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Research Article

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Comparison of antioxidant properties of *Cyathula prostrata* Linn and *Achyranthes aspera* Linn grown in Sri lanka

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Abstract

Cyathula prostrata Linn (Family:Amaranthaceae) is widely used to stop the bleeding of patients suffering from piles (hemorrhoids). *C. prostrata* cannot be found universally and it grows only in areas which has wet climatic condition. *Achyranthes aspera* Linn is also belonging to plant family Amaranthaceae and Sri Lankan Ayurvedic physicians are used this plant as a substitute for *C. prostrata*. Not like *C. prostrata, A. aspera* is found on road sides, field boundaries and waste places as a weed throughout the country. As Ayurvedic pharmacodynamics properties are similar in both *C. prostrata* and *A. aspera* an attempt was made to evaluate the suitability of using *A. aspera* as a substitute for *C. prostrata* in terms of (a) phytochemical screening and (b) *in vitro* antioxidant activities. Results revealed that saponins, phenolic compounds, flavonoids and alkaloids were present in both plants. Antioxidant activities of ABTS [2,2-azino-bis (3 ethylbenzothiazoline-6-sulfonicacid) diammonium salt], DPPH (1,1-diphenyl-2-picrylhydrazyl**)** and FRAP (Ferric reducing antioxidant power) are almost similar in both *C. prostrata* and *A. aspera*. However, total phenolic content, total flavonoid content and ORAC (Oxygen radical absorbance capacity) activities were higher in *C. prostrata* hot water extract than that of *A. aspera*. In conclusion, considering the similarities of phytochemical constituents and antioxidant activities of two plants, *A. aspera* can be used as a substitute for *C. prostrata.*

Introduction

Piles (Hemorrhoids) are swelling or inflammation that develops inside and around the back passage (anal canal) [1]. Etiology of hemorrhoids may be increased straining, hypertension, obesity or pregnancy [2]. In Sri Lanka, decoction of *Cyathula prostrata* Linn (Sinhala name: Rath-Karalheba), which belong to the plant family Amaranthaceae is widely used to stop the bleeding of patients suffering from hemorrhoids. *C. prostrata* is a decumbent herb to 1 m, branched from base; stem striate, stoloniferous, rooting at lower nodes, thickened at nodes reddish or purplish. Leaves are opposite and bisexual flowers are in groups of 3-5 [3]. However, *C. prostrata* cannot be found universally and it grows only in area which has wet climatic condition. *C. prostrata* gets easily spoil with the atmospheric humidity unless it is stored in air tight bags. Therefore, *C. prostrata* is not available in the local market. *Achyranthes aspera* Linn (Sinhala name Gas-Karalheba) is also belonging to plant family Amaranthaceae. Sri Lankan Ayurvedic physicians use this plant as a substitute for *C. prostrata. A. aspera* is found on road sides, field boundaries and waste places as a weed throughout in Sri Lanka. It is a perennial herb, with a few spreading branches, cylindrical, hairy, internodes 8- 12.5 cm long, striate, nodes are bulged, often tinged with pink colour. Leaves are simple, and flowers are arranged in long spikes form in inflorescences with white small flowers [3]. Decoctions made out of whole plants of *C. prostrata* and *A. aspera* are used as a remedy for hemorrhoids. Ayurvedic pharmacodynamics properties are similar in both *C. prostrata* and *A. aspera* for instance both plants have potency of *ushna* (hot), pacify *vata* and *kaphadosha*. Therefore, an attempt was made to evaluate the suitability of using *A. aspera* as a substitute for *C. prostrata* in terms of (a) phytochemical screening and (b) *in vitro* antioxidant activities.

Materials and methods

Collection and identification of plant materials

Whole plants of *C. prostrata* and *A. aspera* were collected from home gardens in Gampaha district, Sri Lanka between October to December 2014. The plant materials were identified and authenticated by Prof. M.H.A. Tissera, Head of Dravyaguna Department, Gampaha Wickramarachchi Ayurveda institute, University of Kelaniya, Sri Lanka. Voucher specimens (CP-1 and AS -1) were deposited in Dravyaguna Department, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Preparation of hot water extract

Each plant material (60 g) was added to a vessel containing 1.92 L of distilled water and reduced the volume up to 240 ml under mild heat. After that, extract was filtered and filtrate was concentrated under vacuum and freeze dried (yields: 10.5 % w/w and 12.2 % w/w for *C. prostrata* and *A. aspera* respectively).

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Phytochemical screening

Phytochemical screening tests for saponins, phenols, flavonoids, glycoside and alkaloids were performed to hot water extracts of *C. prostrata* and *A. aspera* according to the methods described by Yadar and Agarwala [4].

Total flavonoid content (TFC)

Different concentrations (125, 62.5, 31.25, 15.62, 7.81 and 3.90 µg/ml) of *C. prostrata* and *A. aspera* hot water extracts were prepared in methanol. One hundred microliters of 2% aluminium chloride in methanol solution was added to 100 µl of *C. prostrata* or *A. aspera.* The mixture was incubated at room temperature (25 ± 2 °C) for 10 minutes and absorbance was recorded at 415 nm. Pre-plate reading was recorded before adding the aluminium chloride solution. Five different concentrations of quercetin were used to construct the standard curve. TFC of extracts expressed as mg quercetin equivalents per gram of extract [5].

Total polyphenolic content (TPC)

Different concentrations (125, 62.5, 31.25, 15.62, 7.81 and 3.90 µg/ml) of *C. prostrata* and *A. aspera* hot water extracts were prepared in methanol. Twenty microliters of *C. prostrata* or *A. aspera* hot water extract was added to 110 µl of ten times diluted freshly prepared Folin-Ciocalteu reagent and preplate reading was taken. Then seventy microliters of sodium carbonate solution was added to the mixture and incubated at room temperature (25 ± 2 0C) for 30 minutes. The absorbance was recorded at 765 nm. Five different concentrations of gallic acid were used to construct the standard curve. TPC of extracts expressed as mg gallic acid equivalents per gram of extract [6].

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

Reaction volumes of 200 µl of DPPH radical (125 µM) and 50 µl of different concentrations (125,62.5, 31.25, 15.62 and 7.81 µg/ml) of *C. prostrata* or *A. aspera* hot water extract mixed and incubated at 25 ± 2 ^{0}C for 15 minutes and the absorbance was recorded at 517 nm. Five different concentrations of Trolox were used to construct the standard curve. Results were expressed as Trolox equivalents antioxidant capacity in mg Troloxequivalents per gram of extract on dry weight basis [7].

2,2-azino-bis (3 ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) assay

A stable stock solution of ABTS radical cation was produced by reacting 10 mM of ABTS in potassium persulfate at 37 °C for 16 h in dark. Reaction volume of 200 µl, containing 40 µM of $ABTS^+$ radical and 50 µl of 31.25, 15.62, 7.81, 3.90 and 1.95 µg/ml of different concentrations of *C. prostrata* or *A. aspera* hot water extract was incubated at 25 ± 2 °C for 10 min. and the absorbance was recorded at 734 nm. Five different concentrations of Trolox were used to construct the standard curve. Results were expressed as Trolox equivalents antioxidant capacity in mg Trolox equivalents per gram of extract on dry weight basis [8].

Oxygen radical absorbance capacity (ORAC)

In brief, reaction volume of 200 µl, containing 100 µl of 4.8 µM fluorescein and 50 µl of 15.62 and 7.81 µg/ml of different concentrations of *C. prostrata* or *A. aspera* hot water extract was pre-incubated at 37 °C for 10 min. followed by addition of 50 µl of potassium persulphate, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH;40 mg/ml), to each well to initiate the reaction. The plate was placed on the fluorescent microplate reader set with excitation and emission at 494 nm and 535 nm and decay of fluorescein was recorded in 1 minute interval for 35 minutes. Trolox was used as a standard antioxidant. ORAC activities of the extracts were calculated by comparing the net area under curve of fluorescein decay between the blank and the extracts. Results were expressed as ORAC values in mg of Trolox equivalents gram of extract on dry weight basis [9].

Ferric reducing antioxidant power (FRAP)

In brief, reaction volume of 200 µl containing 150 µl working FRAP reagent, 30 µl acetate buffer and 20 µl of 31.25 µg/ml *C. prostrata* or *A. aspera* hot water extract was incubated at room temperature (30 ± 2 °C) for 8 minutes and the absorbance was recorded at 600 nm. Six different concentrations of Trolox were used to construct the standard curve. Results were expressed as Trolox equivalents antioxidant capacity in mg Trolox equivalents per gram of extract on dry weight basis [10].

Data analysis

Data of each experiment were statistically analyzed using SAS software version 6.12. One way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used to determine the differences among treatment means. $P<0.05$ was regarded as significant.

Results and discussion

Antioxidant activities of *C. prostrata* and *A. aspera* hot water extracts were evaluated by using *in vitro* assays: TPC, TFC, FRAP, DPPH and ABTS radical scavenging activity and ORAC. DPPH is a stable radical which gives a dark purple color at 517 nm. When DPPH radical react with a antioxidant, its purple color is disappeared and gives pale yellow color at 517 nm [11]. In ABTS assay, ABTS and potassium per-sulphate produce ABTS radical cation which gives blue green color at 734 nm. The green blue color of the ABTS radical will be converted to colorless when presence of antioxidants [12,13]. The capacity of a compound to scavenge peroxyl radicals was estimated in the ORAC assay. ORAC value is directly proportional to degree of antioxidant power [11]. FRAP assay can be used to measure the antioxidant power of compound/s [14].

According to the results, antioxidant activities of FRAP, DPPH and ABTS are almost similar in both *C. prostrata* and *A. aspera*. However, TPC, TFC and ORAC activities were higher in *C. prostrata* hot water extract than that of *A. aspera*. *In vitro* antioxidant activity of *A. aspera* grown in India was evaluated for different parts of the plant including stems, roots and leaves, roots, stems, leaves, inflorescence and leaves, stems, roots [15-18]. However, antioxidant activity was not evaluated for *A. aspera* grown in Sri Lanka. Similarly, antioxidant activity was evaluated for *C. prostrata* grown in Nigeria using the ethanol and methanol extracts prepared from the whole plant [19,20].

In the present study, FRAP and DPPH scavenging activities of *C. prostrata* and *A. aspera* were not significantly different from each other. Moreover, ABTS scavenging activities of *C. prostrata* and *A. aspera* were almost similar to each other (Table 1). However, ORAC, TFC and TPC of *C. prostrata* was significantly higher than that of *A. aspera* (Tables 1 and 2). Secondary metabolites such as saponins, phenolic compounds, flavonoids and alkaloids were present in both plant extracts and glycosides were not found in either *C. prostrata* or *A. aspera*. In the present study, mg Trolox equivalents per gram of extract were significantly higher in ABTS assay than that of DPPH assay. This may be that DPPH assay is capable of measuring only the hydrophilic antioxidants whereas ABTS assay measures both hydrophilic and

Table1. *In vitro* antioxidant activities of *Cyathula prostrata* Linn and *Achyranthes aspera* Linn

	In vitro antioxidant assays			
	FRAP (mg Trolox equivalents/g of extract)	ORAC (mg Trolox equivalents/g of extract)	DPPH (mg Trolox equivalents/g of extract)	ABTS (mg Trolox equivalents/g of extract)
Cyathula prostrata Linn	25.68 ± 0.97[a]	52.29 ± 1.58[b]	4.71 ± 0.17[d]	36.81 ± 1.80[e]
Achyranthes aspera Linn	20.60 ± 0.95[a]	23.03 ± 0.09[c]	4.24 ± 0.17[d]	32.01 ± 0.24[f]

Data presented as mean ± SEM. FRAP, ORAC, DPPH and ABTS n=4 each

Table 2. Total polyphenolic and Total flavonoid contents of *Cyathula prostrata* Linn and *Achyranthes aspera* Linn

	Total polyphenolic content (mg gallic equivalents/g of extract)	Total flavonoid content (mg quercetin equivalents/g of extract)
Cyathula prostrata Linn	15.1 ± 2.13	9.60 ± 0.10
Achyranthes aspera Linn	3.36 ± 0.20	2.86 ± 0.60

Data presented as mean ± SEM. n=4 each

lipophilic antioxidants [8]. According to Ibrahim and co-workers [20], *C. prostrata* grown in Nigeria had no antioxidant activity in DPPH assay. However, in the present study, *C. prostrata* grown in Sri Lanka has been shown an antioxidant activity in DPPH assay. Furthermore, almost similar quantity of phenolic compounds were present in *C. prostrata* grown in Nigeria (16.95±3.32 mg gallic acid equivalents/g extract) and Sri Lanka (Table 2).

Conclusion

Hot water extracts of *C. prostrata* and *A. aspera* have shown marked *in vitro* antioxidant activities. Antioxidant activities of FRAP, DPPH and ABTS are almost similar in both *C. prostrata* and *A. aspera*. However, TPC, TFC and ORAC activities were higher in *C. prostrata* hot water extract than that of *A. aspera*. Saponins, phenolic compounds, flavonoids and alkaloids were present in both *C. prostrata* and *A. aspera*. When considering the similarities of phytochemical constituents and antioxidant activities of two extracts, *A. aspera* can be used as a substitute for *C. prostrata.*

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