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Free Radical Scavenging Activity of *Cassia spectabilis* and *Cassia fistula*

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Abstract

A selection of medicinal plants Cassia spectabilis and Cassia fistula, collected from Penang Island (Malaysia) was screened for their in vitro antioxidant activity. Different parts (leaves, flowers, stem and pod) of selected plants were dried at room temperature and powdered. Then, the extractions were performed with methanol. The extracts than qualitatively screened for their free radical scavenging activity by employing DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging assays. It was shown that all the methanol extracts of the samples collected were endowed with antioxidant activity, though, as expected, their potency varied according to the different parts. In particular, plants of the Cassia fistula displayed the highest activity and the flower parts of Cassia fistula exhibited the higher antioxidant activity (75.15% at 1.0 mg/ml). In this study, both plants Cassia spectabilis and Cassia fistula were identified as potentially novel sources of free radical scavenging compounds.

Key words: Antioxidant activity, Cassia spectabilis, Cassia fistula, DPPH radical scavenging assay,

INTRODUCTION

Plant-derived material have recently become of great interest owing to their multipurpose applications. Damage mediated by free radicals results in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA, alteration of platelet functions and finally leads to various problems, which have generally been considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis [1-4]. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases [5]. The interest in natural antioxidants from plants has been increasing because of their high capacity in scavenging free radicals related to various diseases [6]. In this respect, and in order to contribute to the knowledge of plants from Malaysia, in the present study, 2 medicinal plants *Cassia spectabilis* and *Cassia fistula* were screened to determine their free radical scavenging and antioxidant activities.

MATERIAL AND METHODS

Plant collection and extraction

Samples of two different plants species of Leguminosae family namely *Cassia spectabilis* and *Cassia fistula* were collected from Penang, Malaysia, in June of 2007. Plants were identified by a botanist of School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Leaves and stems 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.

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay

Quantitative measurement of radical scavenging properties was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (80% (v/v) MeOH as a blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. The commercial antioxidant butylated hydroxytoluene (BHT, Sigma) was used for comparison or as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. DPPH radical's concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = A_o - A_1 / A_o \text{ X100}$$

Where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample [7] (crude extract of Leguminosae plants). The actual decrease in absorption induced by the test compounds was compared with the positive controls.

Statistical analysis

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 extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using the SPSS version 12 programmer.

RESULTS AND DISCUSSION

To find new natural sources of active compounds, we studied the antioxidant potential of different extracts of *Cassia spectabilis* and *Cassia fistula*. Table 1 presents extraction yields (expressed as w/w percentages), obtained for all the extracts. Despite the low values obtained for the extraction yields, the antioxidant contents found were very good, indicating that the extraction was efficient.

Table 1. Extraction yields of 100g *Cassia spectabilis* and *Cassia fistula*

Extraction yield		leaves	flowers	stem	pod
Cassia spectabilis	(%)	5.57	4.98	3.12	3.86
Cassia fistula	(%)	5.27	4.88	3.25	3.75

The antioxidant properties of the methanol extracts of leaves, flowers, stem and pod of *Cassia spectabilis* and *Cassia fistula* were examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Figure 1 and Figure 2 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▪ solution in the absence of extract at 517 nm. From the analysis of Figure 1 and Figure 2, we can conclude that the scavenging effects of all extracts on DPPH radicals were excellent, especially in the case of *Cassia fistula* (75.15% at 1.0 mg/ml for the flower and 70.37% for the pod, at the same concentration). The RSA values were also remarkably good for stem (61.27% at 1.0 mg/ml) and leaves (62.69% at 1.0 mg/ml), but *Cassia spectabilis* revealed a low value of antioxidant activity compared with *Cassia fistula* i.e. 54.29%, 53.28%, 45.17% and 6.18 for flower, stem, leaves and pods respectively at 1.0 mg/ml of extract tested.

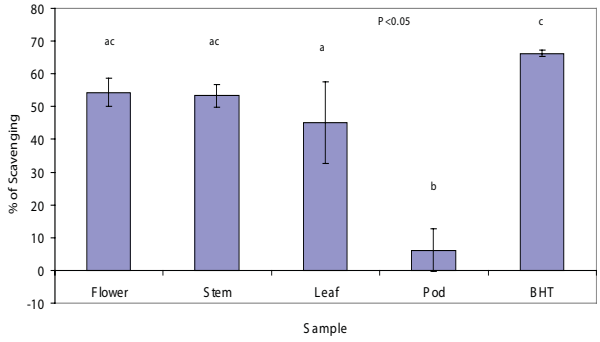

Figure 1. Scavenging effect (%) of crude extract of *Cassia spectabilis* and known antioxidant BHT, at 1.0 mg/ mL

In particular, Flower of *Cassia fistula* displayed the highest activities as antioxidant activity as removal of the stable radical DPPH (Figure 2) and the lowest activity were found in pods of *Cassia spectabilis* (Figure 1). As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT, the reference antioxidant for the *Cassia spectabilis* (Figure 1). Nevertheless the overall activity of the raw extracts was higher than that of commercial antioxidant BHT, for the *Cassia fistula,* except for leaves. In other words, *Cassia fistula* showed the highest antioxidant activity.

In conclusion, the screening of antioxidant activity performed on selected plants from Penang, Malaysia which traditionally used as herbs [8-9] shows that they are endowed with potentially exploitable free radical scavenging activity. Finally, future investigations will concern in-depth analyses (both in terms of chemistry and antioxidant activity) of the isolation and identification of main components and the *in vivo* evaluation of their pharmacology.

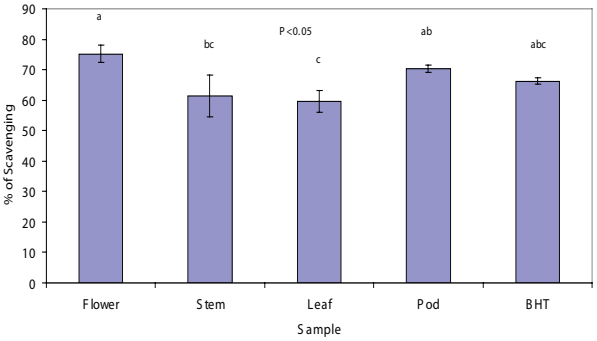

Figure 2. Scavenging effect (%) of crude extract of *Cassia fistula* and known antioxidant BHT, at 1.0 mg/ mL

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