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TOTAL PHENOLICS, TOTAL TANNINS AND ANTIOXIDANT ACTIVITY OF *CASSIA FISTULA* L. EXTRACTS OF BARK, STEM, LEAF AND ROOT UNDER DIFFERENT AGE CLASSES

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ABSTRACT

Indian Laburnum (Cassia fistula L.) bark, stem, leaf and root under different age classes namely Class A (2-3 years), Class B (5-10 years) and Class C (10-15 years) were analyzed for their total phenolic content (TPC), total tannin content (TTC) and antioxidant activity (AA). Both total phenolic content (TPC) and total tannin content (TTC) were determined using Folin-Ciocalteau assays and antioxidant activity (AA) was carried out by Free Radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. From the study, higher TPC, TTC and AA were observed in *Cassia fistula* bark extracts compare to otherportion extracts (stem, leaf and root). Bark extracts from three different age classes, showed total means of 16.67 % TPC and 3.12% TTC. In addition, bark extracts from three different age classes also showed high antioxidant activity (AA) with mean IC_{50} values of 0.04g/ml.

Keywords: Total phenolics; total tannins; antioxidants, age class, *Cassia fistula*

INTRODUCTION

The search for new phenolics and antioxidants from natural sources in order to substitute synthetic chemicals has taken very large attention. Impressive numbers of modern drugs have been isolated from various numbers of plant species which have remarkable pharmacological values, based on their use in traditional medicines. *Cassia fistula* L. belongs to family Leguminosae, their plant organs are well-known for their phenolic compounds[1], which exhibited various pharmacological properties, such as antioxidants[2-4], antifungal[5-6], anti-inflammatory[7], and many other properties. Even though, a wide range of studies have been done on therapeutic and pharmacological values on this plant for different portions over the past few years. Yet, there is less detail study on total phenolic content, total tannin content and antioxidant activity of *Cassia fistula* at different portions (bark, stem, leaf and root) and different age classes till now. Therefore, this study was done in order to determine the total phenolic, total tannins of different portions of *Cassia fistula* L. at different age classes as well as their antioxidant activity.

METHODS AND MATERIALS

Preparation of Cassia fistula Extracts

All the fresh samples (bark, stem, leaf and root) from different age classes and portions were collected from Likas Flora Nursery at Telipok, Sabah. For each age classes, 3 tresswere sampled, therefore total of nine trees were used for this study. Tree samples were separated manually into different portions (bark, stem, leaf and root), followed by sub-portions, namely bottom (B), middle (M) and top (T) for both bark and stem portions only for all nine trees and labeled properly. All samples undergone air-dried for 14 days after washing thoroughly with water. Then, the samples were chipped and ground into powder and sieved around 500 µm. All the samples (powder) were packed properly, sealed and stored in dry places for further study. Fine samples powder of *Cassia fistula* bark, stem, leaf and root (50 g each) from different age classes were extracted with 70% methanol with ratio (1:6 w/v) for 3 hours at 75^{o}C in waterbath based on procedure with modification[8-9].

After extractions, crude extracts were filtrated using Whatman paper No. 1 (150 mm). Then, crude extracts were filled into glass bottle and stored inside the refrigerator under low temperature around 4° C for further analysis.

Total Phenolic Content Determination

Total phenolic compounds of *Cassia fistula* bark, stem, leaf and root extracts from different age classeswere determined according to the procedure using Folin-Ciocalteau reagent[10]. A 0.5 ml of dilute extracts (0.001 g/20 ml) from the different portions and age classes were mixed with Folin-Ciocalteau Reagent (5 ml, 1:10 diluted with distilled water), and aqueous Na_2CO_3 (4 ml, 1M). After incubation for 15 minutes at room temperature, absorbance was measured at 765 nm using UV-spectrophotometer. The phenolic content was calculated using a calibration curve of Gallic Acid (0-25 mg/100 ml). All the experiments were carried out in triplicate and the results averaged.

Total Tannin Content Determination

Total tannin compounds in *Cassia fistula* bark, stem, leaf and root extracts from different age classes were measured and the method was separated into two parts[11]. In Part I, 50 µl of sample extracts (0.01g/20 ml) were packed into a beaker and volume was top up to 1 ml using distilled water. Then, 0.5 ml of Folin-Ciocalteau reagent together with 2.5 ml of $NaCO_3$ (20%) were added into the solutions. The mixture was allowed for 40 minutes at room temperature before measured at 725 nm using UV-spectrophotometer.

In Part II, non-tannins phenols were determined by precipitating tannins with polyvinylpolypyrrolidone (PVPP) which binds tannins. A 200 mg of PVPP was mixed with 2 ml of distilled water and 2 ml of sample extracts in a test tube. The mixture was mixed thoroughly by vortex and before kept for 15 minutes in refrigerator at 4^oC. After that, the mixture was re-vortex and filtered through Whatman filter paper. The 150 µl filtrate was collected in a beaker and again volume was make up to 1 ml by distilled water. As usual, 0.5 ml of Folin-Ciocalteau reagent together with 2.5 ml of $NaCO_3$ (20%) were added into the solutions. After incubation for 40 minutes at room temperature, absorbance was measured using UV-spectrophotometer at 725 nm. Tannic Acid (TA) (0.5 mg/ml) was used to produce a standard calibration curve. The standard must prepare freshly just before used by dissolving 25 mg tannic acid in 50 ml distilled water. Both results in part I (total phenols) and II (non-tannins phenols) were calculated based on standard curve and expressed as mg Tannic Acid/ g dry material. The total tannin content (mg/g) can be calculated by subtracting non-tannin phenols from total phenols and the answer can converted into percentage.

Antioxidant Activity Determination

The antioxidant activity of the *Cassia fistula* bark, stem, leaf and root extracts from different age classes were determined using method reported by Mensor[12]with modification. DPPH reagent was used to investigate free radical scavenging activities of *Cassia fistula* phenolic compounds. Briefly, 0.0118 g DPPH powder was weighted and dissolved in 100 ml of 80% methanol. Three different concentrations of plant extracts (bark, stem, leaf and root) was prepared by dissolving 0.04g, 0.05g, 0.06 g of dry extract (bark, stem, leaf and root) in 10 ml methanol. DPPH reagent (2 ml) plus methanol (1ml) was used as a control. Whereas, 2 ml of DPPH plus 1 ml of plant extracts was considered as sample, and 2 ml of methanol plus 1 ml of plant extracts were served as empty. All the solutions were incubated in dark at room temperature before measured their absorbance using UV-spectrophotometer at 517 nm. Three readings were conducted and the result averaged. The percentage of antioxidant activity (AA) was calculated using equation below, and The IC_{50} stands for the concentration required for the 50 % scavenging activity was calculated as well.

$AA\% = 100-([Abs_{Sample} - Abs_{Empty}]/ Abs_{Control} \times 100)$. Abs= Absorbance

Statistical Analysis

Data analysis was performed on tree age classes, tree portions, total phenolic content, total tannin content, and antioxidant activity using IBM SPSS (Statistical Product and Service Solutions) 21 software.Correlation test was run in order to identify if whether or not significant differences exist.

RESULTS AND DISCUSSION

Total phenolic content (TPC) of Cassia fistula at different portions and age class

The results of Total Phenolic Content (TPC) of *Cassia fistula* bark, stem, leaf and root extracts under different age classes, namely Class A (2-3 years), Class B (5-10 years) and Class C (10-15 years) were shown in Table 1 and Table 2. *Cassia fistula* bark extracts showed the highest content of phenolic compounds, compared to the rest portion extracts (stem, leaf and root). The almost similar trend was observed by previous researcher, showed that highest content of phenolic compounds were present in bark of *Cassia fistula* rather than their fruit pulp, flowers, and leaf[2]. Bark extracts showed 19.21% of TPC higher than stem extracts (5.28%) in age Class A, whereas in age Class B, bark extracts again showed better TPC (16.39%) than stem extracts (6.33%). Meanwhile, in age Class C, bark extracts showed 14.42% of TPC higher than stem extracts (11.31%).

Interestingly, TPC for stem extracts showed an increasing trend from age Class A to C. These most probably because when stem increase with increasing of age, more sapwood in stem will transform into heartwood, which also increase extractives deposits in stem[13], and extractives content has some impact on the concentrations of phenolics[14]. Therefore, it is reasonable to assume that TPC in stem portion extracts might influences by extractives formation within the wood itself during their transition from sapwood into heartwood from age class A to C. Generally, for both bark and stem portion, highest TPC was found at bottom (B) portion, followed by middle (M) and top (T) portion.

Based on Table 2, root extracts showed 10.17% of TPC in age Class A higher than leaf extracts (5.93%), followed by 7.59% of TPC for root extracts and 8.49% of TPC for leaf extracts in age Class B. Whereas in age Class C, root and leaf extracts showed 8.73% and 7.82% of TPC respectively. Statistical analysis showed that, there was significant differences between TPC and tree portions ($r=-0.737$, $p \leq 0.01$). However, no significant differences showed between TPC and age classes.

Table 1: Total Phenolic Content and Total Tannin Content of *Cassia fistula* bark and stem portions at different age classes

Age Class	Portion	Total Phenolic Content (%)		Total Tannin Content (%)	
A 2-3 years	Bark (B)	21.21	19.21	3.416	2.870
	Bark (M)	19.48		2.796	
	Bark (T)	16.95		2.399	
	Stem (B)	5.10	5.28	2.135	2.565
	Stem (M)	4.73		2.342	
	Stem (T)	6.01		3.128	
B 5-10 years	Bark (B)	20.54	16.39	3.499	3.656
	Bark (M)	16.01		3.664	
	Bark (T)	12.61		3.805	
	Stem (B)	9.69	6.33	1.556	2.485
	Stem (M)	4.73		3.069	
	Stem (T)	4.58		2.829	
C 10-15 years	Bark (B)	14.95	14.42	2.490	2.857
	Bark (M)	12.82		2.342	
	Bark (T)	15.49		3.738	
	Stem (B)	9.25	11.31	3.383	2.796
	Stem (M)	15.13		2.920	
	Stem (T)	9.56		2.085	

Table 2: Total Phenolic Content and Total Tannin Content of *Cassia fistula* bark and stem portions at different age classes

Age Class	Portion	Total Phenolic Content (%)	Total Tannin Content (%)
A	Leaf	5.93	1.299
2-3 years	Root	10.17	2.201
B	Leaf	8.49	1.176
5-10 years	Root	7.59	2.333
C	Leaf	7.82	1.325
10-15 years	Root	8.73	1.804

Total Tannin Content (TTC) of Cassia fistula at different portions and age classes

Tannins are widespread in plants, they occur in nearly every plants, in all climates at any parts of plant such as bark, stem, root, leaf, fruit, seed, flower, nut, etc[15]. The results of Total Tannin Content (TTC) of *Cassia fistula* at different portions and age classes were displayed in Table 1 and Table 2. TTC in bark extracts Class A was 2.870%followed by 3.656% in Class B, and Class C bark extracts showed 2.857% of TTC.

Meanwhile, stem extracts showed TTC of 2.565%, 2.485%, 2.796% for age Class A, B, and C respectively (Table 1). Whereas, TTC in root extracts for age Class A, B and C were 2.201%, 2.333% and 1.804% respectively. Under same conditions, leaf extracts showed 1.299%, 1.176% and 1.325% of TTC for age Class A, B and C respectively (Table 2). In this work, bark portion extracts showed highest content of tannins compared to stem, leaf, and root extracts these is especially true as most tress contain plenty of tannins, those tannins were concentrated in the bark layer where it formed a layer against microorganism such as fungi and bacteria[15]. Additionally, *Cassia fistula* bark is actually very rich in tannins content[16]. Therefore, this has probably contributed to high TTC in bark extracts compared to others portion extracts as observed in this study. Besides, stem, root and leaf extracts also showed good promising contents of tannin compounds in general. Previous studied showed that, tannins in bark, stem, root and leaf are actually play a role as "protector" against microorganism (fungi, bacteria, pathogens) and also predators[15].

In this work, no significant differences showed between TTC and age classes ($r=0.840$, $p \geq 0.05$). However, there were significant correlation between TTC and total phenolic content (TPC) ($r=0.475$, $p \leq 0.01$) and TTC with tree portions ($r=-0.593$, $p \leq 0.01$). Since tannins are phenolic compounds, therefore phenolic compounds might somehow influences the tannins content of different portions extracts for *Cassia fistula* under different age classes. However, due to complexity of the tannins itself, further study should be done in order to obtain a more integrated picture relationship between TTC with tree portions, age classes and phenolic contents.

***Antioxidant Activity* (AA)**

DPPH radicals have been applied widely in order to investigate the scavenging activities of natural compounds. Colorof reaction mixture change from purple to yellow when DPPH radicals were scavenged. Table 3 showed the IC_{50} of *Cassia fistula* bark, stem, leaf and root extracts at different age classes by using DPPH Free Radical Scavenging Method.

Generally, bark extracts showed the highest antioxidant activity (AA) compared to stem, leaf and root extracts. Results in Table 3, showed that the highest AA was observed at age Class C, whereby bark portions bottom (B), middle (M) and top (M) showed IC_{50} values of 0.038 g/ml, 0.041 g/ml and 0.037 g/ml respectively. Whereas, stem extracts bottom (B), middle (M) and top (T) at age Class C showed IC_{50} values of 0.039 g/ml, 0.038 g/ml and 0.039 g/ml respectively. Meanwhile, antioxidant activity observed in leaf and root extracts showed an increasing trend from age Class A to C. Both leaf (IC_{50}: 0.040 g/ml) and root (IC_{50}: 0.039 g/ml) showed their highest antioxidant activity at age Class C. However, antioxidant activity of root extract (IC_{50}: 0.039 g/ml) was slightly better than leaf extracts (IC_{50}: 0.040 g/ml) with lower IC_{50} values.

Table 3: IC_{50} of bark, stem, leaf and root extracts of *Cassia fistula* at different age classes

Age Class	Portion	IC_{50} (g/ml)
A 2-3 years	Bark (B)	0.041
	Bark (M)	0.042
	Bark (T)	0.041
	Stem (B)	0.043
	Stem (M)	0.042
	Stem (T)	0.041
	Leaf	0.050
	Root	0.057
B 5-10 years	Bark (B)	0.037
	Bark (M)	0.041
	Bark (T)	0.042
	Stem (B)	0.040
	Stem (M)	0.040
	Stem (T)	0.039
	Leaf	0.048
	Root	0.041
C 10-15 years	Bark (B)	0.038
	Bark (M)	0.041
	Bark (T)	0.037
	Stem (B)	0.039
	Stem (M)	0.038
	Stem (T)	0.039
	Leaf	0.040
	Root	0.039

Statistical analysis indicated that there was significant differences between tree portions (bark, stem, leaf and root) with AA ($r=0.415$, $p \leq 0.01$). Besides, statistical analysis also showed that there was significant correlation between total tannin content (TTC) with AA ($r=0.428$, $p \leq 0.01$). Since tannins are natural antioxidant[17], therefore tannins present in bark, stem, leaf and root of *Cassia fistula* might contribute to their antioxidant activity (AA) as well. As we know that, *Cassia fistula*belongs to family Leguminosae. According to what mentioned by others researcher previously, polyphenolic compounds such as flavonoids, antraquinones, anthrocyanidins, xanthoses showed remarkable antioxidant properties are present quite common in plants of this family[2]. Thus, there is possibility that all this bioactive compounds present in different portions extracts of *Cassia fistula* act either individually, synergy or both in order to give rise to their antioxidant properties based on what's been shown in this work. Meanwhile, previous studied also found that bark of *Cassia fistula* possessed high antioxidant activity due to present of rich phytochemicals constituents with powerful inhibitors properties which may act as primary antioxidants that react with free radicals[2,4].

CONCLUSIONS

The study showed that variation of TPC, TTC and AA of *Cassia fistula* were mainly influenced by the tree portion rather than age classes. Overall, bark portions extracts showed the most promising values for TPC, TTC and AA test as compared to other extracts. Meanwhile, stem, leaf and root extracts also showed remarkable TPC, TTC and AA which compatible with bark extracts as well. Further study should be done in other to determine more about relationship that possible exist between TPC, TTC and AA with others possible factors such as reaction time, solvents concentration, temperature, etc. by using different genus of species.

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