Antioxidant Activity of Thebu (*Costus speciosus*) Rhizome and Leaves

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Abstract
Thebu (*Costus specious*) is an important medicinal plant that is freely available in Sri Lanka. The rhizomes of Thebu plant is a rich source of antioxidant. But due to the damage cause to the plant, use of rhizomes in commercial production of antioxidants is limited. Thus, in the current study, use of tender and mature leaves to extract natural antioxidants was investigated. The antioxidant potential of hexane extracts of tender leaves, mature leaves and rhizomes were evaluated using DPPH radical scavenging and ferric reducing power assays. The total phenolic content and flavonoid contents of each extract were also measured. The total phenolic content of tender leaves, mature leaves and rhizomes from Galle & Colombo districts were more or less similar (0.02 – 0.03 mg gallic acid eq/g extract). On the other hand, the hexane extract of mature leaves from both districts showed significantly higher amount of flavonoid content (6.36 ± 2.66, 6.86 ±1.47 mg quercetin eq/g extraction Galle and Colombo respectively) than tender leaves. Compared to tender leaves and rhizomes, particularly higher amount of DPPH radical scavenging activity was exhibited by mature leaves from both Galle and Colombo districts with 1.37±0.38, 1.71±0.6 mg trolox eq/g extract respectively). The results indicate that mature leaves of *C. specious* are rich in total flavonoid and DPPH radical scavenging activity than the rhizomes. Thus mature leaves of Thebu plant can be used in commercial production of natural antioxidants.

Keywords: Antioxidant activities, *Costus speciosus,* Medicinal value. Natural beverage
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Introduction

Costus speciosus which is locally known as Thebu, is an important medicinal plant with a great importance to the health of people. It is also cultivated as an ornamental plant. The rhizomes and roots of *C.speciosus*is used to improve digestion, and alsoacts as a stimulant herb that clears toxins. In addition to that rhizome is given for curing chronic diseases. (Islam *et al.*, 2010).

It has been shown that the rhizomes of *C.speciosus* are rich in antioxidant components including phenolic compounds. These compounds play a vital role in the body defense mechanism against oxidative stress, arising from the formation of free radicals that leads to various kinds of hazardous diseases. Production of free radicals is balanced by antioxidative defense system in a healthy body. As a consequence of various research processes, synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), are widely used in food industry to prevent oxidative deterioration. However, it is suspected that BHA and BHT are responsible for liver damage and carcinogenesis. In these circumstances, development and utilization of natural antioxidants such as vitamin C, vitamin E, carotenes, phenolic acids, and phytoestrogens are desired (Blois, 1958).
Since this plant is widely available in Sri Lanka, it is imperative to investigate the antioxidant activity of *C. speciosus* plants that grown in Sri Lanka too. Even though the extract of rhizome is rich in antioxidants, use of rhizomes for commercial production of natural antioxidants is destructive to the plant. Therefore, it is beneficial to seek whether the young and mature leaves of *C. speciosus* are also having the same antioxidant effect as in rhizomes which aid human health.

Thus, the current study was conducted to investigate and compare the antioxidant activities of different plant parts (tender leaves, mature leaves, and rhizomes) of *C. speciosus* grown in Sri Lanka through two different antioxidant activity assay methods.

Materials and Methods

C. speciosus were collected from Colombo and Galle districts with six replicates. The sun dried and chopped mature leaves, tender leaves, and rhizome (15g each) were extracted separately with hexane for 3 hr using soxhlet extraction. The extracts were evaporated to dryness, and the stock solution of each extract was prepared by dissolving them in DMSO and 70% ethanol in a final concentration of 20 mg/ml.

The concentrations of total phenolic content in all extracts were determined with Folin-Ciocalteu reagent (FCR) method. 30 µl of each sample was mixed with 110 µl of 10 times diluted FCR and 70 µl from 10% sodium

carbonate solution in micro plate. It was incubated at room temperature for 30 min and absorbance was recorded at 765 nm. Gallic acid was used as the standard. DPPH free radical scavenging assay was performed based on the methods described by Blois (1958). 50 µl from each sample was mixed with 90 µl of methanol in micro plate, and the pre-plate reading was recorded at 517 nm. After adding 60 µl from 20 mg/ml DPPH solution (in methanol), the reaction mix was incubated at 25±2 °C in dark. Ten minutes later, the absorbance was measured at 517 nm.

Ferric reducing antioxidant power (FRAP) assay was performed using the method described by Kucuk *et al.* (2007). 20µl of each sample was mixed with 30 µl of 300mM acetate buffer (pH 3.6), 150 µl of FRAP reagent, in a micro-plate and incubated at room temperature for 8 min. Absorbance was recorded at 600 nm. Trolox was used as the standard. The flavonoids content was determined by aluminum chloride method described by Woisky and Salatino, (1979) with some modifications. 100 µl of each sample was mixed with 100 µl of 2% aluminum chloride in methanol. After getting the pre-plate reading, the reaction mix was incubated at room temperature for 10 min. Finally, the absorbance was measured at 415nm. Quercetin was used as the standard.All data on antioxidant activity tests have included according to the average of triplicate analysis. Collected data was subjected to ANOVA, and the means were compared by Duncan's New Multiple Range Test (DMRT) using SPSS software (SPSS version 16.0 for window, SPSSInc. Chicago, IL, USA).

Results and Discussion

In this study, the antioxidant activity of young leaves, mature leaves, and rhizomes of *C. speciosus* was measured by both DPPH radical scavenging activity and FRAP method. In addition, the total phenolic and flavonoid contents of the extracts were also measured (Table 1).

Total phenolic and flavonoid contents of the Thebu leaf and rhizome extracts are presented in Table 1. Phenolic compounds are known as potential antioxidants and free radical scavengers. Also they play an important role in stabilizing lipid peroxidation. Mature leaves from both districts showed a significantly higher amount of flavonoids, which is a diverse natural phenolic compound with diverse chemical and biological activities including radical scavenging properties.

Free radicals are having a significant effect on oxidation of unsaturated lipids. In DPPH radical scavenging method, DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds. The difference between the extracts and control was statistically significant ($p < 0.05$). Mature leaves of the plant extract show particularly higher amount of DPPH radical scavenging activity (1.37 ± 0.38, 1.71±0.6mg trolox eq/g extract in Galle and Colombo districts respectively) among all the tested extracts, followed by tender leaves (1.05± 0.39, 1.33 ± 0.24 mg trolox eq/g extract in Galle and Colombo districts respectively) and rhizome (0.88 ± 0.23, 1.60 ± 0.76 mg trolox eq/g extract in Galle and Colombo districts respectively). All three extracts of *C. speciosus* from Colombo district showed higher DPPH radical scavenging activity as well as higher phenolic and flavonoid content which could be due to the changes in soil and climatic conditions. However, there are no available literature on comparing antioxidant activities of leaves and rhizome of the *C. speciosus* plant. According to the research findings that compared leaves and rhizome of *Picrorhiza kurroa* (Agnihotri, *et al.*, 2012) indicate that leaves are rich in antioxidant activity compared to rhizome. On the other hand, the high ferrous iron chelating activity of mature leaves may be the reason for the experienced results in this

Table 1: Total phenolic content and total flavonoid content of *C. speciosus* samples collected from Galle and Colombo districts.

Location	Total phenol content (mg gallic acid eq/g extract)		Total flavonoid content (mg quercetin eq/g extract)	
	Galle	Colombo	Galle	Colombo
Tender leaves	0.02^{a}±0.00	0.02^{a}±0.00	2.94^{b}±1.49	2.97^{b}±1.39
Mature leaves	0.02^{a}±0.00	0.03^{a}±0.00	6.36^{a}±2.66	6.86^{a}±1.47
Rhizome	0.03^{a}±0.02	0.03^{a}±0.01	1.53^{b}±0.90	2.25^{ab}±1.65

Values in a column with same superscript letters are not significantly different ($p<0.05$)

study. Unfortunately the ferric reducing power assay (FRAP) method was not success due to formation of turbid compound during assay. Although, it is unable to describe, past studies showed that FRAP assay gives better results for ethanolic extracts than hexane extracts Reference needed.

The results presented in this study are the first information on the antioxidant activities of leaves of *C. speciosus*. According to the results, it is evident that the mature leaves of Thebu plant from both districts are having a higher flavonoid content and DPPH radical scavenging activity compared to rhizome. In conclusion, the results revealed the importance of antioxidants of Thebu mature leaves over rhizomes. Thus, the mature leaves of Thebu plant may helped to protect against lipid peroxidation and free radical damage, and its extracts will probably be a safe food additive. However, future studies on this regard are essential.

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