Identifying secondary metabolite content and antioxidant potential in selected commercially available medicinal plants

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Introduction

Medicinal plants are the most valuable source of biomedicine used in traditional systems of medicine and also it supplies the chemical entities for the synthetic drugs [1]. Plant secondary metabolites are various chemical compounds produced by the plant cell through primary metabolic pathways. At present, traditional medicine, which comprises mostly of herbal components is preferred globally [2]. There are some secondary metabolites abundantly used in traditional medicine. Among these, tannins which are phenolic compounds possessing high molecular weight ranging from 500-3000 Dalton are abundant in vacuole tissues of leaves, wood, roots, fruit and bark of plants [3]. Up to the present, exceeding 4500 varying flavonoids have been found. They are a larger category of phenolic compounds. Flavonoids have been discovered in a lot of plant tissues including plant cell vacuoles. Flavonoids are available as monomers and dimers of oligomers [4]. Antioxidants are important phytoconstituents in medicinal plants. An antioxidant is "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" [5, 6]. Free radicals denote the highly reactive species capable of widespread, indiscriminate oxidation and peroxidation of proteins, lipids and DNA [7]. Rapid production of free radicals leads to oxidative damage to biomolecules and cause serious diseases like degenerative disorders, cancer, diabetes, neural disorders and ageing. Hence antioxidants play a vital role to block free radical production [8, 9]. Antioxidants are known to fulfill their task through several mechanisms including retarding, avoiding or eliminating the oxidative damage to the susceptible molecules [10]. Many research have investigated almost all the fresh herbal medicinal plants and different parts of them to test their phytochemical content, antioxidant potential etc., but the above qualities of commercially available medicinal plants are not that much touched by researchers although it is important. Therefore, the current study was conducted to investigate the secondary metabolite content and in vitro antioxidant activities of six selected commercially available dried medicinal plant parts namely stems of Coscinium fenestratum, flowers of Aegle marmelos, roots of Vetiveria zizanioides, stems of Tinospora cordifolia , rhizomes of Acorus calamus and fruits of Terminalia chebula which are commonly employed in herbal medicine to treat digestive system associated ailments, apart from their medicinal value in the treatment of many other diseases. The plant parts were assessed qualitatively and quantitatively during the current experiment. These plants are widely used in the traditional medicine system of Sri Lanka, due to their vast medicinal properties that immensely contribute to treat diseases.

Methodology

Preparation of crude extracts. A volume of 20 ml of concentrated methanolic crude extracts of the six, commercially available dried plant materials were obtained according to a previously described procedure [11, 12].

Qualitative estimation. The qualitative analysis was done on all plant extracts as triplicates, by conducting at least two standard tests to determine the presence of each of the secondary metabolite of concern; flavonoids, tannins, phenols and alkaloids. The alkaline reagent test and lead acetate test for flavonoids, Wholer's test and ferric

chloride test for tannins, ferric chloride test for phenols and Mayer's test and Dragendorff's test for alkaloids was conducted.

Standard curve preparation. Standard curves were prepared for quantitative estimation of flavonoids, tannins, phenols, alkaloids and ferric reducing power using Rutin, Tannic acid, Gallic acid, Atropine and L-ascorbic acid respectively as standards (Figures not shown here).

Quantitative estimation. The appearance of positive results in the qualitative tests lead to subjecting the plant extracts to a quantitative analysis. The Aluminum chloride, Folin-Denis, Folin-Ciocalteu and BCG spectrometric methods were performed in triplicates to determine the flavonoid, tannin, phenol and alkaloids contents of plant extracts respectively having blanks as controls for each test.

Antioxidant activity. Three standard antioxidant assays namely; FRAP, ABTS and DPPH were conducted spectrometrically in triplicates, on all the plant extract to assess the antioxidant activity through their ferric reducing power and scavenging activity for DPPH and ABTS free radicals keeping blanks as control for each test.

Statistical analysis. The results were expressed as Mean ± SD. Using MINITAB 17 software, one-way ANOVA, pairwise mean separation and also correlation among antioxidant assays and secondary metabolites was obtained by regression analysis.

Results and Discussion

Qualitative screening. The current study gives away information on the presence of some of the secondary metabolites such as alkaloids, phenols, flavonoids and tannins and the antioxidant activity of some selected commercially available herbal medicinal plant parts. The qualitative tests ascertained the presence of different phytochemicals in the commercial plant materials (Table 1).

Table 1. Results of qualitative tests for Tannins, Flavonoids, Phenols and Alkaloids.

Plant		Qualitative test						
		Flavonoids		Tannins		Alkaloids		Phenols
No	Scientific name	Alkaline reagent test	Lead acetate test	Wholer's test	Ferric chloride test	Mayer's test	Dragendorff's test	Ferric chloride test
1	*Coscinium fenestratum*	++	++	+++	++	+++	+++	+++
2	*Acorus calamus*	++	+	+	+	++	++	++
3	*Vetiveria zizanioides*	++	+	++	-	++	-	+++
4	*Tinospora cordifolia*	++	++	++	+	+	-	+++
5	*Aegle marmelos*	+++	+++	++	+++	++	++	+++
6	*Terminalia chebula*	++	++	+++	+++	++	-	+++

(+++) - Very clear precipitate / colour change, (++) - Clear precipitate / colour change,
(+) - Very little precipitate /colour change, (-) No precipitate / colour change

The results of the qualitative screening made some important information. Some plant extracts like V. zizanioides, T. cordifolia and T. chebula showed no precipitate / turbidity to Dragendorff's reagent but showed precipitate / turbidity to Mayer's reagent. This can be attributed to the fact that primary and secondary amines are not detectable by Dragendorff's reagent which is a specific test to detect amines. The reagent reacts mostly with tertiary or quaternary amines [13]. In contrast, the Mayer's reagent reacts with many different types of

alkaloids. In the qualitative analysis of tannins, the ferric chloride tannin test indicated the absence of tannins in V. zizanioides. As a matter of fact, it is tough to see a relationship between the amount of colour change or precipitate formed and the figures obtained for the same phytochemicals during quantitative determination. The selected plants gave positive results for at least one test and that lead to quantitative determination of the extract.

Quantitative estimation. According to the results of the quantitative estimation, all commercial plant materials contained secondary metabolites in different levels (Figure 1). As a whole, phenolic compounds including flavonoids and tannins were available in high quantity in all investigated plants. Flavonoid contents were expressed as mg RE / g dry weight of fine powder by using the standard curve of Rutin (y = 0.84 x + 0.0012, R2 = 0.9676). Tannin contents were expressed as mg TAE /g dry weight of fine powder by using the standard curve of Tannic acid (y = 3.36 x + 0.0382, R2 = 0.9593). Phenol contents were expressed as mg GAE / g dry weight of fine powder by using the standard curve of Gallic acid (y = 6.48 x + 0.0064, R2 = 0.996). Alkaloids contents were expressed as mg AE / g dry weight of fine powder by using the standard curve of Atropine (y = 1.374 x + 0.0305, R2 = 0.9785). The contents of secondary metabolites available in different plants may vary according to the plant part used for the study. Range of contents are summarized in Table 3.

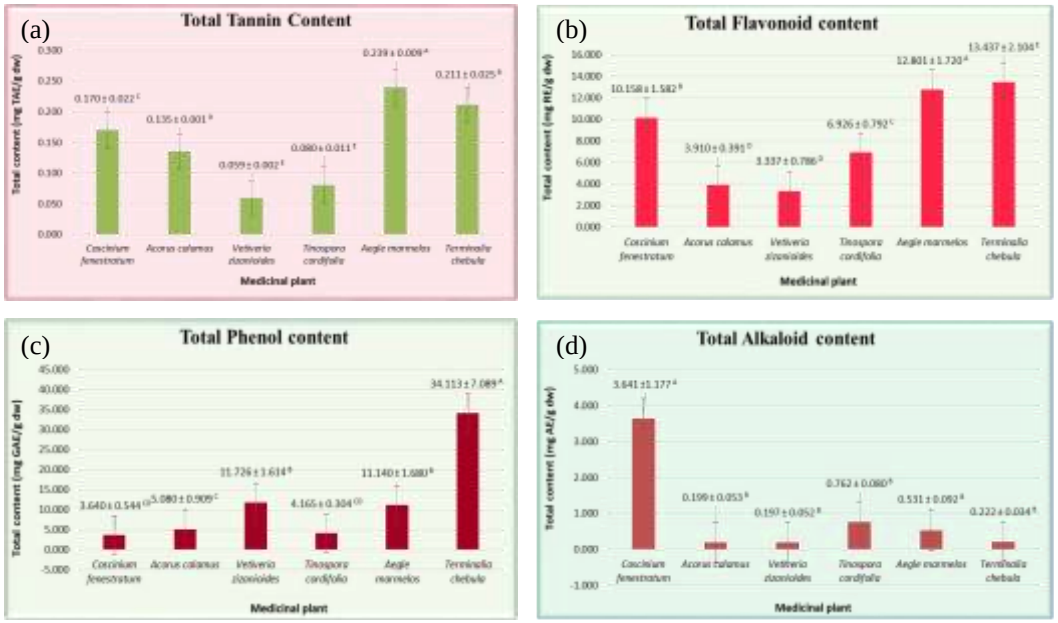

Figure 1. Total contents of the secondary metabolites in the medicinal plants; (a) Tannins (b) Flavonoids (c) Phenols (d) Alkaloids.

Antioxidant activity. The ferric reducing powers were expressed as mg AAE / g dry weight of fine powder by using the standard curve of L-Ascorbic acid (y = 3.39 x + 0.0665, R2 = 0.9757). The ferric reducing antioxidant power of the methanolic extracts of the six plants were varying within the range of (5.243 ± 0.421 - 2.252 ± 0.133) mg AAE /g dw. The ABTS radical scavenging activities were expressed as percentage inhibition of ABTS radical. The percentage inhibition of the methanolic extracts of the six plants were varying within the range (98.86 ± 0.143 - 56.00 ± 5.511) %. The DPPH radical scavenging activities were expressed as percentage inhibition of DPPH radical. The results of free radical scavenging ability of the methanolic extracts of the six plants clearly indicate that percentage inhibition was observed in the range of (95.56 ± 0.634 - 85.92 ± 1.721) % (Figure 2).

Correlation analysis. The correlation among antioxidant assays and secondary metabolite contents were expressed in Figure 3. A strong positive correlation was obtained between total

tannin content and all the three antioxidant assays. Total flavonoid content showed a positive correlation to all three antioxidant assays. The FRAP and ABTS assays showed a significant linear correlation.

The study revealed that the results of different antioxidant tests used here; ABTS, DPPH and FRAP vary notably due to the varying reactions of different antioxidants available in the plant extracts to various radicals in the test solutions.

Table 2. Summarized contents of secondary metabolites for all six commercial medicinal plants.

Secondary metabolite	**Content in all six plants**
Flavonoids	(13.437 ± 2.104 - 3.337 ± 0.786) mg RE /g dw
Tannins	(0.239 ± 0.009 - 0.059 ± 0.002) mg TAE /g dw
Phenols	(34.113 ± 7.089 - 3.640 ± 0.544) mg GAE /g dw
Alkaloids	(3.641 ± 1.177 -0.197 ± 0.052) mg AE /g dw

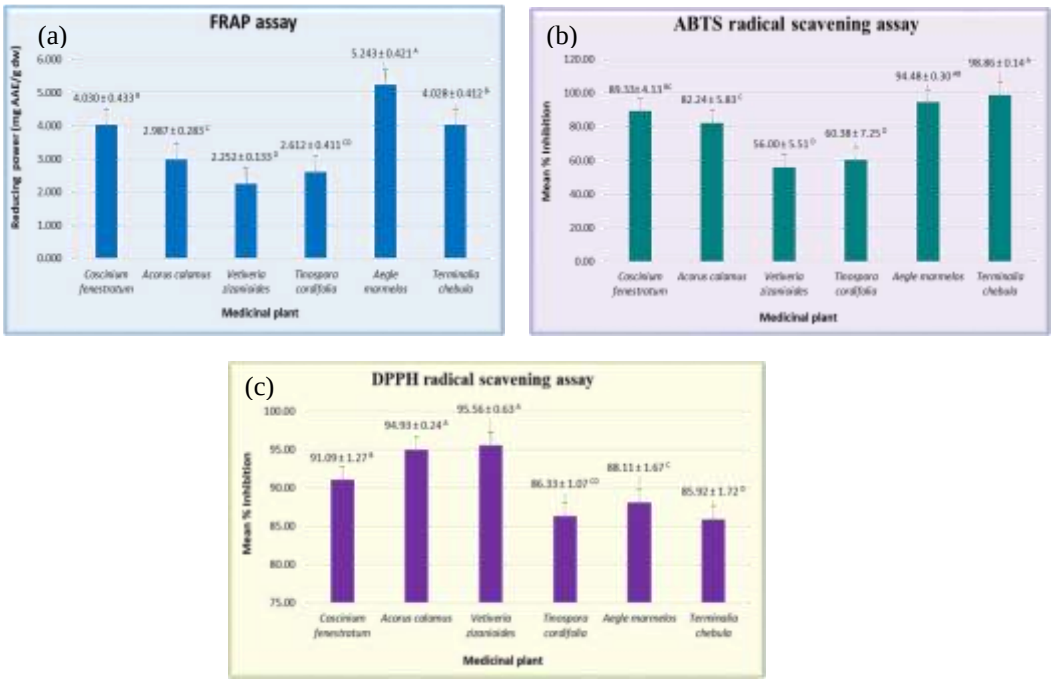

Figure 2. Antioxidant capacity of the six medicinal plants; (a) FRAP assay (b) ABTS assay (c) DPPH assay.

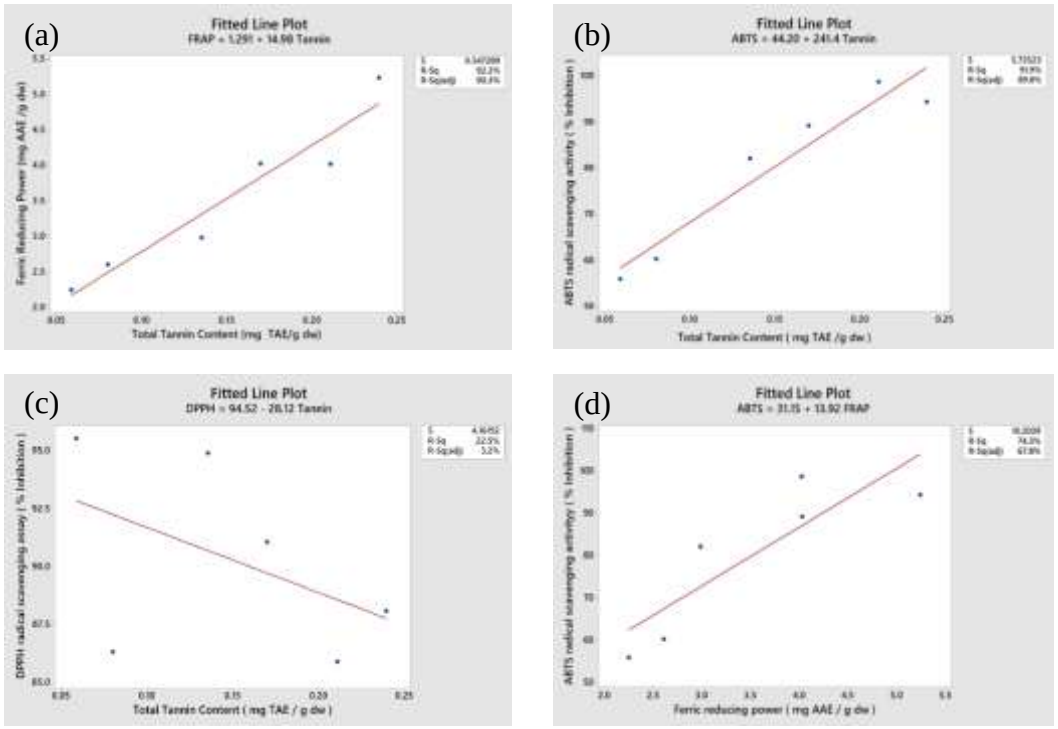

Figure 3. Correlation between; (a) Total tannin content and FRAP assay (b) Total tannin content and ABTS assay (c) Total tannin content and DPPH assay (d) FRAP and ABTS assays.

Conclusion

Among the six commercial medicinal plants evaluated, A. marmelos revealed to be the most promising extract with high phytochemical content and good free radical scavenging activity. The highest contents for tannins, both flavonoids and phenols, alkaloids were shown by A. marmelos, T. chebula and C. fenestratum respectively. The highest performance for antioxidant activity was shown for the three different assays; both FRAP and ABTS, DPPH by A. marmelos and V. zizanioides respectively. The good linear correlation obtained between tannin content to antioxidant potential determined by FRAP and ABTS assays suggest that tannins are mostly responsible for antioxidant properties in the studied plants.

References

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