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Short Communication

TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF THE SRI LANKAN ENDEMIC PLANT GENUS *SCHUMACHERIA*

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ABSTRACT

Objective: *Schumacheria*is a genus endemic to Sri Lanka distributed in lowland rain forests and mountain rain forests. The total polyphenol content and antioxidant and cytotoxic activities of hexane, dichloromethane and methanol extracts of stem-bark, root-bark, leaves and flowers of the three representative plant species*S. castaneifolia*, *S. alnifolia* and *S. angustifolia* belonging to the endemic genus *Schumacheria*are reported.

Methods: Antioxidant activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging assay. The total polyphenol content, expressed as the gallic acid equivalent, was determined using theFolin-Ciocaltue method and the cytotoxic activity was determined using the brine shrimp (*Artemiasalina*) assay.

Results: The methanol extract of *S. castaneifolia* flowers (IC_{50} = 6.8±0.1 ppm)and the methanol extracts of *S. alnifolia* stem-bark (IC_{50} =7.1±0.4 ppm) and leaves (IC_{50} =8.3±0.3 ppm) showed antioxidant activity higher than that of α-tocopherol(IC_{50} = 10.9±4.3 ppm). The methanol extracts of *S. alnifolia* stem-bark (69.3±6.9 mg g^{-1}) and leaf (57.7±0.0 mg g^{-1}) extracts showed the highest polyphenol content closely followed by the methanol extracts of *S. castaneifolia* flowers (71.2±0.6 mg g^{-1}). The highest cytotoxic activity was exhibited by the methanol extract of *S. castaneifolia* flowers (LC_{50} = 1.3±0.7 ppm), and all the other extracts showed comparatively less cytotoxic activity.

Conclusion: *S. castaneifolia* and *S. alnifolia* exhibit potent bioactivitiesvalidating the ethnomedical claims where the former has been used for oral aphthous.

Keywords: *Schumacheria*, Endemic genus, Total polyphenols, Antioxidant, Cytotoxic.

In terms of the richness of its flora, Sri Lanka is a biodiversity hotspot. Out of the 3210 flowering plants recorded from the island, 916are endemic. As for the non-flowering plants such as lichens and mosses, whose accurate numbers are yet to be determined, new species continue to be reported [1, 2]. Considering the potential medicinal value of Sri Lankan plants, thus far there have been only a relatively few attempts at screening for biologically active compounds [3-6]. However, Sri Lankan plants have yielded alkaloids [7, 8], terpenoids [9, 10], iron chelating lichen hydroxybenzoic acids [11, 12],saponins [7], butenolides [13], and phenolic acids [14] all of which have shown potent biological activity.

The genus *Schumacheria* belonging to the family Dilleniaceae is an endemic genus, comprising of three species, namely *S. alnifolia* Hook. f. & Thoms., *S. angustifolia* Hook. f. &Thoms., and *S. castaneifolia* Vahl. All three species of genus *Schumacheria*are considered as relic plant species and their evolution probably started during the late Cretaceous and early territory period in the Gondwanaland about 100 to 120 million years ago [15]. They are erect or scrambling evergreen shrubs or small trees, [16] currently found in lowland-rainforest and mountain-rainforest of Sri Lanka. The two species *S. alnifolia* and *S. angustifolia* are extremely limited in their distribution and are endangered,while *S. castaneifolia* is common,but restricted to the lowland rainforest [17, 18]. As they have not been evaluated previously, it is important that proper systematic bioactivity studies of these three endangered species of *Schumacheria*are carried out. We report herein, the antioxidant and cytotoxic activities and the total polyphenol content of the three representative species of the endemic genus *Schumacheria.*

The plant material of the three species was collected in 2011;*S. castaneifolia* from Thummodara region in Rathnapura; *S. alnifolia* at Fishing-hut in Maskeliya and*S. angustifolia* from Haycock Hillin Hiniduma. The taxonomic identification of plant species was carried out by Dr. D. S. A. Wijesundara, Royal Botanic Gardens, Peradeniya. The specimens have been deposited in the National Herbarium of the Royal Botanical gardens, Peradeniya.

Plant materials were separately cleaned, air dried, ground, sequentially extracted into hexane, dichloromethane and methanol using a bottle-shaker, and the solvent extracts were concentrated to provide the dry crude extract. The methanol extracts (0.5 g) of *Schumacheria* plant specimens were separately dissolved in 70 % methanol-water solution (25.0 ml) and were centrifuged at 1500 g for 10 min. The resulting supernatants were evaluated for the total polyphenol content.

The total polyphenol content was determined by mixing a 10 fold diluted Folin-Ciocaltue reagent (1.0 ml) and the extract(200 µl); after 8 minutes, Na_2CO_3 [7.5 % (m/v)] aqueous solution (800 µl) was added, kept for 1 h and the UV absorbance measured at 765 nm. A calibration plot using standard gallic acid ranging in concentration from 1.0 to 20.0 ppm was obtained. The total polyphenol content of commercially available branded tea was determined and all data were represented equivalent to gallic acid (GAE). A commercially available tea extract (0.5 g) dissolved in 70% methanol-water (25 ml) was used as a positive control.

The antioxidant activity of the plant extracts was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method [19]. The final concentration of DPPH in the test mixture was maintained at 1× 10-4mol dm-3 in methanol, and α-tocopherol was used as a positive control. The IC50 values were determined in triplicate using solutions of 1, 5, 10, 15, 20, 40, 60, 80 and 100 ppm. The absorbance of the test solutions was measured at 517 nm after 30 min using a UV spectrophotometer (Shimadzu, UV-1800). The antioxidant activity was calculated using the formula: % antioxidant activity = [(Ai-Af) / Ai] × 100, where Ai is the initial absorbance of the test mixture following the addition of DPPH and Af is the absorbance after 30 min.

Cytotoxic activity was determined using the brine shrimp assay [20]. From each dry plant extracts, a concentration series was prepared

ranging from 2000, 750, 200, 75.0, 20.0, 7.5, 2.0 and 0.75 ppm in DMSO (1% v/v) in sea water. A final volume of 5.0 ml was maintained for each test sample and 10 one day old shrimp larvae were added to each concentration level. After 24 h, the viable number of shrimps was counted for each concentration level and the percent lethality was determined using Probit analysis. The LC_{50} values were determined with 95 % confidence intervals using software Minitab®16. (4*S*)-4-Methyl-2-(11-dodecynyl)-2 butenolide [21] and DMSO (1.0 % v/v) in sea water were used as a positive control and a negative control, respectively.

All tests were carried out in triplicate. The data was statistically analyzed and expressed as mean±SD.

The IC_{50} values representing the antioxidant activity are given in table 1 where the methanol extract of *S. castaneifolia* flowers and the methanol extracts of *S. alnifolia* stem-bark (IC_{50} = 7.1±0.4 ppm) and leaves (IC_{50} = 8.3±0.3 ppm) showed significant activity (α-tocopherol, IC_{50} = 10.9±4.3 ppm). Importantly, antioxidants play a major role in the neutralising of free radicals formed during the cellular processes. These radicals can lead to various diseases, including cancer and aging by decreasing the efficiency of the living cells [22].

The total polyphenol content of all methanol extracts, the plant extracts and a commercially available branded tea sample were expressed as equivalent to the gallic acid content (GAE) in mg per 1.0 g of dry plant material (table 1). A calibration plot was obtained from a gallic acid standard solution series and the gradient (m = 0.0982) and the graph linearity (R^2 = 0.9993) were obtained. The tea extract showed the highest polyphenol content (GAE, 182.3±15.5 mg g^{-1}). Although the methanol extracts of *S. alnifolia* stem-bark (69.3±6.9 mg g^{-1}), leaves (57.7±0.0 mg g^{-1}) and *S. castaneifolia* flowers (71.2±0.6 mg g^{-1}) exhibited values lower than tea, they were still high enough to be significant. It has been shown that polyphenols possess potent antioxidant properties [23] which agree well with the high polyphenol content and corresponding high antioxidant activities of these two plants.

Cytotoxic activities were determined using the brine shrimp assay where (4*S*)-4-methyl-2-(11-dodecynyl)-2 butenolide was used as a positive control (LC_{50} = 0.15±0.41 ppm). The highest activity was shown by the methanol extract (LC_{50} = 1.3±0.7 ppm) and the dichloromethane extract (LC_{50} = 11.3±5.6 ppm) of the *S. castaneifolia* flowers.

Table 1: Antioxidant and cytotoxic activities and total polyphenol content of the extracts of genus *Schumacheria*

Plant species botanical name	Plant [a] part(s) [#](g)	Type of extract(s) [*] (g)	Antioxidant activity[b] IC_{50} (ppm)	Cytotoxic activity[c] LC_{50} (ppm)	Polyphenol content [d] (mg (GAE) g^{-1})
S. castaneifolia	FL (225)	n-Hexane (5.3)	307.0±37.5	512.8±375.6	
		CH_2Cl_2 (5.0)	262.4±35.2	11.3±5.6	
		CH_3OH (34.2)	6.8±0.1	1.3±0.7	71.2±0.6
S. castaneifolia	SB (600)	CH_2Cl_2 (9.0)	295.3±5.2	73.4±13.4	
		CH_3OH (82.0)	9.8±0.3	903.8±168.2	65.8±0.7
S. castaneifolia	RB (34)	CH_2Cl_2 (2.1)	199.6±6.4	30.9±13.6	
		CH_3OH (13.0)	8.1±0.1	81.6±18.7	44.0±0.1
S. castaneifolia	LF (600)	CH_2Cl_2 (8.4)	266.7±27.0	340.1±80.5	
		CH_3OH (80.5)	10.5±0.4	66.0±17.8	49.2±0.9
S. angustifolia	FL (54)	Hexane (0.8)	686. 4±110.2	156.6±53.3	
		CH_2Cl_2 (0.2)	120.3±5.3	548.7±115.9	
		CH_3OH (6.8)	8.8±0.1	23.6±10.5	59.0±1.2
S. angustifolia	SB (370)	CH_2Cl_2 (2.8)	336.8±8.5	270.3±101.1	
		CH_3OH (25.9)	8.2±0.2	33.7±16.3	36.7±2.3
S. angustifolia	RB (9)	CH_2Cl_2 (0.6)	793.6±53.7	99.8±59.5	
		CH_3OH (3.8)	8.4±0.3	78.2±42.4	22.4±0.2
S. angustifolia	LF (370)	CH_2Cl_2 (6.1)	393.1±41.8	573.6±281.7	
		CH_3OH (25.3)	14.4±0.6	26.4±11.3	19.9±0.3
S. alnifolia	SB (600)	CH_2Cl_2 (11.2)	964.9±40.1	646.9±298.2	
		CH_3OH (70.5)	7.1±0.4	90.2±35.4	69.3±6.9
S. alnifolia	LF (600)	CH_2Cl_2 (10.8)	178.3±14.3	115.5±50.9	
		CH_3OH (77.1)	8.3±0.3	50.5±20.5	57.7±0.0

[a]FL, flowers; SB, stem-bark; RB, root-bark; LF, leaf , [#]Dry weight of each plant part, [*]Dry weight of each extract, [b]IC_{50} values stated as mean±SD, in ppm, were based on different concentrations and three replicates, [c]LC_{50} values stated as mean±SD, in ppm, were based on eight concentrations and three replicates, [d]Polyphenol content equivalent to the gallic acid content (GAE) in milligrams per 1.0 g of dry plant material (mg (GAE) g^{-1}) is based on three replications and is stated as mean±SD.

It can be concluded that the three representative species of the endemic genus *Schumacheria* possess high polyphenol content, antioxidant activity and cytotoxicity. In particular, it is significant that *S. castaneifolia* flowers possess the most potent activities in these three important bioassays giving credence to ethnomedical claims where it has been used, through a long period of oral tradition, for oral aphthous, in the Thummodararegion. Further studies are needed for the three plant species.

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