

Antioxidant, anti-tyrosinase and anti-quorum sensing activities of four mangrove tree species vs. green tea

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ABSTRACT

This study was conducted to validate a claim that mangrove teas have stronger antioxidant and anti-tyrosinase properties than green tea. Different plant parts (leaves, stems and roots) of four mangrove tree species namely *Rhizophora apiculata*, *Rhizophora stylosa*, *Avicennia rumphiana* and *Sonneratia alba* were analysed their antioxidant, anti-tyrosinase and anti-quorum sensing (anti-QS) properties. Strongest antioxidant properties were observed in the stems of *R. stylosa*. Antioxidant properties of the mangrove species (fresh samples) and their teas (dried leaves) were inferior to leaves of *Camellia sinensis* and green tea, respectively. The anti-tyrosinase activity of leaves and stems of *R. apiculata*, and stems of *S. alba* was outstanding but was not detected in green tea. All four mangrove species possessed anti-QS properties with moderate activity displayed by green tea.

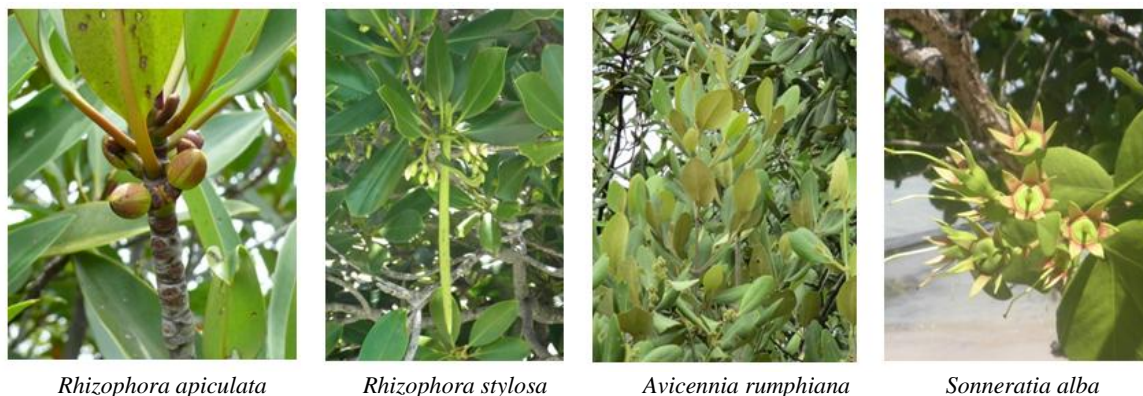
INTRODUCTION

Mangroves are tidal forests of the tropics and sub-tropics that thrive in sheltered coastal areas such as estuaries, accreting shores, bays and lagoons (Giesen *et al.*, 2007). These plant communities also occur in areas protected by sand bars, islands, coral reefs and/or sea grasses. At the seafront, trees such as *Avicennia* and *Sonneratia* with their extensive pneumatophores contribute to land accretion by colonising and stabilising the mudflats. On firmer and more compact sediments further inland, trees of *Rhizophora* with stilt roots and *Bruguiera* with knee roots dominate. Where large rivers occur, mangroves may extend upstream for tens of kilometres, merging into freshwater swamp forests. As a background to this paper, a recent article in the *Asian Pacific Journal of Tropical Medicine* by Suh *et al.* (2014) reported a controversial finding that the antioxidant and anti-tyrosinase properties of two mangrove tree species (*Rhizophora stylosa* Griff.

and *Sonneratia alba* J.E. Smith) sampled from Micronesia were stronger than those of green tea of *Camellia sinensis* (L.) Kuntze. In an attempt to validate this doubtful finding, we selected four mangrove species including *R. stylosa* and *S. alba*, and analysed their antioxidant, anti-tyrosinase and anti-quorum sensing properties. *Rhizophora apiculata*, Bl. and *Avicennia rumphiana* Hallier f. (previously known as *Avicennia lanata* Ridley) were the other two mangrove species studied, and green tea was used as comparison. Anti-quorum sensing was also assessed in this study since the bark extract of *Rhizophora annamalayana* Kathir. (Musthafa *et al.*, 2013), and the leaf extracts of *Rhizophora mucronata* Lamk. and *R. apiculata* (Annapoorani *et al.*, 2013) have been reported to possess positive activity. Of the four species studied, the metabolites and bioactivities of *R. apiculata* and *R. stylosa* have been reviewed (Nebula *et al.*, 2013; Kainuma *et al.*, 2015). Characteristic features are prop or stilt roots of *R. apiculata* and *R. stylosa*, and pneumatophores of *S. alba* and *A. rumphiana*. Flowers of *R. apiculata* are always in pairs, flowers of *R. stylosa* have an elongated style, leaves of *S. alba* are leathery with a rounded apex and leaves of *R. rumphiana* have a dense hairy under-surface.

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*Rhizophora apiculata**Rhizophora stylosa**Avicennia rumphiana**Sonneratia alba***Fig. 1:** The four mangrove species studied.

MATERIALS AND METHODS

Sampling in the field

Bagan Lalang (2°35'N, 101°41'E) is a popular beach resort in the southernmost part of the state of Selangor in West Malaysia. Leaves, stems and roots of *R. apiculata*, *R. stylosa* and *S. alba* were collected from scattered trees growing on sand flats at the seafront, east of the Golden Palm Tree Resort. Samples of *A. rumphiana* were collected from trees growing in clusters further inland. Dr Chan Hung Tuck, a mangrove expert and Executive Committee Member of the International Society for Mangrove Ecosystems (ISME) based in Okinawa, Japan, was present during the field trip to personally confirm the identity of the species. Photographs of these four species are shown in Figure 1. Stems were twigs after removal of leaves. Roots of *R. apiculata*, and *R. stylosa* were aerial stilt roots while those of *S. alba* and *A. rumphiana* were pneumatophores protruding from the ground.

Extraction of fresh plant samples

For antioxidant properties, fresh plant samples (1.0 g) were powdered with liquid nitrogen in a mortar and extracted with 50 ml of methanol with continuous shaking for one hour at room temperature. Extracts were filtered under suction and stored at 4 °C for further analysis. For anti-tyrosinase and anti-quorum sensing activities, fresh samples (10 g) were similarly extracted with 100 ml of methanol, three times for one hour each time of methanol, three times for one hour each time. After swirling continuously in an orbital shaker, the extracts were filtered and stored at 4°C for further analysis.

Extraction of dried leaf samples

Mangrove leaves (20 g) were dried at 50°C in a conventional oven for three hours and in a microwave oven for 40 sec. The oven-dried (OD) and microwave-dried (MD) leaves were extracted using the hot water method (Chan *et al.*, 2011, 2012a). Samples (0.3 g) were ground and extracted with 50 ml of boiling water with continuous swirling at 100 rpm in an

orbital shaker for one hour. The hot water was allowed throughout the extraction process to mimic the tea brewing process. After filtration, the residues were re-extracted again, dried using a freeze dryer and kept in a freezer for further analysis. Similarly 0.3 g of the green tea of *C. sinensis* purchased from Marks and Spencer was extracted with hot water.

Antioxidant properties

Samples were analysed for total phenolic content (TPC) using the Folin-Ciocalteu assay (Chan *et al.*, 2012b, 2012c). Absorbance was measured at 765 nm and TPC was expressed as gallic acid equivalent (GAE) in mg per 100 g of sample. Antioxidant activity of free radical scavenging (FRS) ability of samples was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Absorbance was measured at 517 nm and expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid (AA)/100 g of sample.

Anti-tyrosinase activity

Anti-tyrosinase activity of samples was determined using the modified dopachrome method with L-DOPA as substrate (Masuda *et al.*, 2005; Chan *et al.*, 2008, 2015). The assay was conducted in a 96-well microtiter plate and absorbance was measured at 475 nm with 700 nm as the reference. Results were compared with a control consisting of 50% DMSO and tyrosinase inhibition was calculated as $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100\%$. The concentration of extracts used for determining tyrosinase inhibition was 0.25 mg/ml.

Anti-quorum sensing activity

Following the procedures described by Blosser and Gray (2000), the violacein inhibition assay was used to assess anti-quorum sensing (anti-QS) activity of samples against *Chromobacterium violaceum* (wild type, ATCC 12472). Sample extracts were dissolved in methanol to produce stock solutions of 5.0 mg/ml. Stock solutions (0.5 ml) were transferred onto sterile Petri dishes with 4.5 ml of fresh nutrient broth inoculated with *C.*

violaceum (OD₇₂₀ 0.100 A, which corresponded to 1.5×10^7 cfu/ml). The Petri dishes were incubated for 24 h at 26°C with gentle swirling at 100 rpm before the cell density was measured at OD₇₂₀. A streak plate of each Petri dish was done to ensure no contamination and that the optical density was a valid representation of cell density. To measure violacein production, 2.0 ml of broth culture was centrifuged at 13000 rpm for 15 min to recover the *C. violaceum* cells. Violacein was then extracted using 2.0 ml of butanol with sonication and absorbance of the extracted violacein was measured at 577 nm (A₅₇₇) against a blank solution of butanol. Violacein production was expressed in terms of violacein units (VU) calculated as the ratio of A₅₇₇/OD₇₂₀. The lower the ratio value, the stronger is the anti-QS activity.

Statistical analysis

All experiments were done in triplicate (n = 3) and results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was analysed using the Tukey Honestly Significant Difference (HSD) test with significant difference at $p < 0.05$.

RESULTS AND DISCUSSION

Based on TPC and AEAC of different plant parts of the four mangrove species, the ranking was *R. stylosa* > *R. apiculata* > *S. alba* > *A. rumphiana* (Table 1). At the species level, highest values were obtained from the stems of *R. stylosa*, roots of *R. apiculata* and *S. alba*, and leaves of *A. rumphiana*.

Table 1: Total phenolic content and free radical scavenging activity of four mangrove tree species (fresh weight).

Species	Plant Part	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
<i>Rhizophora apiculata</i>	L	2790±94c	3320±193c
	S	3320±29b	3750±170b
	R	3860±212a	4250±245a
<i>Rhizophora stylosa</i>	L	2090±176c	2570±148c
	S	6440±387a	11660±547a
	R	3950±231b	7350±329b
<i>Avicennia rumphiana</i>	L	1300±21a	1040±69a
	S	432±65b	485±15b
	R	352±51b	289±5c
<i>Sonneratia alba</i>	L	1660±175b	2770±166b
	S	1400±129b	2880±212b
	R	2210±200a	4320±303a

Data on phenolic contents in fresh weight are means ± standard deviations. Abbreviations: L = leaves, S = stems, R = roots, TPC = total phenolic content (mg GAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity, GAE = gallic acid equivalent and AA = ascorbic acid. Within each column of each species, different letters (a–c) are significant at $p < 0.05$ using the Tukey HSD test.

The antioxidant properties of the stems of *R. stylosa* are noteworthy in that their TPC and AEAC (6440 mg GAE/100 g and 11660 mg AA/100 g) are significantly higher than leaves of

Lagerstroemia speciosa L. (4150 mg GAE/100 g and 6120 mg AA/100 g) and *Anacardium occidentale* L. (3890 mg GAE/100 g and 6620 mg AA/100 g), respectively. Both *L. speciosa* (banaba) and *A. occidentale* (cashew) are known to have very strong antioxidant properties (Chan and Wong, 2015). The AEAC value of *R. stylosa* stems (11660 mg AA/100 g) is comparable to that of the highland tea plant of *C. sinensis* (11380 mg AA/100 g) reported by Chan *et al.* (2007).

The potent antioxidant properties of stems of *R. stylosa* could be attributed to flavanols (Li *et al.*, 2007; Takara *et al.*, 2008) and pentacyclic triterpenoids (Li *et al.*, 2008). Amongst the flavanols isolated, proanthocyanidin B2, epicatechin + catechin and cinchona in Ib had DPPH radical scavenging ability with IC₅₀ values of 4.3, 6.5 and 7.8 µg/ml, respectively (Li *et al.*, 2007). Proanthocyanidin B2 (4.3 µg/ml) with the strongest free radical scavenging activity was 4.2 times stronger than butylated hydroxytoluene (18 µg/ml), the positive control. Flavanols from the stems of *R. stylosa* had strong radical scavenging activity with EC₅₀ values of 4.6–9.3 µM (Takara *et al.*, 2008). In general, the antioxidant properties of the four mangrove species were significantly weaker than those of *C. sinensis*. TPC and AEAC values are 7590 mg GAE/100 g and 12820 mg AA/100 g for the highland tea plants, and 7280 mg GAE/100 g and 11380 mg AA/100 g for the lowland tea plants, respectively (Chan *et al.*, 2007).

Table 2: Phenolic content and free radical scavenging activity of hot water tea infusions of dried mangrove leaves in comparison with green tea (dry weight).

Species	Drying method	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
<i>Rhizophora apiculata</i>	MD	2820±509a	4130±263a
	OD	3070±60a	3750±99a
<i>Rhizophora stylosa</i>	MD	2650±141a	3410±263b
	OD	2360±114a	3980±167a
<i>Avicennia rumphiana</i>	MD	3940±55a	6280±505a
	OD	2250±149b	2930±11b
<i>Sonneratia alba</i>	MD	2270±93b	3860±313b
	OD	3570±80a	6920±647a
Green tea		6550±338	11740±619

Data on phenolic contents in dry weight are means ± standard deviations. Abbreviations: MD = microwave-dried, OD = oven-dried, TPC = total phenolic content (mg GAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity, GAE = gallic acid equivalent and AA = ascorbic acid. Within each column of each species, different letters (a–b) are significant at $p < 0.05$ using the Tukey HSD test.

Microwave and oven drying had variable effects on the antioxidant properties of the hot water tea infusions of mangrove leaves (Table 2). At the species level, TPC and AEAC values of MD and OD leaves were comparable for *R. apiculata* and *R. stylosa*, higher in MD leaves for *A. rumphiana* and higher in OD leaves for *S. alba*. The highest values of TPC (3940 mg GAE/100 g) observed in MD leaves of *A. rumphiana* and AEAC (6920 mg AA/100 g) observed in OD leaves of *S. alba* were both 1.7 times

lower than those of green tea with TPC of 6550 mg GAE/100 g and AEAC of 11740 mg AA/100 g. Finding from this study therefore contradicts the observation by Suh *et al.* (2014) that mangrove teas have stronger antioxidant properties than green tea, despite differences in the methods of tea preparation and in the brands of green tea used.

Of the four species, anti-tyrosinase activity was not observed all the plant parts of *R. stylosa* and *A. rumphiana* (Table 3). Strongest activity was displayed in the leaves (70%) and stems (70%) of *R. apiculata*, and in the stems (62%) of *S. alba*. Expectedly, tyrosinase inhibitory activity was not detected in green tea of *C. sinensis*. If tea leaves were to possess endogenous tyrosinase inhibitors, they would prevent fermentation of leaves into black tea by polyphenol oxidase. It was noted that the values of *R. apiculata* and *S. alba* superseded those of leaves of *Hibiscus tiliaceus* L. (42%) and *Psidium guava* L. (41%) (Wong *et al.*, 2010), roots of *Eurycoma longifolia* Jack (45%) (Wan Hassan *et al.*, 2015a), and pericarps of *Garcinia mangostana* L. (Wan Hassan *et al.*, 2015b), which have strong anti-tyrosinase activity. Contrary to findings of this study, Suh *et al.* (2014) reported that leaves, stems and roots of *R. stylosa* inhibited tyrosinase with the strongest activity in the stems (90%).

Table 3: Tyrosinase inhibition of four mangrove tree species.

Species	Plant part	Tyrosinase inhibition (%)
<i>Rhizophora apiculata</i>	L	70±4a
	S	70±7a
	R	52±7b
<i>Rhizophora stylosa</i>	L	ND
	S	ND
	R	ND
<i>Avicennia rumphiana</i>	L	ND
	S	ND
	R	ND
<i>Sonneratia alba</i>	L	48±6b
	S	62±6a
	R	55±6ab
Green tea	L	ND

Data on tyrosinase inhibition are means ± standard deviations. Abbreviations: L = leaves, S = stems, R = roots and ND = not detected. The concentration of extracts used for determining tyrosinase inhibition was 0.25 mg/ml. Within the same column, different letters (a–b) are significantly different at $p < 0.05$, as measured by the Tukey's HSD test. ANOVA does not apply between species.

Data on the anti-QS activity of the four mangrove species based on optical density of inhibition of *C. violaceum* growth and violacein production are shown in Table 4. Violacein production expressed as violacein units (VU) was used to indicate quorum sensing. A good anti-QS agent should have low VU with minimal reduction in bacterial growth. Species with significantly lower VU values than the control were stems and roots of *R. apiculata*, leaves and roots of *A. rumphiana*, and roots of *S. alba*.

However, stems and roots of *R. apiculata*, and roots of *S. alba* also exhibited growth inhibition of *C. violaceum* as shown by their low optical density at 720 nm, and thus VU reduction might not be solely caused by QS inhibition. Leaves and roots of *A. rumphiana* showed the greatest promise as anti-QS agents because of their low VU values and minimal impact on bacterial growth. In

comparison, the anti-QS activity of green tea was moderate. Among mangrove species, anti-QS activity was reported in the bark extract of *R. annamalayana* by Musthafa *et al.* (2013) using the violacein inhibition assay against *C. violaceum* and the anti-bioluminescence assay against *Vibrio harveyi*. At 1.0 mg/ml, the extract reduced QS-dependent factor production with no inhibitory effect on bacterial growth. Anti-QS activity against *Pseudomonas aeruginosa* was also reported in the leaf extracts of *R. apiculata* and *R. mucronata* (Annaporani *et al.*, 2013).

Table 4: Anti-quorum sensing activity of four mangrove tree species based on optical density of inhibition of *Chromobacterium violaceum* growth and violacein production.

Species	Plant part	A ₅₇₇ (violacein concentration)	OD ₇₂₀ (CV cell density)	A ₅₇₇ /OD ₇₂₀ (violacein unit)
<i>Rhizophora apiculata</i>	L	1.10±0.04	0.77±0.03	1.37±0.06
	S	0.47±0.01	0.57±0.01	0.82±0.01*
	R	0.46±0.01	0.52±0.02	0.87±0.05*
<i>Rhizophora stylosa</i>	L	1.98±0.38	1.24±0.17	1.32±0.08
	S	1.34±0.28	0.67±0.17	1.62±0.35
	R	1.14±0.27	0.53±0.08	2.03±0.22
<i>Avicennia rumphiana</i>	L	2.58±0.29	3.00±0.29	0.86±0.06*
	S	1.76±0.42	0.74±0.07	2.34±0.32
	R	2.01±0.33	1.93±0.51	1.07±0.12*
<i>Sonneratia alba</i>	L	0.95±0.04	0.50±0.08	1.94±0.39
	R	0.59±0.19	0.66±0.08	0.91±0.30*
Green tea		1.50±0.06	1.33±0.01	1.13±0.05*
Control		1.37±0.02	0.95±0.01	1.45±0.01

Abbreviations: L = leaves, S = stems, R = roots, CV = *Chromobacterium violaceum*, A = absorbance and OD = optical density. Values are means ± standard deviations ($n = 3$). Violacein concentration and CV cell density were measured at absorbance at 577 nm (A₅₇₇) and optical density at 720 nm (OD₇₂₀), respectively. Violacein production, represented in terms of violacein units (VU), was calculated as A₅₇₇/OD₇₂₀. Lower VU values suggest stronger anti-quorum sensing activity. The viable cell count for the control was 2.8×10^9 cfu/ml. The concentration of extract used for determining violacein inhibition was 0.5 mg/ml. In the VU column of each species, values with the asterisk are significant stronger than the control.

CONCLUSION

Strongest antioxidant properties were observed in the stems of *R. stylosa*. Antioxidant properties of the four mangrove species and their teas were strong but inferior to those of *C. sinensis* leaves and green tea. The anti-tyrosinase activity of leaves and stems of *R. apiculata*, and stems of *S. alba* was outstanding. All four species possess anti-QS properties with moderate activity displayed by green tea. These aspects, notably the anti-tyrosinase and anti-QS properties, warrant further study.

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