ISSN 1880-7682
Volume 13, No. 4 **August 2015**

ISME/GLOMIS Electronic Journal

An electronic journal dedicated to enhance public awareness on the importance of mangrove ecosystems

Botany, uses, chemistry and bioactivities of mangrove plants I: *Rhizophora stylosa*

Mami Kainuma[1], Mio Kezuka[1], Tomomi Inoue[2], Eric W.C. Chan[3], Joseph Tangah[4], Shigeyuki Baba[1] & Hung Tuck Chan[1]

[1] *Secretariat, International Society for Mangrove Ecosystems, c/o Faculty of Agriculture, University of the Ryukyus, Okinawa, 903-0129 Japan (e-mail: isme@mangrove.or.jp)*

[2] *Centre for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Onogawa, Tsukuba 305-0053, Japan*

[3] *Faculty of Applied Sciences, UCSI University, Cheras 56000, Kuala Lumpur, Malaysia*

[4] *Forest Research Centre, Sabah Forestry Department, Sandakan 90009, Sabah, Malaysia*

Introduction

Mangrove species of *Rhizophora* (*R. apiculata*, *R. mucronata* and *R. mangle*) are endowed with chemical constituents of terpenoids, flavonoids, phenolic acids and steroids (Wu *et al*., 2008; Nebula *et al*., 2013). They possess antioxidant (Bunyapraphatsara *et al*., 2003; Sánchez, *et al*., 2006; Rahim *et al*., 2008; Ravikumar & Gnanadesigan, 2012), antibacterial (Melchor *et al*., 2001; Howlader *et al*., 2013; Annapoorani *et al*., 2013), anti-viral (Premanathan *et al*., 1999), anti-ulcer (Perera, *et al*., 2001; de Armas, *et al*., 2005; Berenguer *et al*., 2006; de-Faria, *et al*., 2012), analgesic (Rohini & Das, 2009; Howlader, *et al*., 2013), anti-inflammatory (Rohini & Das, 2009, 2010; Prabhu *et al*., 2012), cytotoxic (Prabhu *et al*., 2012; Howlader, *et al*., 2013), anti-diabetic (Ramanathan *et al*., 2008; Alikunhi *et al*., 2012), diuretic (Howlader, *et al*., 2013), hepato-protective (Ravikumar & Gnanadesigan, 2012.), anti-diarrheal (Rohini & Das, 2010), and wound healing (Fernandez *et al*., 2002; de Armas *et al*., 2005) properties.

Metabolites and bioactivities of Rhizophoraceae including *Rhizophora stylosa* have only been reviewed by Nebula *et al*. (2013). In a review of natural products from mangrove flora by Wu *et al*. (2008), information on *R. apiculata* and *R. mucronata* was presented but not on *R. stylosa.* In this note, the chemistry and bioactivities of *R. stylosa*, which are lesser known, are updated with some description of its botany and uses. Results of a DNA analysis of *R. stylosa* from Kiribati are included.

Botany and Uses

Rhizophora stylosa Griff. occurs on sand flats or rocky promontories fronting the sea (Chan, 1996; Chan & Wong, 2009; Nilus *et al*., 2010; Ng & Chan, 2012a). Under such exposed conditions, trees have gnarled growth with no clear bole and extensive prop roots looping outwards (Figure 1, left).

In young trees, the bark is reddish brown while in old trees, the bark is greyish black and fissured. The leaf blade is broadly elliptic, apex with prominent mucronate spike, pale green midrib and clear black dots at the under surface. (Figure 1, right). Inflorescences are branched 2−4 times with 4−8 cream-colored buds borne on elongated peduncle. Flowers have four white petals with woolly margins and elongated style of 4−5 mm. Fruits are ovate and brown when ripe. Hypocotyls are cylindrical, warty with pointed tip, 30 cm in length, and collars are bulbous and yellow in colour.

Figure 1 Tree (left), and leaves and reproductive parts (right) of *Rhizophora stylosa*

Natural hybrids of *R. stylosa* are not uncommon. Examples are *Rhizophora* x *lamarckii*, a cross between *R. stylosa* and *R. apiculata* (Chan, 1996; Ragavan *et al*., 2011; Ng & Chan, 2012b), and *Rhizophora* x *mohanii*, a cross between *R. stylosa* and *R. mucronata* (Ragavan *et al*., 2015).

Uses of *R. stylosa* in traditional medicine have not been reported. The dyeing cotton fabric (*kusaki-zome*) using tannin extracted from the bark of *R. stylosa* (Figure 2) remains an important cottage industry on Iriomote island, Japan (Baba, 2004; Baba *et al*., 2013). On Iriomote and Ishigaki islands, seedlings of *R. stylosa* are sold in souvenir shops to tourists as ornamentals. In the island countries of the Pacific, the species is used as fuelwood. In Australia, the aborigines use its wood to make boomerangs, spears and ceremonial items (Giesen *et al*., 2007).

Figure 2 Bark of *Rhizophora stylosa* (left) and dyed cotton fabric (right)

During a visit to the Republic of Kiribati in October 2013, several ISME staff members and Japanese volunteers encountered trees of *R. stylosa* with golden yellow buds and flowers, growing amongst normal *R. stylosa* trees on Tarawa atoll (Figure 3, left). Tentatively named *Rhizophora stylosa* var. *aureus*, the variety has golden yellow flowers including sepals, petals, ovary and style. Leaves are yellowish green with a pale yellow midrib, while retaining features of the elongated style of flowers, prominent mucronate spike at the leaf apex and clear black dots at the under surface of leaves (Figure 3, right). Propagules have slightly shorter hypocotyls and inconspicuous reddish collars.

Figure 3 Buds and flowers (left), and leaves and reproductive parts (right) of *Rhizophora stylosa* var. *aureus* in comparison with *R. stylosa*

From Tarawa, leaf samples of *R. stylosa* and *R. stylosa* var. *aureus* were collected for DNA analysis. Leaves of *R. stylosa* were also sampled from Butaritari, another atoll of Kiribati, for comparison. Genomic DNA was extracted from leaves of *R. stylosa* and *R. stylosa* var. *aureus* from Tarawa, using the Qiagen DNeasy Plant Mini Kit following the manufacture's protocol.

The starch-branching enzyme II (SBE 2) gene, known to distinguish *R. stylosa*, *R. mucronata* and *R. apiculata* (Inomata *et al*., 2009; Ng *et al*., 2013), were amplified by PCR and sequenced following the protocol developed by Ng & Szmidt (2013) with modification. Results showed that the SBE 2 sequences were 100% identical, supporting the morphological information that they are likely of the same species (accession # KT 198728 and KT 198730). Likewise, the sequences of the phenylalanine ammonia lyase 1 (PAL 1) gene were also identical (data not shown). More study is needed to determine the genetic determinants for the colour difference.

The alignment of differential nucleotide positions of the SBE 2 gene from *Rhizophora* species of Kiribati and the Malay Peninsula are shown in Table 1. In the Indo-West Pacific region, 1175-G, a nucleotide that is specific to *R. stylosa*, has been used to distinguish the species from *R. mucronata* and *R. apiculata* with 1175-T nucleotides (Ng *et al*., 2013, 2015). However, we have found that *R. stylosa* from Tarawa had T in position 1175 of the SBE 2 gene sequences. The result was consistent with *R. stylosa* from Butaritari. In addition, the SBE 2 gene of the *R. stylosa* genome from Kiribati cannot be PCR-amplified by the 1175-T containing primer. This demonstrates localized genetic variation between *R. stylosa* from Southeast Asia and the Pacific islands, and this warrants further study of more genetic loci.

Table 1 SBE 2 gene position of *Rhizophora* species from Kiribati and Peninsular Malaysia

	336	387	389	428	429	498	603	635	637	677	732	911	1013	1042	1122	1123	1175	1253
[1]	T	C	T	T	G	C	G	A	A	C	A	T	G	G	G	C	T	T
[2]	T	C	T	T	G	C	G	A	A	C	A	T	G	G	G	C	T	T
[3]	T	C	T	T	G	C	G	A	A	C	A	T	G	G	G	C	T	T
[4]	T	C	T	T	G	C	G	A	A	C	A	T	G	G	G	C	G	T
[5]	T	C	G	G	G	C	G	A	A	C	A	T	G	G	T	C	T	T
[6]	C	T	G	T	A	T	T	G	T	T	G	C/G	A	A	T	T	T	A

[1] = *R. stylosa*, Tarawa, Kiribati (this study)
[2] = *R. stylosa* var. *aureus*, Tarawa, Kiribati (this study)
[3] = *R. stylosa*, Butaritari, Kiribati (this study)
[4] = *R. stylosa*, Peninsular Malaysia (Ng *et al*., 2013)
[5] = *R. mucronata*, Peninsular Malaysia (Ng *et al*., 2013)
[6] = *R. apiculata*, Peninsular Malaysia (Ng *et al*., 2013, 2015)

Chemistry

Stems and twigs: From the chloroform-methanol extract of stems and twigs of *R. stylosa* collected from Hainan Island in China, a new acetylated flavanol of 3,7-*O*-diacetyl(–)-epicatechin, and seven known flavanol derivatives of (+)-catechin, (–)-epicatechin, 3,3',4',5,7-*O*-pentaacetyl(–)-epicatechin, 3-*O*-acetyl(–)-epicatechin, (+)-afzelechin, proanthocyanidin B2 and cinchonain Ib were isolated (Li *et al*., 2007). From the stems and twigs, Li *et al*. (2008) also reported two new pentacyclic triterpenoids of 3β-*O*-(*E*)-coumaroyl-15α-hydroxy-β-amyrin, 15α-hydroxy-β-amyrin, and five known pentacyclic triterpenoids of 3β-taraxerol, 3β-taraxerol formate, 3β-taraxerol acetate, 3β-*O*-(*E*)-coumaroyl-taraxerol and 3β-*O*-(*Z*)-coumaroyl-taraxerol. From Iriomote Island in Japan, the methanol stem extract of *R. stylosa* yielded two flavan-3-ols of glabraosides A & B, together with seven flavanol derivatives of (+)-catechin, (–)-epicatechin, cinchonains Ia, Ib, IIa & IIb, and (+)-catechin 3-*O*-α-L-rhamnoside (Takara *et al*., 2008).

Leaves and flowers: From leaves of *R. stylosa*, triterpenoids of taraxerone, taraxerol, careaborin and cis-careaborin, flavonoids of astilbin and rutin, phenolic acids of isovanillic acid, protocatechuic acid, and steroids of β-sitosterol and β-daucosterol have been isolated (Yang *et al*., 2008). Among the isolated compounds, astilbin and rutin were reported in this plant for the first time. The scent of *R. stylosa* flowers is mainly attributed to essential oils of 1,2-dimethoxybenzene, eugenol, 2,3-butanediol and linalool (Azuma *et al*., 2002).

Bioactivities

Stems and twigs: Of the eight flavanols isolated from stems and twigs of *R. stylosa*, proanthocyanidin B2 exhibited the strongest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with IC_{50} value of 4.3 μg/mL, followed by (–)-epicatechin and (+)-catechin both with IC_{50} values of 6.5 μg/mL, and cinchonain Ib with IC_{50} value of 7.8 μg/mL (Li *et al*., 2007). Of the nine flavonoids isolated from stems of *R. stylosa*, strongest DPPH radical scavenging activity was displayed by glabraosides A & B, and cinchonains Ia, IIa & IIb with EC_{50} values of 4.6, 8.6, 9.3, 7.3 and 6.5 μM, respectively (Takara *et al*., 2008).

Leaves: Of the compounds isolated from leaves of *R. stylosa*, taraxerol inhibited the growth of HeLa and BGC-823 human cancer cells with IC_{50} of 73.4 and 73.3 μmol/L, respectively (Yang *et al*., 2008). Cis-careaborin inhibited BGC-823 and MCF-7 human cancer cells with IC_{50} of 45.9 and 116 μmol/L, respectively. For the first time, astilbin and rutin were reported to markedly stimulate the proliferation of mice splenic lymphocytes.

Different parts: A comparison of different parts of *R. stylosa* sampled from Micronesia showed that stems had the highest phenolic content, DPPH radical scavenging and anti-tyrosinase activity followed by leaves and roots (Suh *et al*., 2014). Values of stems and leaves of *R. stylosa* were higher than green tea. This finding warrants verification.

Conclusion

A golden yellow variety of *R. stylosa* in Kiribati was tentatively named *R. stylosa* var. *aureus*. DNA analysis showed that they are likely of the same species. Compared to *R. apiculata*, *R. mucronata* and *R. mangle*, much less research efforts have been devoted to the phytochemistry and pharmacology of *R. stylosa*. Chemical constituents of triterpenoids, flavonoids, phenolic acids and steroids were reported in leaves, stems and twigs of *R. stylosa*. Pharmacological properties of antioxidant, anti-tyrosinase and cytotoxic activities have been documented.

Acknowledgements

The authors are grateful to Prof. Richard Whitkus of the Sonoma State University (USA) for use of his laboratory to conduct the DNA analysis, and to Ms. Rasita (Kiribati) for assisting in collecting the leaf samples of *R. stylosa* and *R. stylosa* var. *aureus* from Kiribati.

References

Alikunhi, N.M., Kandasamy, K., Manoharan, C. & Subramanian, M., 2012. Insulin-like antigen of mangrove leaves and its anti-diabetic activity in alloxan-induced diabetic rats. *Natural Product Research* 26(2): 1161-1166.

Annapoorani, A., Kalpana, B., Syed Musthafa, K., Pandian, S.K. & Veera Ravi, A., 2013. Anti-pathogenic potential of *Rhizophora* spp. against the quorum sensing mediated virulence factors production in drug resistant *Pseudomonas aeruginosa*. *Phytomedicine* 20: 956-963.

Azuma, H., Toyota, M., Asakawa, Y., Takaso, T. & Tobe, H., 2002. Floral scent chemistry of mangrove plants. *Journal of Plant Research* 115: 47-53.

Baba, S., 2004. Keynote presentation: What we can do for mangroves. In: Vannucci, M. (ed.) *Mangrove Management and Conservation: Present and Future*, p. 8-36. United Nations University Press, Tokyo, Japan.

Baba, S., Chan, H.T. & Aksornkoae, S., 2013. *Useful Products from Mangrove and other Coastal Plants*. ISME Mangrove Educational Book Series No. 3. ISME and ITTO, Japan.

Berenguer, B., Sanchez, L.M., Quilez, A., Lopez-Barreiro, M., de Haro, O., Galvez, J. & Martin, M.J., 2006. Protective and antioxidant effects of *Rhizophora mangle* L. against NSAID-induced gastric ulcers. *Journal of Ethnopharmacology* 103: 194-200.

Bunyapraphatsara, N., Jutiviboonsuk, A., Sornlek, P., Therathanathorn, W., Aksornkoae, S., Fong, H.H.S., Pezutto, J.M. & Kosmeder, J., 2003. Pharmacological studies of plants in the mangrove forest. *Thai Journal of Phytopharmacy* 10(2): 1-12.

Chan, E.W.C. & Wong, S.K., 2009. Chemical constituents of leaves of *Rhizophora x lamarckii*, *R. apiculata* and *R. stylosa*. *ISME/GLOMIS Electronic Journal* 7(1): 1-2.

Chan, H.T., 1996. A note on the discovery of *Rhizophora x lamarckii* in Peninsular Malaysia. *Journal of Tropical Forest Science* 9: 128-130.

de Armas, E., Sarracent, Y., Marrero, E., Fernández, O. & Branford-White, C., 2005. Efficacy of *Rhizophora mangle* aqueous bark extract in the treatment of aphthous ulcers: a pilot study. *Current Medical Research and Opinion* 21(11): 1711-1715.

de-Faria, F.M., Alves Almeida, A.C., Luiz-Ferreira, A., Dunder, R.J., Takayama, C., da Silva M.S., Vilegas, W., Rozza, A.L., Pellizzond, C.H., Toma, W. & Souza-Brito, A.R.M., 2012. Mechanisms of action underlying the gastric antiulcer activity of *Rhizophora mangle* L. *Journal of Ethnopharmacology* 139: 234-243.

Fernandez, O., Capdevila, J.Z., Dalla, G. & Melchor, G., 2002. Efficacy of *Rhizophora mangle* aqueous bark extract in the healing of open surgical wounds. *Fitoterapia* 73: 564-568.

Giesen, W., Wulfraat, S., Zieren, M. & Scholten, L., 2007. *Mangrove Guidebook for Southeast Asia*. FAO, Bangkok, Thailand and Wetlands International, Wageningen, Netherlands.

Howlader, M.S.I., Ahmed, M.J., Hamidul Kabir, A.N.M., Uddin, M.G. & Hossain, M.K., 2013. Antibacterial, cytotoxic, analgesic and diuretic activities of *Rhizophora mucronata* Lam. bark. *Indian Journal of Natural Products and Resources* 4(3): 229-232.

Inomata N., Wang X.R., Changtragoon S. & Szmidt, A.E., 2009. Levels and patterns of DNA variation in two sympatric mangrove species, *Rhizophora apiculata* and *R. mucronata* from Thailand. *Genes & Genetic Systems* 84: 277-286.

Li, D.L., Li, X.M., Peng, Z.Y. & Wang, B.G., 2007. Flavanol derivatives from *Rhizophora stylosa* and their DPPH radical scavenging activity. *Molecules* 12: 1163-1169.

Li, D.L., Li, X.M. & Wang, B.G., 2008. Pentacyclic triterpenoids from the mangrove plant *Rhizophora stylosa*. *Natural Product Research* 22(9): 808-813.

Melchor, G., Armenteros, M., Fernandez, O., Linares, E. & Fragas, I., 2001. Antibacterial activity of *Rhizophora mangle* bark. *Fitoterapia* 72: 689-691.

Nebula, M., Harisankar, H.S. & Chandramohanakumar, N., 2013. Metabolites and bioactivities of Rhizophoraceae mangroves. *Natural Products and Bioprospecting* 3: 207-232.

Ng, W.L. & Chan, H.T., 2012a. Survey of *Rhizophora stylosa* populations in Peninsular Malaysia. *ISME/GLOMIS Electronic Journal* 10(2): 4-6.

Ng, W.L. & Chan, H.T., 2012b. Further observations on a natural *Rhizophora* hybrid population in Malaysia. *ISME/GLOMIS Electronic Journal* 10(1): 1-3.

Ng, W.L., Chan, H.T. & Szmidt, A.E., 2013. Molecular identification of natural mangrove hybrids of *Rhizophora* in Peninsular Malaysia. *Tree Genetics and Genome* 9: 1151-1160.

Ng, W.L., Onishi, Y., Inomata, N., Teshima, K.M., Chan, H.T., Baba, S. Changtragoon, S., Siregar, I.Z. & Szmidt, A.E. 2015. Closely related and sympatric but not all the same: genetic variation of Indo-West Pacific *Rhizophora* mangrove across the Malay Peninsula. *Conservation Genetics* 16: 137-150.

Ng, W.L. & Szmidt, A.E., 2013. A simple and inexpensive molecular assay for species identification of Indo-West Pacific *Rhizophora* mangroves for conservation and management. *Conservation and Genetics Resources* 5: 1059-1061.

Nilus, R., Chung, A.Y.C., Pereira, J.T., Sugau, J.B., Tangah, J., Sabran, S. & Chong, R.F.Y., 2010. *Mangroves of Sabah: An Introduction to the Flora and Fauna*. Sabah Forestry Department, Malaysia.

Perera, L.M.S., Ruedas, D. & Gómez, B.C., 2001. Gastric antiulcer effect of *Rhizophora mangle* L. *Journal of Ethnopharmacology* 77: 1-3.

Prabhu, V.V. & Guruvayoorappan, C., 2012. Anti-inflammatory and anti-tumour activity of the marine mangrove *Rhizophora apiculata. Journal of Immunotoxicology* 9(4): 341-352.

Premanathan, M., Arakaki, R., Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N. & Nakashima, H., 1999. Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus. *Antiviral Research* 44(2): 113-122.

Ragavan, P., Saxena, M., Coomar, T. & Saxena, A., 2011. Preliminary study on natural hybrids of genus *Rhizophora* in India. *ISME/GLOMIS Electronic Journal* 9(5): 13-19.

Ragavan, P., Saxena, A., Jayaraj, R.S.C., Ravichandran, K. & Saravanan, S., 2015. *Rhizophora* x *mohanii*: A putative hybrid between *Rhizophora mucronata* and *Rhizophora stylosa* from mangroves of the Andaman and Nicobar Islands, India. *ISME/GLOMIS Electronic Journal* 13(2): 3-7.

Rahim, A.A., Rocca, E., Steinmetz, J., Kassim, M.J., Ibrahim, M.S. & Osman, H., 2008. Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. *Food Chemistry* 107: 200-207.

Ramanathan, T., Hariharan, B. & Ganesan, K., 2008. Antidiabetic activity of a coastal mangrove leaves of *Rhizophora mucronata*. *Plant Archives* 8(2): 931-933.

Ravikumar, S. & Gnanadesigan, M., 2012. Hepatoprotective and antioxidant properties of *Rhizophora mucronata* mangrove plant in CCl_4 intoxicated rats. *Journal of Experimental and Clinical Medicine* 4(1): 66-72.

Rohini, R.M. & Das, A.K., 2009. A comparative evaluation of analgesic and anti-inflammatory activities of *Rhizophora mucronata* bark extracts. *Pharmacologyonline* 1: 780-791.

Rohini, R.M. & Das, A.K., 2010. Antidiarrheal and anti-inflammatory activities of lupeol, quercetin, β-sitosterol, adene-5-en-3-ol and caffeic acid isolated from *Rhizophora mucronata* bark. *Der Pharmacia Lettre* 2(5): 95-101.

Sánchez, J., Melchor, G., Martínez, G., Escobar, A. & Faure, R., 2006. Antioxidant activity of *Rhizophora mangle* bark. *Fitoterapia* 77: 141-143.

Suh, S.S., Hwang, J., Park, M., Park, H.S. & Lee, T.K., 2014. Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia. *Asian Pacific Journal of Tropical Medicine* 2014: 531-535.

Takara, K., Kuniyoshi, A, Wada, K., Kinjyo, K. & Iwasaki, H., 2008. Antioxidative flavan-3-ol glycosides from stems of *Rhizophora stylosa*. *Bioscience, Biotechnology & Biochemistry* 72(8): 2191-2194.

Wu, J., Xiao, Q., Xu, J., Li, M.Y., Pan, J.Y. & Yang, M., 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. *Natural Products Reports* 25: 955-981.

Yang, X.H., Li, H.B., Chen, H., Li, P. & Ye, B.P., 2008. Chemical constituents in the leaves of *Rhizophora stylosa* and their biological activities. *Acta Pharmaceutica Sinica* 43: 974-978.

Please cite this article as Kainuma, M., Kezuka, M., Inoue, T., Chan, E.W.C., Tangah, J., Baba, S. & Chan, H.T., 2015. Botany, uses, chemistry and bioactivities of mangrove plants I: *Rhizophora stylosa*. *ISME/GLOMIS Electronic Journal* 13(4): 12-17.