Branched 1-2 times with 2-4 buds borne on short peduncle	Always with single pair of buds, borne on stout peduncle	Branched 2-4 times, 4-8 buds borne on elongated peduncle
Flower	Petals slightly hairy, style 2-3 mm	Petals glabrous, style 1 mm	Petals hairy, elongated style 4-5 mm
Fruit	Sterile	Brown when ripe, 2-2.5 cm	Brown when ripe, ovate, 2 cm
Propagule	Sterile	Hypocotyl cylindrical, club-shaped with blunt tip, up to 30 cm, collar red in colour	Hypocotyl cylindrical, warty with pointed tip, up to 30 cm, collar yellow in colour

## Results and discussion

Results showed that the chromatogram (280 nm) of leaves of *R. x lamarckii* had great similarity with those of *R. apiculata* and *R. stylosa* (Fig. 2). Based on areas of four peaks, values of the hybrid were generally intermediate to those of its parents (Table 2). Total values were 14,276, 6,771 and 20,309 mAU\*s for *R. x lamarckii*, *R. apiculata* and *R. stylosa*, respectively.

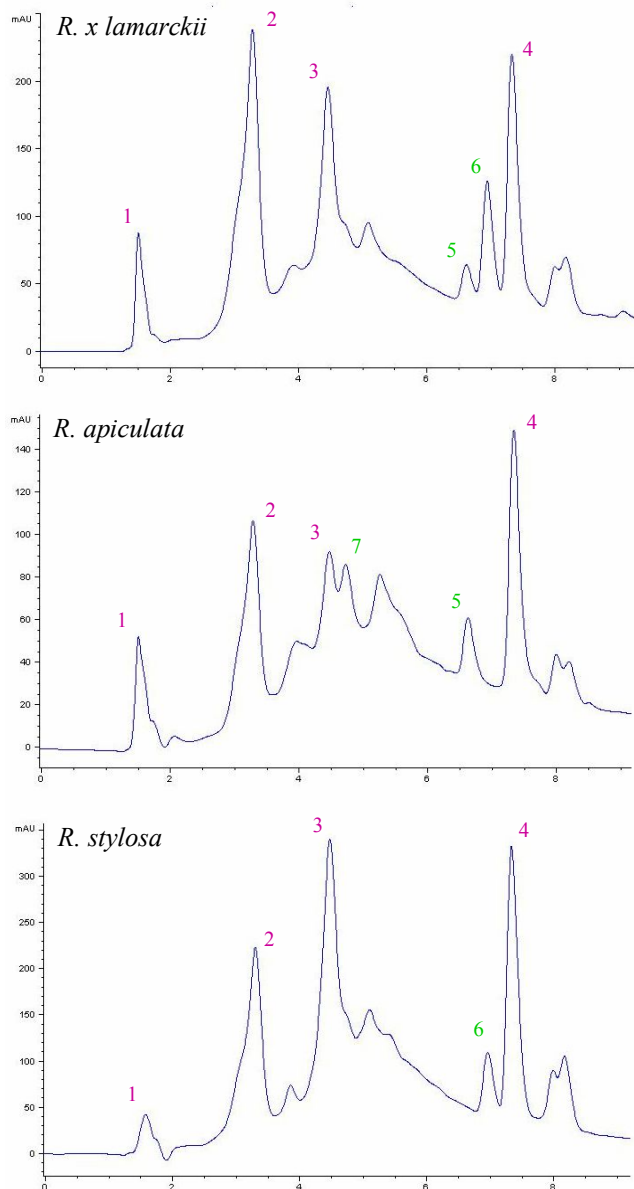


Fig. 2. Chromatograms (280 nm) of leaves of *Rhizophora x lamarckii*, *R. apiculata* and *R. stylosa*

It is interesting to note that peak 5 of *R. x lamarckii* was found in *R. apiculata* but not in *R. stylosa* and vice versa for peak 6. *R. apiculata* had an additional peak 7 that was not detected in *R. x lamarckii* and *R. stylosa*.

Table 2. Peak areas of leaf chromatograms of *Rhizophora x lamarckii*, *R. apiculata* and *R. stylosa*

Peak	Area (mAU*s)		
	<i>R. x lamarckii</i>	<i>R. apiculata</i>	<i>R. stylosa</i>
1	927	665	737
2	5,334	2,352	5,689
3	5,068	1,594	8,910
4	2,947	2,160	4,973
Total	14,276	6,771	20,309

Spiking with commercial phenolic compounds showed that peak 4 was rutin (quercetin rutinoside). Using NMR spectroscopy, Kandil *et al.* (2004) had earlier reported that leaves of *R. mangle* contained at least seven flavonoid glycosides. Five of them were based on quercetin of which one of them was rutin.

## Conclusion

There was great similarity in chemical constituents of leaves of the hybrid compared to those of its parents. Peak areas were intermediate in *R. lamarckii* with higher values in *R. stylosa* and lower values in *R. apiculata*. Two peaks in the hybrid were shared with only one of the parents. One of the compounds identified in *R. x lamarckii*, *R. apiculata* and *R. stylosa* was rutin. Mangrove hybrids are rare and every effort has to be made to conserve Pulau Burung which is regularly visited by tourists.

## References

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