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Prevalence of secondary metabolites and antioxidants in selected commercially available medicinal plants of Sri Lanka

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Abstract

Medicinal plants have been utilized by human since time immemorial and even a long time before historical records were kept about the role of plants in medicinal preparations. The plant kingdom of is a treasure trove of probable medicine. The secondary metabolites present in the plants and their contribution as antioxidants are responsible for their therapeutic properties. The methanolic crude extracts of the selected commercially available medicinal plants were subjected to a qualitative and quantitative analysis to investigate the presence of tannins, flavonoids, phenols and alkaloids. The antioxidant capacity of the plants was determined through FRAP, ABTS and DPPH assays. The highest contents for tannins, both flavonoids and phenols, alkaloids were shown by the medicinal plants *Aegle marmelos*, *Terminalia chebula* and *Coscinium fenestratum* respectively. The highest performance for antioxidant assays was shown by *Aegle marmelos* for both FRAP and ABTS, and *Vetiveria zizanioides* for DPPH. A significant correlation was obtained among the total tannin content and the antioxidant assays ($p=0.05$). A high correlation was found among FRAP and ABTS assays. The results of the study indicated that these commercially available medicinal plants have secured a considerable level of secondary metabolites and free radical scavenging capacity. It proves that the plant secondary metabolites analyzed and some other compounds available in the plants studied, contribute to their free radical scavenging potential.

Keywords: Antioxidants, commercially available medicinal plants, Secondary metabolites, Alkaloids, Tannins, Phenols, Flavonoids, Antioxidants, Free radicals

1. Introduction

Herbal medicines are the most valuable bio asset of medicine used in indigenous practices of medicine, pharmaceutical drugs, dietary supplements and they also supply the chemical entities for the engineered drugs [1]. These herbal plants constitute some chemical compounds that have a specific action in plant physiology. Plant secondary metabolites are various bioactive compounds produced by the plant cell through secondary metabolic pathways which are derived from the primary metabolic pathways. It includes alkaloids, tannins, saponins, flavonoids, phenolic compounds. These secondary metabolites are commonly used in agriculture, human treatment etc. Over 80% of people prefer conventional medication, which comprises mostly medicinal plants [2].

Nature has been the provider of herbal compounds since time immemorial. The significance of medicinal herbs in the treatment of illnesses in human is essential. The plant kingdom harbors a never-ending asset of active compounds highly valuable in the treatment of many illnesses that are difficult to cure [3]. The utilization of natural drugs for the treatment of illnesses and infections is a protected and normal treatment [4].

Plant secondary metabolites are classified into two categories in the view of the chemical constitution; the presence of ring structures, the presence of sugar molecules, etc., based on

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composition; whether nitrogen is present or not, or based on the dissolving in different solvents [5]. Secondary metabolites provided by plants can be recognized as an assert of drugs, flavouring compounds, industrial products and food additives [6].

Antioxidants are important phytoconstituents in medicinal plants. An antioxidant can be defined as "any substance that, when available at very minute concentrations than that of an oxidizable material, which notably impedes or halts oxidation of that material" [7]. Free radicals denote the exceptionally reactive compounds capable of extensive, non-selective oxidation and peroxidation of proteins, lipids and DNA [8]. Rapid generation of free radicals leads to oxidative damage to biomolecules and causes serious health issues like degenerative disorders, cancer, diabetes, neural disorders, and ageing, and thereby antioxidants play an imperative role to obstruct the creation of free radicals [9, 10].

The current study was focused to determine the prevalence of secondary metabolites and in vitro antioxidant activities of six selected commercially available medicinal plants. The plant parts were subjected to both qualitative and quantitative estimation during the current experiment. These plants are broadly utilized in conventional medications of Sri Lanka, due to their vast medicinal properties that immensely contribute to treating diseases (Table 1). The results of this investigation will be of high value for the medical sector, pharmaceutical industries and general consumers as well.

2. Materials and Methodology

Plant materials

The commercially available medicinal plants required for the study were purchased in desired amounts from the local market as dried forms of the plant parts.

Preparation of crude extract

Commercially available dried plant materials were ground well using a mechanical grinder [11]. The coarse powder was sieved using a 212-micron sieve to collect fine powders. All the powders were stored in properly labeled airtight plastic bottles covered by black paper and left inside the refrigerator at 10°C.

A mass of 10 g of each powdered plant material was soaked in 100 ml of 80% methanol and they were kept in a mechanical shaker at 350rpm for 24 hours in the dark. The plant extracts were subjected to a filtration process using filter paper Whatman No.1 (125 mm). The filtrates were evaporated under reduced pressure using a rotary evaporator at 40°C to obtain a concentrated extract of 20 ml. This was repeated until triplicate measurements were made [12].

Qualitative screening of plant extracts

- Tannins

Wohler's test - A volume of 1 ml of plant extract was diluted by adding 9ml of distilled water. From that 1.6 ml was obtained into a separate test tube. It was combined with a few drops of 1% lead acetate. The formation of a white precipitate was taken as a piece of evidence for the presence of tannins [13].

Ferric chloride test - A volume of 1ml of plant extract was diluted up to 10ml by adding distilled water in a boiling tube. It was mixed well by boiling. The solution was filtered. A few drops from

the filtrate were taken to a separate test tube. It was combined with 1% ferric chloride. The appearance of bluish-black or bluish green colour was taken as a positive result for tannins [14].

- Flavonoids

Alkaline reagent's test - Two to three drops of NaOH (2N) were added to a test tube containing 1 ml of the plant extract. The appearance of intense yellow colour that disappears when dilute HCl was added, giving a clear solution was taken as a positive result for flavonoids [15,16].

Lead acetate test - A 3ml of 10% lead acetate was put to a test tube containing diluted plant extract. The extract was diluted by adding 2ml of distilled water to 1 ml of the plant extract. The formation of a bulky white precipitate was taken as a positive result for flavonoids [17].

- Phenols

Ferric chloride test - Three to four drops of 5% ferric chloride were added to 2ml of plant extract taken into a test tube. The appearance of bluish-black or bluish green colour was taken as a sign of the presence of phenols in the extract [13, 15].

- Alkaloids

Mayer's test - A few drops of diluted HCl were mixed with 1 ml of plant extract inside a test tube. A few drops of freshly prepared Mayer's reagent were added to it. The appearance of white precipitate or turbidity was taken as a positive result for alkaloids [13, 16].

Dragendorff's test - A volume of 1 ml of the plant extract was mixed with a few drops of diluted HCl. Two to three drops of Dragendorff 's reagent were added to it. The formation of reddish-brown precipitate or turbidity was taken as an indication of alkaloids [12, 18].

Quantitative screening

i. Estimation of Total Tannin Content

The spectrophotometric method proposed by Folin-Denis was used to measure the tannin content. A volume of 0.5ml of plant extract was put into a separate beaker and 0.25ml of Folin Denis reagent was added to each beaker. Then 0.5ml of saturated Na_2CO_3 followed by 3.5ml of distilled water was added to each beaker. Then the absorbance was measured at 700nm within 30minutes. The blank was prepared as above without adding plant extract. Finally, the tannin concentration was taken by using the standard curve, then the total tannin content in extracts was expressed as tannic acid equivalents (mg of TAE/g of extract) [19, 20].

ii. Estimation of Total Flavonoid Content

The aluminum chloride spectrophotometric method was employed to determine the flavonoid content. A volume of 0.5ml of plant extract was taken into a separate beaker. Similar to the preparation of the standard curve, 2ml of distilled water and 0.15ml of $NaNO_2$ were added to it. After 6 minutes, 0.15ml of $AlCl_3$ was added to it and it was left to stand for 6 minutes. Next 2ml of NaOH was added to it. The final volume was made up to 5ml by adding 0.2ml of distilled water. After 15 minutes, the absorbance was measured at 510nm [19, 21]. The blank was made as above excluding plant extract. This was repeated until triplicate measurements were performed. Finally, the flavonoid concentration was measured by using the standard curve, then the total flavonoid content in extracts was expressed as Rutin equivalents (mg of RE/g of extract) [11].

iii. Estimation of Total Phenol Content

The spectrophotometric method put forward by Folin-ciocalteu was used to measure the total phenolic content. A volume of 0.5ml of plant extract was added into a 50ml volumetric flask. Distilled water was added carefully to make the volume up to the mark. From that 0.5ml was taken into a different beaker. 5ml of 10% Folin-ciocalteu reagent and 4ml of 1M sodium carbonate were added to it. The absorbance of it was measured after 30 minutes at 760nm [22]. The blank was made as above excluding the addition of the plant extract. This was repeated until triplicate measurements were made. Finally, the phenol concentration was quantified by using the standard curve, then the total phenolic content in extracts was expressed as Gallic acid equivalents (mg of GAE/g of extract)

iv. Estimation of Total Alkaloid Content

A volume of 0.5ml of plant extract was added into a separatory funnel. Then, 5ml of phosphate buffer (pH 4.7) and 5ml of BCG were added to it. Next 5ml of chloroform was put to it. Then it was shaken vigorously. The chloroform layer was slowly taken out of the funnel into a beaker and its absorbance was taken at 470nm. The blank was made as above excluding the addition of plant extract. Repetitions were made until triplicate measurements are made [18, 23]. Finally, the alkaloid concentration was obtained by using the atropine standard curve, then the total alkaloid content in extracts was expressed as atropine equivalents (mg of AE/g of extract).

Antioxidant assays

i. Ferric Reducing Antioxidant Power assay

The ferric reducing power assay was undertaken using a previously described procedure [24]. A volume of 0.5ml of plant extract was combined with 2.5 ml of phosphate buffer (pH 7.4) and 2.5 ml of aqueous potassium ferricyanide solution (1%, w/v). This mixture was kept at 50±2°C in the water bath for 20 minutes. It was let to cool down and 2.5 ml of 10% (w/v) trichloroacetic acid was added. The centrifuge was set for 3000 rpm and the resulting mixtures were centrifugated for 5 minutes. A volume of 2.5ml of the upper layer of the solution was combined with 2.5 ml of distilled water and 0.5 ml freshly prepared ferric chloride solution (0.1%, w/v). The absorbance was measured 15 minutes past the initial mixing at 700nm in a UV-VIS spectrophotometer. The blank was made in the same manner without a sample. The standard was L-Ascorbic acid. The results were expressed in terms of L-ascorbic acid equivalent (mg/g dry weight of leaves).

ii. ABTS Radical Scavenging Assay

ABTS•+ cation radical was produced by the reaction between equal amounts of ABTS (7 mM, 5 ml) and potassium persulfate (2.45 mM, 5 ml) solutions. It was stored in the darkness at room temperature for 12-16 h before use. ABTS•+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm.

ABTS Radical Scavenging Assay was done using a previously described procedure [25]. A volume of 5 microliter of plant extract was put to the 3.995 ml of diluted ABTS•+ solution. The absorbance was taken 30 minutes after the initial mixing at 734 nm with a UV-VIS spectrophotometer. Methanol was used as a blank. The following equation was utilized to obtain the percentage inhibition of the radicals due to the free radical scavenging activity of leaf extracts.

% inhibition = { (Ab control - Ab sample) / Ab control } X 100

Where, Ab control was the absorbance of ABTS' and Ab sample was the absorbance of ABTS•+ plus plant extract [25].

iii. DPPH radical scavenging assay

DPPH solution (0.1 mM) was made in methanol by dissolving 2 mg of DPPH in methanol and the remaining volume was made up to 100 ml with methanol. The solution was left in the dark for 30 minutes to complete the reaction [26].

DPPH radical scavenging assay was performed using a previously described procedure [27]. A volume of 100 µL of plant extract was mixed with a 3.9 ml freshly prepared methanolic solution of DPPH (0.1 Mm). The resulting solution was mixed using the vortex for 15 seconds and then left at room temperature for 30 min in unilluminated conditions. The absorbance value of the resulting mixture was taken at 517 nm through a UV-VIS spectrophotometer. Methanol was used as a blank. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was obtained using the following formula.

% inhibition = { (Ab control – Ab sample) / Ab control } x 100

Where Ab control was the absorbance of the DPPH solution with nothing added and Ab sample was the absorbance of DPPH radical plus plant extract [28].

Statistical analysis

All the analyses were carried out in triplicates and the results were expressed as Mean ± SD.

Mean separation method

The one-way analysis of variance (ANOVA) was done to determine whether the mean of a response variable is the same in two or more unrelated, independent groups of a predictor variable. Then the pairwise mean separation was conducted using the Fisher method in MINITAB 17.

Method for correlation analysis

Correlation among the antioxidant capacities exposed by the DPPH radical scavenging assay, ABTS radical scavenging assay, Ferric reducing antioxidant power assay and Total phenolic content was analyzed. For that, correlation coefficient (R) and coefficient of determination (R^2) was obtained and regression analysis was conducted by using MINITAB 17.

3. Results and Discussion

The current investigation gives away information about 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. In the present arena, plant secondary metabolites or Phyto-constituents, which had uninvestigated medicinal uses in the past, are gaining huge importance as a reservoir of pharmaceutical material [29]. Compounds of plant origin have presently attracted much enthusiasm due to their multifaceted uses. Herbal plants are a wealth of indispensable pharmacological products and bioactive compounds that are multipurpose and can be utilized in indigenous medicine, western medicine, and artificial medicine manufacturing industries [30].

Choosing of the most suitable solvent when conducting a phytochemical extraction is most essential phenomena as quantity and the speed of extraction as the of phytochemicals including phenols are directly affected by the solvent used [31]. In the present investigation, 80 % methanol was used as the solvent to make the plant extracts. According to available literature solvents like methanol, ethanol, chloroform, water, DMSO have been used to prepare plant extracts for phytochemical screening experiments and antioxidant assays. Usually, organic solvents like ethanol, methanol and acetone are chosen in terms of extracting phenols. A combination of an organic solvent with water as well is known to give good results [32].

The rather easily replaceable and short aliphatic group present in methanol compared to ethanol makes it easier to combine with phenols containing carbons in their aromatic ring [33]. Methanol has higher effectiveness to extract low molecular weight polyphenols [31]. Organic solvents such as methanol, ethanol, or these mixed with varying amounts of water, are usable for the extraction of flavonoids from medicinal plants [34]. To extract alkaloids from medicinal plants, there needs to be a suitable solvent such as alcohol, benzene (organic solvents). Salts of alkaloids are extremely dissolved in water and alkaloid bases dissolve in organic solvents [35, 36]. Overall, the best extraction yield of phenols, alkaloids, flavonoids, tannins were reported by using methanol as solvent. It can be related to the better solubility of these secondary metabolites in methanol [37].

i. Qualitative analysis of plants

During a phytochemical screening experiment, including those done on medicinal plant parts, it is first subjected to a qualitative screening to get a clue of the presence of the target secondary metabolites. In the present study, the qualitative tests ascertained the availability of phytochemicals shown available in the fresh samples of the same commercial plant materials. The data are shown in Table 2. All the secondary metabolite groups were tested using two well-established tests for more reliability. The selected plants gave positive results for at least one test and that lead to the quantitative determination of the plant extract.

The observations of the qualitative analysis made some important revelations. Some plant extracts like *V. zizanioides*, *T. cordifolia* and *T. chebula* showed null response to Dragendorff's reagent but showed moderate to weak response to Mayer's reagent. This can be attributed to the fact that primary and secondary amines are not detectable by Dragendorff's reagent. The reagent reacts mostly with tertiary or quaternary amines [38]. In contrast, 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*. 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.

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	*Cosciniu m*	++	++	+++	++	+++	+++	+++

	fenestratum							
2	*Acorus calamus*	++	+	+	+	++	++	++
3	*Vetiveria zizanioides*	++	+	++	-	++	-	+++
4	*Tinospora cordifolia*	++	++	++	+	+	-	+++
5	*Aegle marmelos*	+++	+++	++	+++	++	++	+++
6	*Terminalia chebula*	++	++	+++	+++	++	-	+++

+++ Strong positive reaction,

++ Moderate positive reaction,

+ Weak positive reaction,

- No reaction

ii. Quantitative analysis of plants

According to the results of the quantitative estimation all commercial plant materials contained plant secondary metabolites in different levels as depicted in the Table 3 displayed below. As a whole, phenolic compounds including flavonoids and tannins were available in high quantity in all investigated plants.

Table 2: Total contents of secondary metabolites in the studied medicinal plants

Plant	**Total Alkaloid content mg AE / g dw**	**Total Flavonoid content RE/g dw**	**Total Tannin content mg TAE/g dw**	**Total Phenol content mg GAE/g dw**
Coscinium fenestratum	3.641 ± 1.177	10.158 ± 1.582	0.170 ± 0.022	3.640 ± 0.544
Acorus calamus	0.199 ± 0.053	3.910 ± 0.391	0.135 ± 0.001	5.080 ± 0.909
Vetiveria zizanioides	0.197 ± 0.052	3.337 ± 0.786	0.059 ± 0.002	11.726 ± 1.614

Tinospora cordifolia	0.762 ± 0.080	6.926 ± 0.792	0.080 ± 0.011	4.165 ± 0.304
Aegle marmelos	0.531 ± 0.092	12.801 ± 1.720	0.239 ± 0.009	11.140 ± 1.680
Terminalia chebula	0.222 ± 0.034	13.437 ± 2.104	0.211 ± 0.025	34.113 ± 7.089

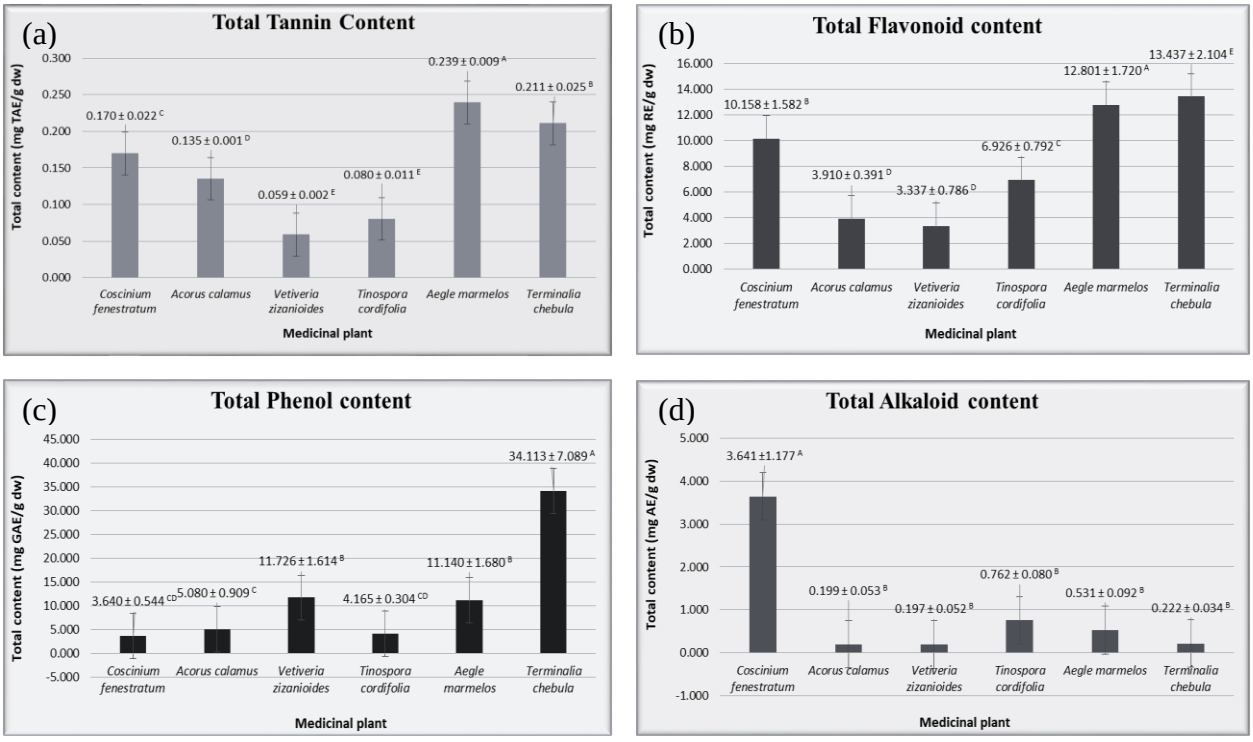

Figure 1: Total contents of the secondary metabolites; (a) Tannins (b) Flavonoids (c) Phenols (d) Alkaloids

Total Flavonoid content

A spectrophotometric assay based on aluminum complex generation is one of the most commonly utilized procedures for the determination of total flavonoid content in food or medicinal plant samples [39]. There are two ways of performing this test; with and without adding $NaNO_2$. The method that includes the addition of $NaNO_2$ in an alkaline medium is specially used for rutin, luteolin and catechins. The total flavonoid content in the plant extracts can be determined by a UV-Vis spectrophotometer using the aluminum chloride method [40].

For determining the number of flavonoids present in a plant extract, the ability of flavonoids to form complexes with aluminum chloride can be exploited and the so formed complex can be quantified using a UV-Vis spectrophotometer. The principle behind this method is the complex formation of aluminum ions with flavonoid molecules with the presence of basic conditions. Red coloured chelates of aluminium and flavonoids are the output of the reaction and they are absorbed at a wavelength of 510 nm. The number of hydroxyl groups attached to the flavonoid molecules together with the characters of aluminium ions affects the coloration and sensitivity of the method [41, 42].

Plant-derived flavonoids often surpass the performance of popular free radical scavengers including ascorbic acid and alpha tocopherol, in laboratory conditions due to their high capability to grant electrons and protons. The plant kingdom serves as the only natural provider of flavonoids which are recognized as products possessing both nutritional and medicinal value in a single pack [43].

The concentration of flavonoids of six different plant extracts was expressed as mg RE / g dry weight of fine powder by using the standard curve of Rutin ($y = 0.84 x + 0.0012$, $R^2 = 0.9676$). The flavonoid content of the methanolic extracts of the six plants were varying within the range (13.437 ± 2.104 - 3.337 ± 0.786) mg RE /g dw of fine powder. It was revealed through the results that *Terminalia chebula* showed the maximum concentration of flavonoids (13.437 ± 2.104 mg RE/g dw).

Total Tannin Content

In the plant world, both angiosperms and gymnosperms harbour a vast array of different tannins which are phenolic compounds possessing high molecular weight ranging from 500-3000 Dalton [44]. Tannins are abundant in vacuole tissues of leaves, wood, roots, fruit and bark of plants [45]. This test for tannins was first put forward by Folin and Denis in 1912 [46] and discovered that a combination of Phosphotungstic and Phosphomolybdic acid can be reduced by aromatic hydroxyl groups and produce a blue colouration in basic solution [47]. Tannins in the plant extracts subject the Phosphotungstomolbdic acid to the reduction in alkaline medium and produce an intense blue colouration. The intensity is a measure of the amount of tannins present in the sample [48]. There are several other methods used for quantification of tannins among which Folin-Ciocalteu is the frequently utilized method because of its high practicability and low cost although it has an advanced chemical structure and its reaction with phenols [32].

The concentration of tannins of six different plant extracts was expressed as mg TAE /g dry weight of fine powder by using the standard curve of tannic acid ($y = 3.36 x + 0.0382$, $R^2 = 0.9593$). The tannin content of the methanolic extracts of the six plants were varying within the range of (0.239 ± 0.009 - 0.059 ± 0.002) mg TAE /g dw of fine powder. It was revealed through the results that *Aegle marmelos* showed the maximum concentration of tannins (0.239 ± 0.009 mg TAE/g dw)

Total Phenol Content

The total phenol content of the plant extracts was measured by the folin ciocalteu spectrometric method. The folin ciocalteu reagent comprises Phosphotungstic acid ($H_3PW_{12}O_{40}$) and Phosphomolybdic acid ($H_3PMo_{12}O_{40}$); a combination of two acids that can react with phenolic compounds and non-phenolic reducing compounds and it forms chromogens. The Oxotungstate and Oxomolybdate that are originated from the redox reaction showed a blue colour. The intensity of the blue colour is proportional to the number of polyphenols present in the plant extract sample. Therefore, it can be determined in alkaline conditions using a UV spectrophotometer [49].

The advantages of FC assay are, it is a subtle method to conduct and the consistency of results and straightforwardness [50]. The Folin-Ciocalteu method is a very fast and most frequently used assay to determine the total phenolic content although not all phenolic compounds show the same reaction to the FC method [51].

The concentration of phenol of six different plant extracts was expressed as mg GAE / g dry weight of fine powder by using the standard curve of Gallic acid ($y = 6.48 x + 0.0064$, $R^2 = 0.996$). The phenol content of the methanolic extracts of the six plants were varying within the range of (34.113

± 7.089 - 3.640 ± 0.544) mg GAE /g dw of fine powder. It was revealed through the results that *Terminalia chebula* showed the maximum concentration of phenols (3.641 ± 1.177 mg GAE/g dw)

Total Alkaloid Content

In the BCG spectrometric method implemented for the quantification of alkaloids, BCG forms a yellow coloured complex with the alkaloids in the plant extract. The yellow coloured complex formed is completely extractable by chloroform at the pH value of 4.7. The absorption spectrum of the atropine-BCG complex at pH 4.7 shows the maximum absorption at 470 nm [52]. The advantages of this method are the simplicity and sensitivity of the procedure. At the same time, there is no requirement for sophisticated instruments. It is also performed during a short time period [53]. The limitation of this test is that Bromocresol green makes a complex with only alkaloids of certain types; alkaloids to which nitrogen is attached to their structure. Amine or amid alkaloids do not react with BCG [18, 53].

The concentrations of alkaloids of six different plant extracts were expressed as mg AE / g dry weight of fine powder by using the standard curve of atropine ($y = 1.374 x + 0.0305$, $R^2 = 0.9785$). The alkaloid content of the methanolic extracts of the six plants were varying within the range of (3.641 ± 1.177 -0.197 ± 0.052) mg AE /g dw of fine powder. It was revealed through the results that *Coscinium fenestratum* showed the maximum concentration of alkaloids (3.641 ± 1.177 mg AE/g dw)

The contents of secondary metabolites available in different plants may vary according to the plant part; bark, wood, leaves, fruit, flower, etc. used for the study.

Antioxidant activity

Antioxidants follow many different mechanisms in showing their antioxidative property, including impeding, keeping away, or removing the oxidative destruction to the vulnerable compounds. The activity of free radical scavengers can be summarized as extinguishing free radicals, metal chelation, subduing free radical producing enzymes and reviving free radical scavenging enzymes of the body [54]. The radical scavenging activity or antioxidant capacity of the plants were assessed by implementing three main assays; FRAP (Ferric Reducing Antioxidant Power), ABTS and DPPH. These are the most routinely used assays to investigate the antioxidant power of plants. The results are depicted in Table 4.

Table 3: Antioxidant activities of the studied medicinal plants

Plant	FRAP assay (mg AAE / g dw)	DPPH assay (% Inhibition)	ABTS assay (% Inhibition)
Coscinium fenestratum	4.030± 0.433	91.09 ± 1.267	89.33 ± 4.131
Acorus calamus	2.987 ±0.283	94.93 ± 0.239	82.24 ± 5.830
Vetiveria zizanioides	2.252 ± 0.133	95.56 ± 0.634	56.00 ± 5.511

Tinospora cordifolia	2.612 ± 0.411	86.33 ± 1.069	60.38 ± 7.245
Aegle marmelos	5.243 ± 0.421	88.11 ± 1.673	94.48 ± 0.297
Terminalia chebula	4.028 ± 0.412	85.92 ± 1.721	98.86 ± 0.143

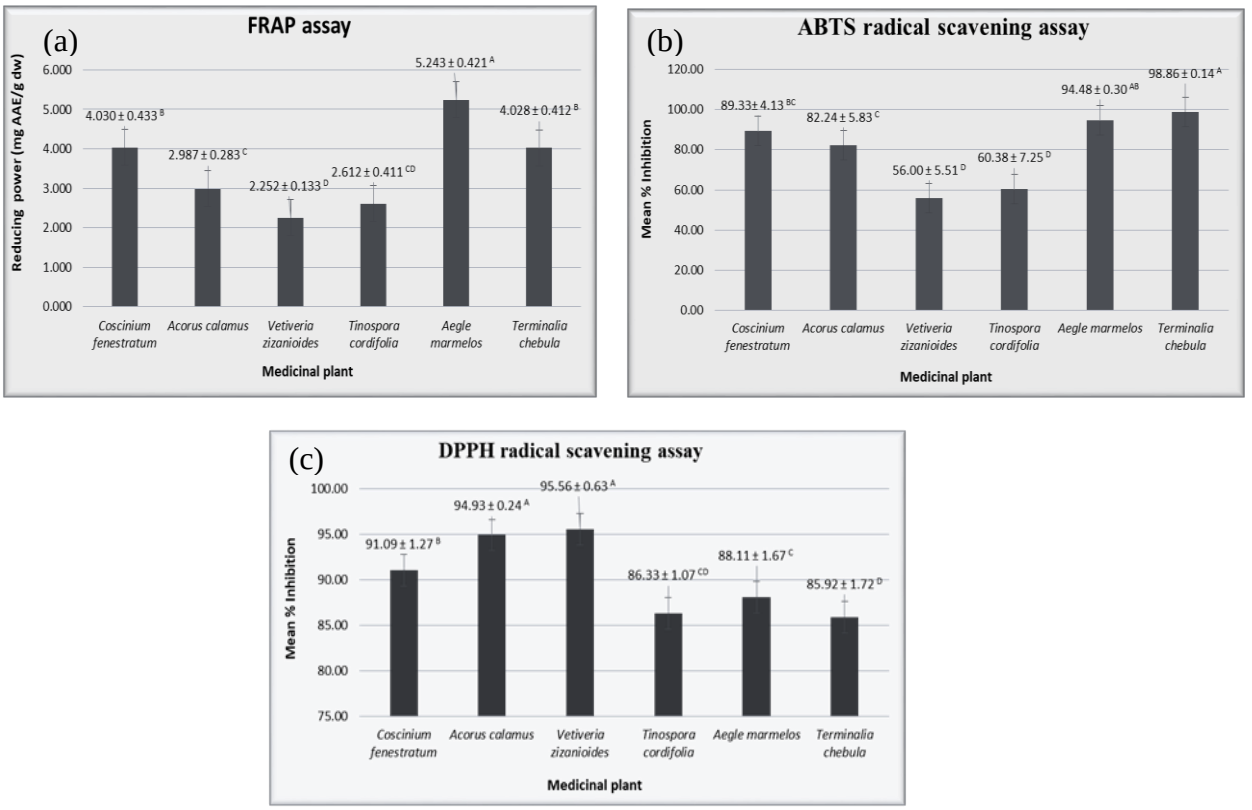

Figure 2: Antioxidant capacity; (a) FRAP assay (b) ABTS assay (c) DPPH assay

Ferric reducing antioxidant power assay (FRAP)

The Ferric reducing antioxidant power assay was conducted to determine the antioxidant potential of the plant extracts. The principle behind this method is the reduction reaction of ferric-tripyridyltriazine complex to its coloured form known as ferrous tripyridltriazine complex in the presence of antioxidants [55]. A disadvantage of the FRAP method was that it was time-consuming in terms of preparation of chemicals and requires lot of effort [56]. FRAP assay deals with toxic chemicals and it is a disadvantage to the researcher.

The ferric reducing power assay of six different plant extracts were expressed as mg AAE / g dry weight of fine powder by using the standard curve of L-Ascorbic acid ($y = 3.39 x + 0.0665$, $R^2 = 0.9757$). 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 of fine powder. It was revealed through the results that *Aegle marmelos* showed the best-reducing power (5.243 ± 0.421 mg AAE/g dw)

ABTS radical scavenging assay

The ABTS radical scavenging activity was measured using the spectrometric method. ABTS forms a stable blue-green coloured free radical and it changes colour in the non-radical form. Here an already formed ABTS radical solution is mixed with the plant extract containing antioxidants. Antioxidants in the plant extracts cause the solution of free radicals to decolorize by interrupting with the ABTS•+ radical generation [54].

$ABTS^{\bullet+} + AH \rightarrow ABTS^{+} + A^{\bullet}$ [54]

ABTS assay takes a long time to complete because the radical formation itself requires 12-16 hours to succeed the reaction between ABTS and potassium persulphate. But after producing the radical it is a trivial test [56]. Another advantage is the solubility of ABTS radical in both organic and inorganic solvents because it can be used to test the radical scavenging capacity of both water loving and lipid loving compounds [58].

The ABTS radical scavenging activity of six different plant extracts were expressed based on the 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) %. It was revealed through the results that *Terminalia chebula* showed the highest ABTS radical scavenging activity (98.86 ± 0.143) %.

DPPH radical scavenging assay

Most frequently, the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical is applied to determine the antioxidative properties of numerous plant materials, due to its stability [59]. After reacting with compounds or extracts that can donate hydrogen atom, the DPPH radical is transformed into a stable DPPH radical, shown by a color change from purple to yellow [60]. The reduction of DPPH radical is determined by obtaining the absorbance of extracts at a 517 nm wavelength. During the reaction with hydrogen atom donating compounds in the plant extracts or samples, the DPPH radical turns into a stable DPPH radical. The colour of the solutions transform from purple to yellow [61, 60]. Out of the purchasable organic nitrogen radicals, DPPH is a stable structure. The advantages of implementing the DPPH assay are its validity, accuracy and subtle and comparatively economical nature and also there is no need to produce radical separately [62].

The DPPH radical scavenging activity of six different plant extracts were expressed based on the 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) %. It was revealed through the results that *Vetiveria zizanioides* showed the highest DPPH radical scavenging activity (95.56 ± 0.634) %.

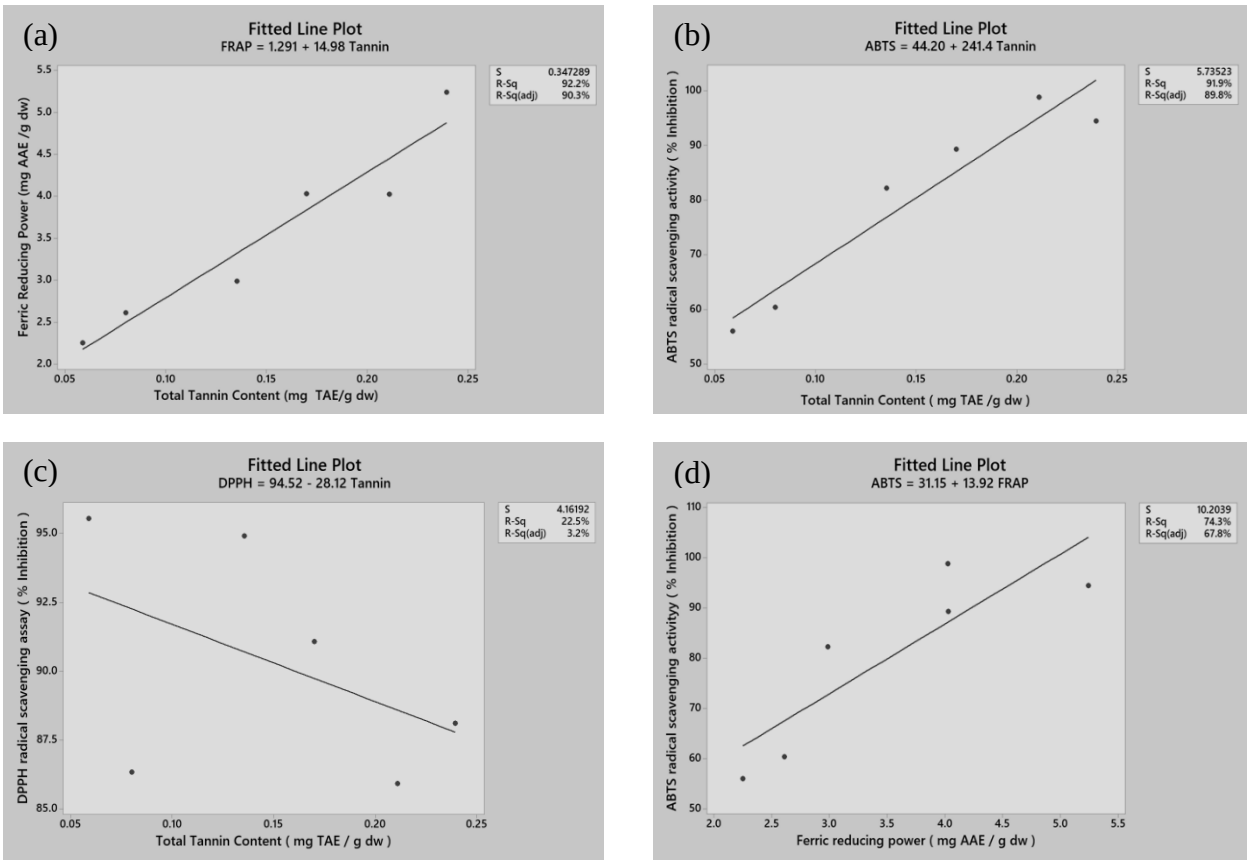

Figure 3: Correlation between; (a) Total tannin content and FRAP assay (b) Total tannin content and ABTS assay (c) Total tannin content and DPPH assay (d) FRAP and ABTS assays

The correlation among different antioxidant assays and secondary metabolite contents were found by performing a regression analysis (Figure 3). A positive correlation was obtained between total tannin content and all three antioxidant assays (Figure 3) as well as total flavonoid content and all three antioxidant assays. Out of them, a strong correlation was seen between total tannin content and FRAP, ABTS assays. This is due to the contribution of tannins [63] and flavonoids [64] to the antioxidant activity of plant extracts. Tannins and flavonoids belong to phenolic compounds in general [65].

The secondary metabolites present in the plants contribute to their antioxidant activity. The mechanism of antioxidant action varies with the type of secondary metabolite. Flavonoids act as free radical scavengers and involve in chelation processes to perform their antioxidant function [66, 67]. The mechanism of action used in the antioxidant activity of phenols is the termination of free radicals [68]. Tannins also act as metal ion chelators during antioxidant action [69].

The ferric reducing antioxidant power assay and the ABTS radical scavenging assay showed a significant linear correlation (R= 0.8234, R^2 = 0.678) as illustrated in Figure 3. No significant correlations were found among DPPH versus ABTS assays, DPPH versus FRAP assays, and total phenol content versus antioxidant assays.

There was no important correlation between phenolic content to ABTS and DPPH assays. This may be due to, the different reactivity of different phenol compounds with ABTS and DPPH. It may be due to their structure and other physical properties. Another reason may be that phenolic

compounds are not the only reason for free radical scavenging activity and it may be because of some other secondary metabolite. Also, the quantity of phenols detected by Folin-Ciocalteu method may not be the most precise value [70]. All types of phenols cannot be assessed solely by the results of the Folin-Ciocalteu assay.

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

4. Conclusion

Among the six commercial medicinal plants evaluated, *A. marmelos* was found out to be the most favourable extract with high content of phytochemicals and fine antioxidant capacity. It supports the fact that different plant secondary metabolites are responsible for the free radical scavenging activity of plants.

The good linear correlation received between tannin content to antioxidant potential determined by FRAP and ABTS assays suggests that Tannins are mostly responsible for antioxidant properties in the studied commercially available medicinal plants.

The highest contents for tannins, both flavonoids and phenols, alkaloids were shown by the medicinal plants *A. marmelos*, *T. chebula* and *C. fenestratum* respectively. The highest performance for antioxidant activity for the three different assays; both FRAP and ABTS, DPPH was shown by the medicinal plants *A. marmelos* and *V. zizanioides* respectively.

Assays conducted in the study proved different plants with highest free radical scavenging activity. So, it clearly stated that apart from the tested phytochemicals some other molecules of plants as well play a role in antioxidant activities.

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