ANTIOXIDANT PROPERTIES OF *HIBISCUS*: SPECIES VARIATION, ALTITUDINAL CHANGE, COASTAL INFLUENCE AND FLORAL COLOUR CHANGE

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WONG SK, LIM YY & CHAN EWC. 2009. Antioxidant properties of *Hibiscus*: species variation, altitudinal change, coastal influence and floral colour change. Antioxidant properties (AOP) of leaves and flowers of six *Hibiscus* species were screened. For leaves of *Hibiscus rosa-sinensis* and *Hibiscus tiliaceus*, the effects of altitudinal change and coastal influence respectively were assessed. Flowers of *Hibiscus mutabilis,* during colour change, were analysed. AOP evaluated were total phenolic content (TPC), total anthocyanin content (TAC), radical-scavenging activity expressed as ascorbic acid equivalent antioxidant capacity (AEAC), ferric-reducing power (FRP) and ferrous ion-chelating (FIC) ability. TPC, AEAC and FRP of leaves and flowers of *H. tiliaceus* were the greatest. The red flowers of *H. rosa-sinensis* and *Hibiscus schizopetalus* yielded the highest TAC and FIC ability. The AOP of leaves of highland populations of *H. rosa-sinensis* were greater than those of lowland. There was no distinct variation in AOP of leaves of inland and coastal populations of *H. tiliaceus*. The AOP of red flowers of *H. mutabilis* were greater than those of pink and/or white flowers. Overall ranking of flowers during colour change was red > pink > white.

Keywords: Phenolic, anthocyanin contents, UV-B effects, geographical variation, flower colours

WONG SK, LIM YY & CHAN EWC. 2009. Ciri antioksida *Hibiscus*: variasi spesies, perubahan altitud, pengaruh pesisiran pantai dan pertukaran warna bunga. Ciri antioksida (AOP) daun dan bunga enam spesies *Hibiscus* dikaji. Bagi daun *Hibiscus rosa-sinensis* dan *Hibiscus tiliaceus*, kesan perubahan altitud dan pengaruh pesisiran pantai masing-masing dinilai. Pertukaran warna bunga *Hibiscus mutabilis* dianalisis. AOP yang dikaji ialah jumlah kandungan fenol (TPC), jumlah kandungan antosianin (TAC), aktiviti menyerang radikal yang dinyatakan sebagai kebolehan antioksida asid askorbik (AEAC), kuasa menurun ferik (FRP) dan pengkelatan ion ferum (FIC). Nilai-nilai TPC, AEAC dan FRP paling tinggi bagi daun dan bunga *H. tiliaceus*. Bunga merah *H. rosa-sinensis* dan *Hibiscus schizopetalus* menunjukkan nilai TAC dan FIC tertinggi. AOP daun *H. rosa-sinensis* bagi populasi gunung lebih tinggi daripada populasi tanah pamah. Tidak ada perbezaan dalam AOP daun populasi *H. tiliaceus* yang jauh dari pantai dengan populasi pesisiran pantai. AOP bunga merah *H. mutabilis* lebih tinggi daripada bunga merah muda dan/atau putih. Secara amnya, nilai AOP bunga ketika pertukaran warna ialah merah > merah muda > putih.

INTRODUCTION

The genus *Hibiscus* (Malvaceae) comprises about 275 species in the tropics and subtropics (Dasuki 2001). Within the Malesian region, 43 species are found. Most *Hibiscus* species have a remarkable colour pattern with the base of the corolla forming a deep-coloured heart (Lowry 1976). Another feature is flower colour change, of which the most spectacular is in *H. mutabilis*. Leaves of *Hibiscus* are simple, lobed, alternate or spiral and have paired stipules (Ng 2006). Flowers are radially symmetrical with cup-shaped calyx, five petals joined at the base, style bearing many stamens and stigma with five hairy lobes.

Plants protect themselves from oxidative damage due to ultraviolet (UV) exposure by producing antioxidative phenolic compounds (Larson 1988). Enhanced UV-B radiation induces greater production of phenolic compounds such as flavonoids in plant tissues (Bassman 2004). These compounds play an important role as filters absorbing and effectively reducing the UV flux reaching plant tissues (Caldwell *et al.* 1989). Enzymes associated with synthesis of phenolics are produced in greater quantities or show increased activity (Jansen *et al.* 1998, Chalker-Scott & Scott 2004). Leaves of tropical

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forest plants produce more antioxidants when exposed to elevated light conditions (Frankel & Berenbaum 1999).

Low temperatures have also been shown to enhance synthesis of phenylalanine ammonia lyase (PAL) in plants leading to increased production of phenolics, including flavonoids (Chalker-Scott & Scott 2004). Decreased temperatures trigger greater biosynthesis of phenolics in some plant species, even in the absence of UV-B radiation (Bilger *et al.* 2007).

Flavonoids are widely recognized as antioxidants with health-promoting properties in human diets. They also exhibit a wide spectrum of biological functions including the interaction between plants and their environment (Koes *et al.* 1994, Pourcel *et al.* 2007). However, the antioxidant function of flavonoids in plants remains unclear and is being debated (Hernandez *et al.* 2009).

Solar UV radiation increases with elevation and with proximity to the sea. UV-B increases 14–18% per 1000 m rise in elevation, with greater increase in the tropics compared with the temperate (Caldwell *et al.* 1989). There is greater UV radiation in coastal areas due to reflection of sunlight from sand and sea surfaces (Kawanishi *et al.* 1994). With greater UV radiation in higher altitudes and in coastal areas, one would expect highland and coastal plants to have greater antioxidant properties (AOP). However, there are very few studies comparing the AOP between highland and lowland, and between coastal and inland plant populations.

Floral colour change in plants is known to provide visual discrimination to pollinators (Harborne 2001) and to influence the pattern of visitation by pollinators (Delph & Lively 1989). Pollinators use colour as a cue to the availability of floral rewards. By selectively visiting flowers based on colour cue, pollinators increase their foraging efficiency. The retention of spent or post-reproductive flowers has been attributed to the increase in overall attractiveness of plants to pollinators (Gori 1989). There is little research done on the chemical constituents and the properties of flowers during colour change.

In this study, the AOP of leaves and flowers of six *Hibiscus* species were screened. For leaves of *H. rosa-sinensis* and *H. tiliaceus*, the effects of altitudinal change and coastal influence were assessed respectively. Flowers of *H. mutabilis* during colour change were analysed. This study represents the most comprehensive study on AOP of leaves and flowers of *Hibiscus* species, as affected by some ecological factors.

MATERIALS AND METHODS

Species studied, sampling locations and collection procedures

Species studied were *H. mutabilis, H. rosa-sinensis, H. sabdariffa, H. schizopetalus, H. taiwanensis* and *H. tiliaceus*. Their common names, sections and sampling locations are listed in Table 1. Voucher specimens of species studied were deposited in the herbarium of the Monash University Sunway Campus Malaysia. Altitudes and co-ordinates of sampling locations were determined using a Casio altimeter (Model PRG-70-1VDR) and Google Earth 4.2 respectively.

Leaves and flowers for screening of AOP were collected a day before extraction and kept in sealed plastic bags in a refrigerator. For more critical studies, such as monitoring AOP of

Table 1 Common names, sections and sampling locations of *Hibiscus* species studied

Species	Common name	Section	Sampling location
Hibiscus mutabilis	Confederate rose	Trionum	Kepong (KL), Hibiscus Garden (KL)
Hibiscus rosa-sinensis	China rose	Lilibiscus	Kepong (KL), Selayang Baru (S), Genting Sempah (P), Genting Highlands (P)
Hibiscus sabdariffa	Roselle	Furcaria	Kepong (KL), Sungai Buloh (S)
Hibiscus schizopetalus	Coral hibiscus	Lilibiscus	Hibiscus Garden (KL)
Hibiscus taiwanensis	Cream hibiscus	Trionum	Hibiscus Garden (KL)
Hibiscus tiliaceus	Sea hibiscus	Azanza	Jalan Kuching (KL), Selayang Baru (S), Taman Tun Dr Ismail (S), Pantai Jeram (S), Pantai Remis (S)

KL = Kuala Lumpur, S = Selangor, P = Pahang

colour change in petals of *H. mutabilis*, flowers were sampled in the morning of the day of study. Plant samples were transported to the laboratory in a chilled ice-box. From each location, three individual plants per species were sampled.

Hibiscus species were chosen for study because shrubs of *H. rosa-sinensis* are planted in lowlands and highlands, trees of *H. tiliaceus* occur naturally along coastal areas and are planted in inland areas, and planted shrubs of *H. mutabilis* display spectacular flower colour change. The Hibiscus Garden in Kuala Lumpur has a good collection of species, providing materials for study.

Extraction of leaves and petals

For antioxidant analysis, fresh leaves and petals (1 g) were separately powdered with liquid nitrogen in a mortar and extracted using methanol (50 ml), with continuous swirling for 1 hour at room temperature using an orbital shaker. Extracts were filtered under suction and stored at -20 °C for further use.

To test the efficiency of methanol extraction, second and third extractions were conducted on *H. rosa-sinensis* and *H. tiliaceus* leaves and flowers. After filtration, residues along with the filter paper were transferred back into the extraction vessel and extracted again, each time with 50 ml of methanol.

Phenolic contents

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay (Kahkonen *et al.* 1999). Samples (300 µl; triplicate) were introduced into test tubes followed by 1.5 ml of Folin-Ciocalteu's reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorbance at 765 nm was measured. TPC was expressed as gallic acid equivalent (GAE) in mg per 100 g material. The calibration equation for gallic acid was $y = 0.0111x - 0.0148$ ($r^2 = 0.9998$), where y is absorbance and x is concentration of gallic acid in mg/l.

Total anthocyanin content (TAC) was determined by the pH differential method (Teow *et al.* 2007). Potassium chloride solution (2 ml, 1 M and pH 1) was added to 1 ml of sample in triplicate. Measurements were blanked against sodium acetate buffer (2 ml, 1 M and pH 4.5) with the same amount of sample. Absorbance was measured at 520 and 700 nm, and TAC was expressed as cyanidin-3-glucoside equivalent (CGE) in mg per 100 g of sample. The molar extinction coefficient of cyanidin-3-glucoside was 26 900.

Antioxidant activities

Antioxidant activities of extracts measured included radical-scavenging activity, ferric-reducing power (FRP) and ferrous ion-chelating ability (FIC). The methods were based on procedures described by Chan *et al.* (2007).

Radical-scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different dilutions of extracts (1 ml) were added to 2 ml of DPPH (5.9 mg/100 ml methanol). Absorbance was measured at 517 nm after 30 min. Radical-scavenging was calculated as IC_{50} and expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid /100 g:

$$\text{AEAC (mg AA/100 g)} = IC_{50(\text{ascorbate})}/IC_{50(\text{sample})} \times 10^5$$

The IC_{50} of ascorbic acid used for calculation of AEAC was 0.00387 mg/ml.

The FRP was measured by adding different dilutions of extracts (1 ml) to 2.5 ml phosphate buffer (0.2 M and pH 6.6 and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid solution (2.5 ml and 10% w/v) was added to stop the reaction. The mixture was then separated into aliquots of 2.5 ml and diluted with 2.5 ml of water. To each diluted aliquot, 0.5 ml of ferric chloride solution (0.1% w/v) was added. After 30 min, absorbance was measured at 700 nm. FRP was expressed as mg GAE/g. The calibration equation for gallic acid was $y = 16.767x$ ($r^2 = 0.9974$), where y is absorbance and x is concentration of gallic acid in mg/ml.

The FIC ability was assessed by mixing $FeSO_4$ (0.1 mM and 1 ml) with different dilutions of extracts (1 ml), followed by ferrozine (0.25 mM and 1 ml). Absorbance (A) was measured at 562 nm after 10 min. Measurements were compared with a control comprising solvent in place of sample. The ability of extracts to chelate ferrous ions was calculated as:

$$\text{Chelating effect \%} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

RESULTS AND DISCUSSION

Description of *Hibiscus* species

Leaves of *H. mutabilis* are broadly ovate with mostly five triangular lobes. Flowers are white in the morning, turning pink in the afternoon, and red in the evening. Temperature may be an important factor affecting the rate of colour change as white flowers kept in the refrigerator remain white until they are taken out to warm, whereupon they slowly turn pink (Ng 2006). Leaves *H. rosa-sinensis* are ovate with serrated margins. Flowers are red with a long and slender style, anthers yellow and stigma red. Leaves of *H. sabdariffa* are broadly ovate with 3–5 lobes and stems are reddish. Flowers open pinkish in the morning, turning orange in the evening. Leaves of *H. schizopetalus* resemble those of *H. rosa-sinensis*. Flowers are red, pendulous with a long extended style and petals are finely dissected. Leaves of *H. taiwanensis* are finely toothed and flowers are yellow with a prominent deep brown centre. Leaves of *H. tiliaceus* are heart-shaped and flowers are bell-shaped with maroon-coloured heart and stigma. Flowers are yellow in the morning, turning orange-red in the evening.

Methanol extraction efficiency

Based on TPC of three successive extractions of *H. rosa-sinensis* and *H. tiliaceus* leaves and flowers, the extraction efficiency of methanol in the first extraction averaged 90 ± 3%. The averages of second and third extractions were only 6 ± 2 and 3 ± 2% respectively. As the extraction efficiency was high, all subsequent analyses in this study were done on samples extracted with methanol. Methanol is the most suitable solvent in the extraction of phenolic compounds from plant tissues due to its ability to inhibit the action of polyphenol oxidases that cause the oxidation of phenolic compounds and its ease of evaporation compared with water (Waterman & Mole 1994, Yao *et al.* 2004).

AOP of leaves and flowers of *Hibiscus* species

Leaves of *H. tiliaceus* showed outstanding AOP with TPC and AEAC values of 2080 mg GAE /100 g and 2370 mg AA/100 g respectively (Table 2). Values were 2.4 and 2.7 times higher than those of *H. mutabilis* which ranked second. Ranking based on TPC and AEAC was *H. tiliaceus* > *H. mutabilis* > *H. sabdariffa* > *H. taiwanensis* > *H. schizopetalus* ≈ *H. rosa-sinensis*. Leaves of *H. schizopetalus*, *H. sabdariffa* and *H. rosa-sinensis* had better FIC ability than those of *H. mutabilis*, *H. tiliaceus* and *H. taiwanensis* (Figure 1a). Leaves of species with higher TPC and AEAC values had lower FIC ability for *H. tiliaceus* and *H. mutabilis*, and vice versa for *H. schizopetalus* and *H. rosa-sinensis*. This suggests the presence of compounds in leaves of *H. schizopetalus* and *H. rosa-sinensis* with relatively weak radical-scavenging activity

Table 2 Total phenolic content (TPC), total anthocyanin content (TAC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing power (FRP) of leaves and flowers of *Hibiscus* species (fresh weight)

Species	Leaf		Flower			
	TPC (mg GAE /100 g)	AEAC (mg AA /100 g)	TPC (mg GAE /100 g)	TAC (mg CGE /100 g)	AEAC (mg AA /100 g)	FRP (mg GAE /g)
H. tiliaceus	2080 ± 419 a	2370 ± 539 a	2420 ± 167 a	64 ± 5 a	3180 ± 678 a	14 ± 1.3 a
H. mutabilis	861 ± 92 b	877 ± 137 b	495 ± 23 b	16 ± 2 b	562 ± 37 b	2.4 ± 0.1 b
H. sabdariffa	523 ± 61 c	351 ± 40 c	264 ± 61 c	43 ± 12 c	230 ± 60 c	1.5 ± 0.3 c
H. taiwanensis	403 ± 3 d	233 ± 22 d	580 ± 79 b	92 ± 10 d	761 ± 98 d	3.6 ± 0.4 de
H. schizopetalus	336 ± 50 e	94 ± 26 e	516 ± 30 b	192 ± 21 e	520 ± 50 b	3.0 ± 0.2 d
H. rosa-sinensis	301 ± 21 e	96 ± 35 e	735 ± 46 d	284 ± 17 f	640 ± 56 bd	4.0 ± 0.3 e

Values of TPC, TAC, AEAC and FRP are means ± SD (n = 3). For each column, values followed by the same letter are not statistically different at $p < 0.05$ as measured by the Tukey's HSD test. Values of *H. mutabilis* flowers were based on pink petals. For each species, samples were collected from the same location. GAE = gallic acid equivalent, AA = ascorbic acid, CGE = cyanidin-3-glucoside equivalent.

but good metal-chelating ability that can prevent the generation of hydroxyl radicals via Fenton's reaction.

Flowers of *H. tiliaceus* had TPC, AEAC and FRP values of 2420 mg GAE/100 g, 3180 mg AA/100 g and 14 mg GAE/g respectively, which were significantly higher than values of all other species (Table 2). Ranking based on TPC was *H. tiliaceus* > *H. rosa-sinensis* > *H. taiwanensis* ≈ *H. schizopetalus* ≈ *H. mutabilis* > *H. sabdariffa*. The red flowers of *H. rosa-sinensis* and *H. schizopetalus*, which yielded the highest TAC, displayed high FIC ability (Figure 1b). Species with low TAC such as *H. mutabilis* and *H. sabdariffa* displayed low or no FIC ability. TAC appears to be positively correlated with FIC ability in flowers of *Hibiscus* species. Anthocyanins with potent metal-chelating activity have been reported in peels of egg plant (*Solanum melongena* var. *esculentum*) (Noda *et al.* 2000) and leaves of *Perilla pankinensis* (Gulcin *et al.* 2005).

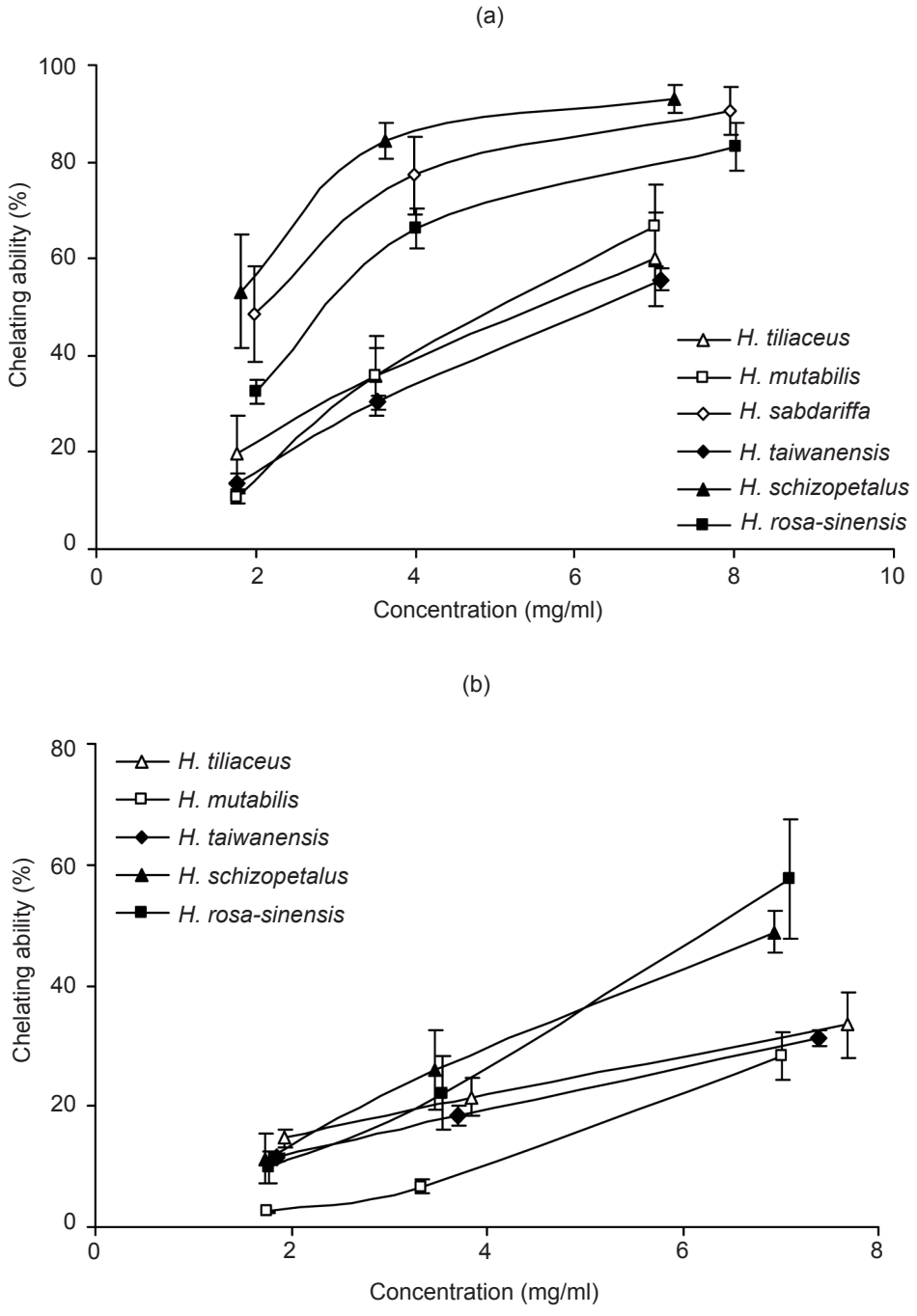

Figure 1 Ferrous ion-chelating (FIC) ability of leaves (a) and flowers (b) of *Hibiscus* species

Of the six *Hibiscus* species screened, leaves and flowers of *H. tiliaceus* had the strongest AOP. A likely explanation is that *H. tiliaceus* is the only indigenous tree species while the other species are exotic shrubs and herbs. Being long-lived, trees have to produce a wide range of chemical defenses against herbivores and infections. Trees have higher antioxidant activity than shrubs and herbs (McCune & Johns 2007).

Based on AOP of leaves and flowers, the six *Hibiscus* species screened can be divided into three categories. They are species with comparable values in leaves and flowers *(H. tiliaceus)*, species with significantly higher values in leaves than flowers (*H. mutabilis* and *H. sabdariffa*), and species with significantly higher values in flowers than leaves (*H. taiwanensis*, *H. rosa-sinensis* and *H. schizopetalus*).

AOP of leaves of *H. rosa-sinensis* at various altitudes

The AOP of leaves of two highland populations of *H. rosa-sinesis* were compared with two lowland populations of the same mountain range. The same variety of *H. rosa-sinensis* was sampled. Based on four populations sampled for analysis, TPC and AEAC of highland populations were found to be significantly higher than those of lowland. Values of highland populations were 511 mg GAE/100 g and 224 mg AA/100 g at Genting Highlands (1580 m asl), and 470 mg GAE/100 g and 217 mg AA/100 g at Genting Sempah (550 m asl) respectively (Table 3). Values of lowland populations were 328 mg GAE/100 g and 100 mg AA/100 g at Selayang Baru (80 m asl), and 301 mg GAE/100 g and 96 mg AA/100 g at Kepong (60 m asl) respectively. Plants sampled at 1580 m asl in Genting Highlands were stunted and their leaves were generally smaller and more crinkled than those sampled from lower altitudes.

Higher altitudes appear to trigger an adaptive response in leaves of *H. rosa-sinensis*. Higher leaf TPC and AEAC of highland populations may be due to environmental response to higher UV-B radiation and lower air temperature. Higher leaf TPC and AEAC of highland populations have been reported in four *Etlingera* species (Zingiberaceae) (Chan *et al.* 2007). Flowering heads of *Crepis capillaris*, *Hieracium pilosella* and *Hypochaeris radicata* (Asteraceae) collected from various altitudes showed positive correlation with contents of flavonoid and phenolic acid (Zidorn *et al.* 2005). Analysis of altitudinal variation of phenolic content in flowering heads of *Arnica montana* cv. ARBO (Asteraceae) showed positive correlation with DPPH radical-scavenging activity but not with total flavonoid content (Spitaler *et al.* 2008). This observation is consistent with the increase in caffeic acid derivatives at higher altitudes. Caffeic acid derivatives are potent non-flavonoid radical scavengers. Recently, Albert *et al.* (2009) reported lower air temperature rather than enhanced UV-B radiation as the key factor influencing the altitudinal variation of phenolics in *A. montana*.

Table 3 Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves of *Hibiscus rosa-sinensis* at various altitudes (fresh weight)

Location	Habitat	Co-ordinate	Altitude (m)	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Kepong, Kuala Lumpur	Lowland	N 3° 13' 16" E 101° 38' 15"	60	301 ± 21 a	96 ± 35 a
Selayang Baru, Selangor	Lowland	N 3° 15' 26" E 101° 9' 22"	80	328 ± 13 a	100 ± 8 a
Genting Sempah, Pahang	Highland	N 3° 21' 07" E 101° 47' 31"	550	470 ± 91 b	217 ± 63 b
Genting Highlands, Pahang	Highland	N 3° 25' 23" E 101° 47' 40"	1580	511 ± 60 b	224 ± 42 b

Values of TPC and AEAC are means ± SD (n = 3). For each column, values followed by the same letter are not statistically different at $p < 0.05$ as measured by the Tukey's HSD test. GAE = gallic acid equivalent, AA = ascorbic acid.

AOP of leaves of coastal and inland populations of *H. tiliaceus*

The AOP of three coastal populations of *H. tiliaceus* at Pantai Jeram I, Pantai Jeram II and Pantai Remis in Selangor were compared with those of three inland populations at Jalan Kuching in Kuala Lumpur, Selayang Baru in Selangor and Taman Tun Dr Ismail in Selangor. The former are trees growing naturally on sandy beaches fronting the sea. The latter are trees planted along roads in urban areas.

The TPC and AEAC values of leaves of coastal populations ranged from 1760 to 2220 mg GAE/100 g and from 1650 to 2300 mg AA/100 g respectively (Table 4). Values of leaves of inland populations ranged from 1800 to 2670 mg GAE /100 g and from 2010 to 3890 mg AA/100 g respectively. Leaves of the inland population at Taman Tun Dr Ismail had the greatest AOP, with TPC significantly higher than one inland and one coastal populations, and with AEAC significantly higher than all other populations. None of the coastal populations were significantly higher than the inland populations.

It is generally believed that seashore plant species, which are exposed to full sunlight, possess strong antioxidant activity (Masuda *et al.* 1999). With greater UV radiation in coastal areas, due to reflection of sunlight from sand and sea surfaces, one would expect higher AOP in coastal than inland plant populations.

Results from this study did not show any distinct variation between AOP of coastal and inland populations of *H. tiliaceus*. Similarly, Hashiba *et al.* (2006) found inconsistent variation in leaf flavonoid contents of coastal and inland populations of *Campanula punctata* (Campanulaceae). However, Keiko *et al.* (2005) found higher flavonoid and phenolic acid contents in inland populations of *Adenophora triphylla* var. *japonica* (Campanulaceae) than in coastal populations. Unlike the effect of altitudinal change, coastal influence on AOP of plant populations may be more complex and variations could be due to the species and other environmental factors besides greater sunlight and UV-B exposure.

AOP of flowers of *H. mutabilis* during colour change

Flowers of *H. mutabilis* are white in the morning, pink during noon and red in the evening of the same day. The red flowers remain on plants for several days before abscission occurs. Weight of a single detached flower of *H. mutabilis* was 15.6 g when white, 12.7 g when pink and 11.0 g when red. As there was an overall weight loss in flowers, white petals from newly opened flowers were weighed and kept in sealed Petri dishes to prevent desiccation and allowed to change colour. Measurements were made in the morning (Day 1), afternoon (Day 1) and morning

Table 4 Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves of coastal and inland populations of *Hibiscus tiliaceus* (fresh weight)

Location	Habitat	Co-ordinate	Distance from shore	TPC (mg GAE / 100 g)	AEAC (mg AA / 100 g)
Pantai Jeram I, Selangor	Coastal	N 3° 13' 48" E 101° 18' 16"	15 m	2220 ± 751 ab	2300 ± 598 a
Pantai Jeram II, Selangor	Coastal	N 3° 13' 14" E 101° 18' 18"	10 m	1760 ± 271 a	1650 ± 222 a
Pantai Remis, Selangor	Coastal	N 3° 12' 09" E 101° 18' 22"	20 m	1940 ± 465 ab	1830 ± 525 a
Jalan Kuching, Kuala Lumpur	Inland	N 3° 12' 23" E 101° 40' 15"	42 km	2080 ± 419 ab	2370 ± 539 a
Selayang Baru, Selangor	Inland	N 3° 15' 26" E 101° 9' 22"	40 km	1800 ± 296 a	2010 ± 425 a
Taman Tun Dr Ismail, Selangor	Inland	N 3° 08' 33" E 101° 37' 23"	33 km	2670 ± 300 b	3890 ± 437 b

Values of TPC and AEAC are means ± SD (n = 3). For each column, values followed by the same letter are not statistically different at $p < 0.05$ as measured by the Tukey's HSD test. GAE = gallic acid equivalent, AA = ascorbic acid.

(Day 2). Under laboratory conditions, colour change of petals was slower than that of flowers under outdoor conditions.

The red flowers of *H. mutabilis* had TPC, TAC, AEAC and FRP values of 540 mg GAE/100 g, 43 mg CGE/100 g, 594 mg AA/100 g and 2.5 mg GAE/g which were higher than white and/or pink flowers respectively (Table 5). Increase in TAC of *H. mutabilis* flowers was prominent. TAC of red flowers (43 mg CGE/100 g) was 2.7 times that of pink flowers (16 mg CGE/100 g) and 7.7 times that of white flowers (5.6 mg CGE /100 g). Overall ranking of AOP of *H. mutabilis* flowers was red > pink > white.

Subramanian and Nair (1970) postulated that anthocyanins in pink and red flowers of *H. mutabilis* are synthesized independently since there is no reduction in phenolic content. However, Lowry (1976) suggested that anthocyanins are formed through direct conversion from flavonols as they have structural similarities. This study showed a significant increase in TPC during colour change.

CONCLUSIONS

Among the six *Hibiscus* species screened, AOP in terms of TPC, AEAC and FRP were significantly greatest in leaves and flowers of *H. tiliaceus*. Leaves of species with high TPC and AEAC had low FIC ability and vice versa. The red flowers of *H. rosa-sinensis* and *H. schizopetalus* yielded the greatest TAC and FIC ability. AOP of leaves of highland populations of *H. rosa-sinensis* were greater than those of lowland. There was no distinct variation in TPC and AEAC of leaves between coastal and inland populations of *H. tiliaceus*. The AOP of red flowers of *H. mutabilis* were greater than pink and/or white flowers. Overall ranking of flowers during colour change was red > pink > white.

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Table 5 Total phenolic content (TPC), total anthocyanin content (TAC), ascorbic acid equivalent antioxidant capacity (AEAC) and ferric-reducing power (FRP) of petals of *Hibiscus mutabilis* during colour change (fresh weight)

Day (time)	Colour	TPC (mg GAE/100 g)	TAC (mg CGE/100 g)	AEAC (mg AA/100 g)	FRP (mg GAE/g)
1 (9 a.m.)	White	456 ± 11 a	5.6 ± 1.7 a	517 ± 13 a	2.1 ± 0.1 a
1 (4 p.m.)	Pink	495 ± 23 b	16 ± 1.9 b	562 ± 37 ab	2.4 ± 0.1 b
2 (10 a.m.)	Red	540 ± 9 c	43 ± 3.4 c	594 ± 32 b	2.5 ± 0.1 b

Values of TPC, TAC, AEAC and FRP are means ± SD (n = 3). For each column, values followed by the same letter are not statistically different at $p < 0.05$ as measured by the Tukey's HSD test. GAE = gallic acid equivalent, AA = ascorbic acid, CGE = cyanidin-3-glucoside equivalent.

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