Antioxidant Activity of Methanolic Extracts of *Cassia Surattensis*

S. Sangetha[a], S. Sasidharan[b,*], Z. Zuraini[a], and S. Suryani[a]

[a]*School of Distance Education, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia.*
[b]*Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Jalan Bedong-Semeling, Batu 3 ½, Bukit Air Nasi, Bedong 08100, Kedah, Malaysia.*

E-mail: srisasidharan@yahoo.com

Summary

In this study, the antioxidant properties of *Cassia surattensis* (flowers, leaves, stem and pod) extracts were evaluated through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity and Xanthine oxidase inhibition assay. The IC_{50} values were calculated for the above methods in order to evaluate the antioxidant efficiency of each *C. surattensis* extracts. The phenol contents were also obtained. *C. surattensis* flowers revealed the best antioxidant property, presenting much lower IC_{50} values (423.32µg/ml for DPPH assay and 11.1 µg/ml for xanthine oxidase assay). Furthermore, the highest antioxidant contents (polyphenols) were found for these extracts (657.2392 $\pm$ 2.0321mg GAEs/g extract). The antioxidative activities were correlated with the total phenol. This study suggests that possible mechanism of this activity may be due to free radical-scavenging and antioxidant activities which may be due to the presence of polyphenols in the extracts.

KEY WORDS: Antioxidant activity, *Cassia surattensis,* DPPH, xanthine oxidase

Introduction

There has been interest in the contribution of free radical reaction participating in reactive oxygen species to the overall metabolic perturbation that result in tissue injury and disease. Reactive oxygen such as superoxide anion, hydrogen peroxide, and hydroxyradical are generated in specific organelles of cells (Mitochondria and Microsomes) under normal physiological condition. These reactive oxygen species can damage DNA, so as to because mutation and chromosomal damage oxidize cellular thiols and abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (1, 2). Recently, various phytochemicals and their effect on health, especially the suppression of active oxygen species by natural antioxidant from tea, spices and herbs, have been intensively studied (3, 4)

Cassia surattensis belong to the family of Fabaceae. They are distributed throughout Malaysia. This flowering plants are native to southern Asia, from southern Pakistan east through India to Myanmar and south to Sri Lanka. This *C. surattensis* widely grown as an ornamental plant in tropical and subtropical areas. This plant species has been tradionally used in many countries for food and medicinal use. No local used was known for *C. surattensis,* but the bark and leaves are said to be antiblenorrhagic (5). A property also mention for a decoction of the roots (6). The Balinese rub the leaves of *C. surattensis* into both internal and external cooling medicine (5). Because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest for the use of plant extracts (7). Hence, in this work we studied the antioxidant potential of different extracts of *C. surattensis* (flowers, leaves, stem and pod).

Material and methods

Standards and reagents

Standards; BHT (Butylated hydroxytoluene) and (+)-catechin, were purchased from Sigma (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH_) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were obtained from Sigma

Chemical Co. (St. Louis, MO, USA). Methanol was obtained from Pronalab (Lisbon, Portugal).

Plant collection and extraction

Samples were collected from Penang, Malaysia, on June of 2007. Plants were identified by a botanist of School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Flowers, leaves, stem and pod of selected plants were dried (room temperature) and powdered with a mortar.

Preparation of plant extracts

Some 100g of dried and powdered plant material were extracted at room temperature with 500 mL of methanol under constant shaking for 24 h. After filtration, the methanolic (MeOH) solutions were evaporated to dryness in a rotary evaporator for the antioxidant assays.

Total phenolic content

Contents of total phenolics in the extracts were estimated by a colorimetric assay based on procedures described by Singleton and Rossi (8) with some modifications. Basically, 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (0.250–2.500 mM; Y = 0.2903X; R2 = 0.999) and the results were expressed as mg of gallic acid equivalents/g of extract (GAEs).

DPPH radical-scavenging activity

Various concentrations of *C. surattensis* extracts (50.0 µl) were mixed with 5.0 ml of methanolic solution containing DPPH radicals (0.004% w/v). The mixture was shaken vigorously and left to stand for 30 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of

DPPH discoloration, using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution (9). The extract concentration providing 50% of radical scavenging activity (IC_{50}) was calculated from the graph of RSA percentage against extract concentration. BHT was used as standard.

In vitro xanthine oxidase inhibitory activity

All the 4 extracts of *C. surattensis* were used for the in vitro xanthine oxidase inhibitory **(**XOI) activity. The XOI activity was assayed spectrophotometrically under aerobic conditions (10). The assay mixture consisted of 1ml of test solution (10, 25, 50 and 100 µg/ml), 2.9 ml of phosphate buffer (pH 7.5), and 0.1 ml of enzyme solution (0.01 units/ml in phosphate buffer, pH 7.5), which was pre-pared immediately before use. After preincubation at 25 ^{o}C for 15 min, the reaction was initiated by the addition of 2ml of substrate solution (150mM xanthine in the same buffer). The assay mixture was incubated at 25 ^{o}C for 30 min. The reaction was then stopped by the addition of 1ml of 1N hydrochloric acid, and the absorbance was measured at 290 nm using an UVspectrophotometer. A blank was also prepared in the same way, but the enzyme solution added to the assay mixture after adding 1N hydrochloric acid. The assay was done in triplicate. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid per min at 25 ^{o}C. XOI activity was expressed as the percentage inhibition of XO in the above assay system, calculated as:

$$\% \text{ Inhibition} = \left\{ \frac{(A - B) - (C - D)}{A - B} \right\} \times 100\%$$

where *A* is the activity of the enzyme without test extract, *B* the control of *A* without test extract and enzyme, *C* and *D* are the activities of the test extract with and without XO. Allopurinol (10, 25, 50 and 100µg/ml), a known inhibitor of XO, was used as a positive control. IC_{50} values were calculated from the mean values of data.

Statistical analysis

For all the experiments three samples were analysed and all the assays were carried out in triplicate. The results are expressed as mean values and standard error or standard deviation (SD). The differences between the *C. surattensis* extracts were analysed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with a = 0.05. This treatment was carried out using the SPSS v. 12.0 programme. The regression analysis between phenol contents and IC_{50} values for antioxidant activity used the same statistical package.

Results and Discussion

Table 1 presents polyphenols contents and extraction yields (expressed as w/w percentages), obtained for all the *C. surattensis* extracts. Among all of the extracts analysed, a significant content of total phenolics (>100 mg/g of extract) and good radical-scavenging activity were found for all extracts, except for pod. It became clear that *C. surattensis* flower, stem and leaves present the highest antioxidant activity (Figure 1) compared with reference antioxidant BHT. Despite the low values obtained for the extraction yields, the antioxidant contents found were very good, indicating that the extraction was efficient. Polyphenols was found in all the samples and in the following order: flower > stem > leaves > pod. The coefficients of variation (CV; calculated by the ratio between standard deviation and mean) are also presented. CV values for polyphenol contents, revealed high reproducibility, ranging from 0.202% (stem) to 0.406% (leaves).

Table 1. Extraction Yields, and Contents of Total Phenolics in the Extracts of *Cassia surattensis*, and Corresponding Coefficients of Variation

		Flower	**Stem**	**Leaves**	**Pod**
Extraction yield	(%)	5.57	4.87	3.37	3.87
Polyphenols	(mg/ml)	657.2392 $\pm$ 2.0321[a]	627.3383 $\pm$ 1.265388[b]	367.1327 $\pm$ 1.489704[c]	299.8799 $\pm$ 0.768062[d]
CV	(%)	0.309	0.202	0.406	0.256

In each line, different letters mean significant differences ($p < 0.05$).

The antioxidant properties of the methanol extracts of leaves, flowers, stem and pod of *C. surattensis* were examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Fugure 1 as comparable with known antioxidant BHT. The radical-scavenging activity (RSA) values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of $DPPH^{\bullet}$ solution in the absence of extract at 517 nm. From the analysis of Figure 1, we can conclude that the scavenging effects of flowers, stem and leaves extracts on DPPH radicals were excellent ($P<0.05$), especially in the case of *C. surattensis* flower (93.54% at 1.0 mg/ml). The RSA values were also remarkably good for stem (81.97% at 1.0 mg/ml) and leaves (66.28% at 1.0 mg/ml), but *C. surattensis* pod (45.72% at 1.0 mg/ml) revealed a low value of antioxidant activity compared with BHT (66.23% at 1.0 mg/ml). Nevertheless the overall activity of the raw extracts was higher than that of commercial antioxidant BHT, for the *C. surattensis,* except for pod. Table 2 shows antioxidant activity with IC_{50} values of *C. surattensis* flower, stem, leaves and pod measured by DPPH radical-scavenging assays. Overall, *C. surattensis* flower revealed the best antioxidant properties (significantly lower IC_{50} values = 423.32μg/ml; $p < 0.05$) and the *C. surattensis* pod revealed a very poor antioxidant activity (significantly lower EC_{50} values = 1078.34μg/ml; $p < 0.05$). The obtained results are in agreement with the phenol contents determined for each sample and shown in Table 1. Xanthine oxidase is a flavoprotein, which catalyses the oxidation of hypoxanthine to xanthine and generated superoxide and uric acid (11). It has exhibited that xanthine oxidase inhibitors may be useful for the treatment of hepatic disease and gout, which is caused by the generation of uric acid and superoxide anion radical (12). All extracts exhibit an anti-superoxide activity in a concentration dependent manner (Figure 2; Table 3). At concentrations of 10.00–100.0 μg/mL, all extracts inhibited the superoxide formation with an inhibition rate range from 36.6 to 76.8%. Interestingly, Extract from flower displayed an inhibition effect greater than that of other parts. From 10.00–100.0 μg/mL mg/mL, the xanthine oxidase inhibition effect of all samples was in the order of allopurinol > flower > stem > leaves > Pod. In the xanthine-xanthine oxidase system, the IC_{50} values of all extracts were found to range from 2.7 to 46.9 μg/mL (Table VI). Hence, flower (IC_{50} = 11.1

µg/mL) also showed a stronger antioxidant activity than other parts after allopurinol (IC_{50} = 2.7 µg/mL).

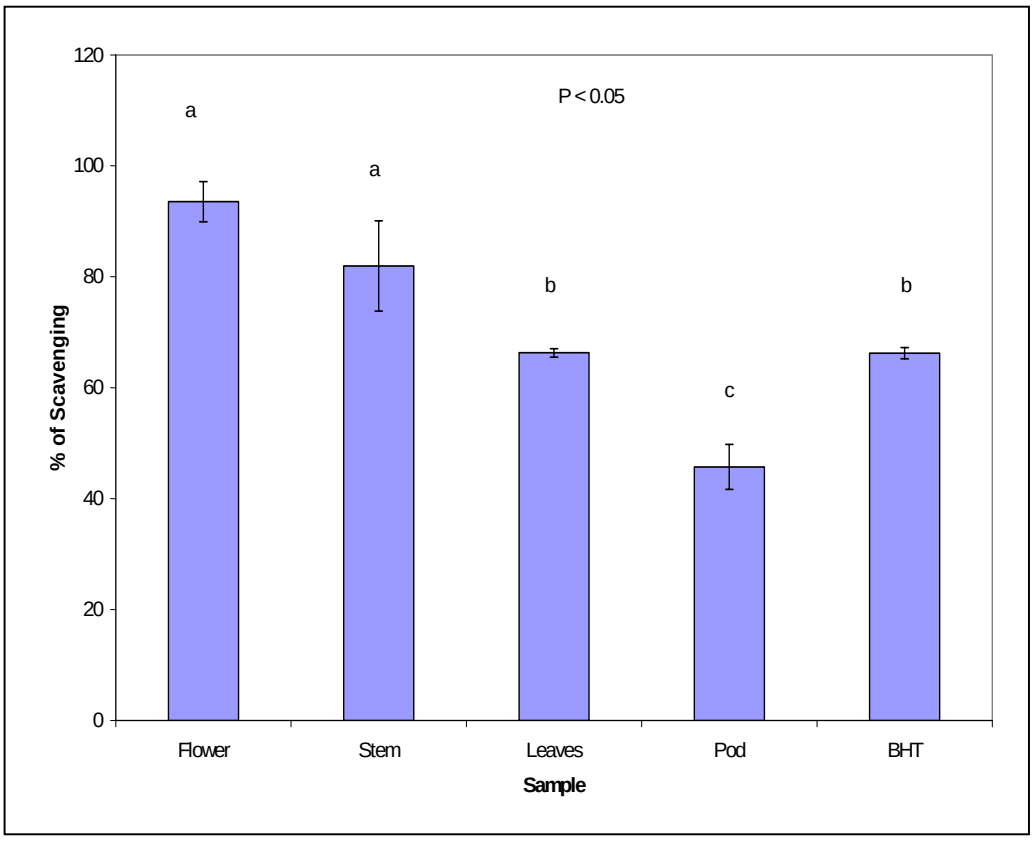

Figure 1. Scavenging effect (%) of extract of *Cassia surattensis* and known antioxidant BHT, at 1.0 mg/ mL

Table 2. IC_{50} Values of the Crude Extract of *Cassia surattensis* for DPPH assay

Extract Concentration µg/mL	**% of Scavenging**			
	Flower	**Stem**	**Leaves**	**Pod**
1200	92.2	85.0	61.6	53.9
900	70.8	60.9	48.8	42.5
600	59.1	46.7	35.0	36.4
300	44.9	24.4	21.8	21.0
*IC_{50}	423.3[a]	684.8[a]	933.8[a]	1078.3[a]

* The IC_{50} was obtained by linear regression equations.
[a] µg/ml

There are many previous works report a significantly negative linear correlation between the polyphenol contents and IC_{50} antioxidant activity values (13-15). This negative linear correlation proves that the samples with highest polyphenol contents show lower EC_{50} values, confirming that phenolics are likely to contribute to the antioxidant activity of the extracts, as has been reported in other species (16). In the present study, despite the high coefficient of correlation values (R^2=0.9015) obtained, proving the existence of correlation showed statistical significance were those gathered for IC_{50} radical-scavenging activity and polyphenols, as can be seen in Table 4.

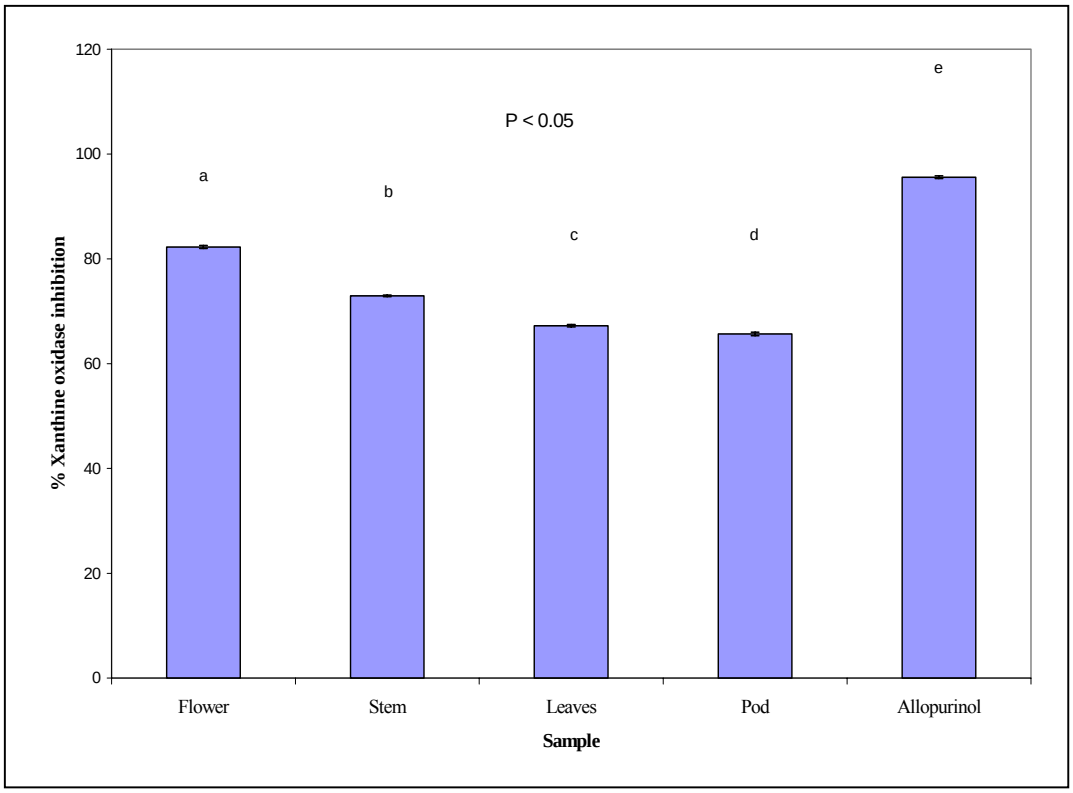

Figure 2. Xanthine oxidase inhibition (%) of extract of *Cassia surattensis* and known antioxidant Allopurinol, at 100.0 µg/ mL

Table 3. IC_{50} Values of the Crude Extract of *Cassia surattensis* for xanthine oxidase assay

Extract Concentration µg/mL	% of Xanthine inhibition				
	Flower	**Stem**	**Leaves**	**Pod**	**Allopurinol**
100	76.8	72.9	67.2	65.7	95.6
50	66.4	70.3	57.7	53.6	73.2
25	62.4	60.5	47.4	43.2	69.1
10	45.7	43.0	42.4	36.6	51.8
*IC_{50}	11.1 [a]	17.1 [a]	32.9 [a]	46.93 [a]	2.7 [a]

* The IC_{50} was obtained by linear regression equations.
[a] µg/ml

Table 4. Correlations Established Between Total Polyphenols with Antioxidant Activity IC_{50} Values (df = 3)

Assay	Polyphenols		
	Equation, R^2	***F***	***P***
RSA	y = -0.5959x + 952.73, 0.9015	18.296	0.05
XOI	y = -0.087x + 69.429, 0.9487	25.791	0.02

Conclusion

As far as we know, this is the first report concerning the antioxidant activity of four (flowers, leaves, stem and pod) different parts extracts of *C. surattensis*. The work herein indicates that the flower of *C. surattensis* present the highest antioxidant activity values. The results obtained indicate a high potential of application for these *C. surattensis* flower extracts as an antioxidant. It can be included in foods with notable benefits for mankind or animal health.

References

1. Halliwell B, Gutteridge JMC. The chemistry of oxygen radicals and other oxygen-derived species. In: Free Radicals in Biology and Medicine. Oxford University Press, New York 1985; pp. 20-64.
2. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. PNAS 1993; 90**:** 7915-7922.
3. Sies H, Stahl W. Vitamins E and C, b-carotene and other carotenoids as antioxidants. Am. J. Clin. Nutr. 1995: 62**:**1315S-1321S.
4. Elmastas M, Gulcin I, Beydemir S, Kufrevioglu OI, Aboul-Enein HY. A study on the in vitro antioxidant activity of Juniper (*Juniperus communis* L.) fruit extracts. Anal Lett. 2006; 39**:** 47–65.
5. Perry LM. 1980. Medicinal Plants of East and Southeast Asia. The MIT Press, Cambridge, MA, 210-211 pp.
6. Burkill IH. A Dictionary of the Economic Products of the Malay Peninsula**.** Government of Malaysia and Singapore by the Ministry of Agriculture and coorperatives, Kuala Lumpur. 1935; p. 481.
7. Calliste CA, Trouillas P, Allais DP, Duroux JL. *Castanea sativa* Mill. leaves as new sources of natural antioxidant: An electronic spin resonance study. J. Agr. Food Chem. 2005; 53**:** 282–288.
8. Singleton VL, Jr. Rossi JA. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 1965; 16**:** 144–158.
9. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extract. Lebensm Wiss Technol. 2003; 36**:** 263-271.
10. Owen PL, Johns T. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. J. Ethnopharmacol. 1999; 64**:** 149–160.
11. Chiang HC, Chen YY. Xanthine oxidase inhibitors from the roots of eggplant (*Solanun melongena* L.). J. Enzyme Inhibit. 1993; 7: 225–235.
12. Lin CC, Huang PC, Lin JM. Antioxidant and hepatoprotective effects of *Anoectochilus formosanus* and *Gynostemma pentaphyllum*. Am. J. Chin. Med. 2000; 28**:** 87-96.
13. González EM, de Ancos B, Cano MP. Relation between bioactive compounds and free radical-scavenging capacity in berry fruits during frozen storage. J. Sci. Food Agr. 2003; 83**:** 722 – 726.
14. Llobera A, Cañellas J. Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. Food Chem. 2007; 101**:** 659-666.
15. Makris DP, Boskou G, Andrikopoulos NK, Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. J. Food Comp. Anal. 2007; 20**:** 125-132.
16. Lecumberri E, Mateos R, Izquierdo-Pulido M, Rupérez P, Goya L, Bravo L. Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). Food Chem. 2007; 104**:** 948-954.