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# Qualitative and quantitative screening of secondary metabolites in selected medicinal plants of Sri Lanka

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**Abstract**
Medicinal plants are valuable source of bioactive compounds such as secondary metabolites (SMs). Sri Lanka is a bio diversity hotspot of flora specially with untouched diverse nature of medicinal plants. The present study includes four endemics and one non endemic but a rare medicinal plant of the country. The methanolic crude extracts of these medicinal plants were qualitatively and quantitatively tested for alkaloids, phenols, flavonoids and tannins. The total phenolic compounds was found significantly higher amount in all tested plants specially in both leaves and flowers, followed by flavonoids, alkaloids and tannins. The highest amount of total phenolic compounds (80.28 ± 2.99 mg GAE/g dw) and tannin (30.81± 1.37 mg TAE/g dw) was resulted in O. *octandra*. Similarly, a significant high amount of total alkaloid content was recorded in B. *ceylanica* (3.55 ± 0.08 mg AE/g dw) and followed by in leaves (2.54±0.29) and flowers (0.07 ± 0.04) of W. *antidysenterica* which belongs to the family Apocynaceae. The total flavonoid content was highest in P. *zatarhendi* var *tomentosa* (147.37± 29.38mg RE/g dw) compare to all others. The results of this study prove that these endemic and native medicinal plants are rich reservoirs of therapeutically important SMs and can be explored them for maximum utilization in traditional and alternative medicine.

**Keywords:** Secondary metabolites, medicinal plants, alkaloids, tannins, phenols, flavonoids

## Introduction
Medicinal plants that contain one or many SMs that can used to heal diseases [1, 2]. Medicinal plants were used to treat human diseases from the beginning of human history. During that time, the active compounds of plants were not identified. But the discovery of morphine (widely used as a pain killer) from Opium poppy plant by Friedrich Serturner in 1804 was an eye opening of scientific community for exploring the varieties of SMs for using as therapeutic agents [3]. Scientists found out that some phytochemicals in medicinal plants such as phenols, alkaloids, flavonoids and tannins have an influence on the human physiology [2, 4, 5]. Such compounds are known as bioactive compounds. Since then, medicinal plants are predominantly analyzed for their bioactive compounds. In present, 74% of natural based drugs are produced from plant derived chemicals [6]. In addition, the knowledge of SMs in medicinal plants are valuable to identify economic plant products i.e. oils, gums, dyes, aromatic compounds to produce perfumes, bio pesticides, precursors to produce complex chemicals etc.
SMs are low molecular weight organic chemical compounds produced inside plant cells or organelles which are not growth essentials but they are found to be implicated with plant defense in instance with phytopathogens and herbivores [7]. Also, they support the plants to withstand unfavorable environmental conditions [8]. SMs are either by-products or intermediates of plant metabolic pathways. The quantity of SMs present in plants vary between the plants of the same species [9, 10]. There are around 100,000 SMs in plant kingdom. They are categorized into three major groups according to their biochemical synthetic pathways: Nitrogen and sulfur containing molecules (alkaloids, cyanogenic glycosides and glucosinolates), Phenolics (flavonoids and tannins), and terpenoids [8]. SMs are known to cause the distinct aroma, taste, dyes and colours in plants [3]. Most importantly they have the therapeutic properties on human and animals which were a revolutionized finding alternative medicines in the past few decades [11, 12].
80% of world population and 70% of Sri Lankans still believe and use indigenous herbal medicine for their health requirements [13, 14]. Ayurveda (Sanskrit word meaning science of life) is the herbal medicine practice in India and Sri Lanka over 5000 years [2].

Ayurveda medicine strongly relies on the medicinal plants of the region. Sri Lanka as a tropical island with different topographic and climatic regions, has a rich endemism in medicinal plants. It has been highlighted that the Sri Lankan endemic medicinal plants should be investigated more for their medicinal values before they get extinct [14]. It is high time to study the Sri Lankan medicinal plants and identifying unique SMs that can be utilized to innovate new drugs to treat human diseases and to produce other economic products. Accordingly, five medicinal plants which are having many medicinal values for different types of human diseases were selected for the current experiments for analyzing the presence of SMs qualitatively and quantitatively (Table 1). The results of this study will be valuable for applying in fields such as pharmacognosy, pharmacology, phytochemistry, natural products, ethnobotany etc.

**Table 1:** Medicinal value of selected plants.

| Plant | Medicinal Value | References |
|---|---|---|
| *Berberis ceylanica* Schneider | The stem extractions are prepared as tinctures (dissolving the drug in alcohol) and made it drink for the patients to treat fever, snake bites, syphilis, menorrhagia, jaundice and enlargement of liver and spleen. | [15, 16] |
| *Walidda antidysenterica* (L) M.Pichon | Leaves and flowers are used for the treatment of ailments after delivery, abscess in genital organs, gonorrhea. The bark and leaves are used together to cure tonsillitis and bronchitis. The bark alone used for snake bites such as Russel's viper bite. The roots are used to treat epilepsy, chronic fever, cough, hemorrhoids and jaundice. The latex is used for toothy and gum ailments. | [17] |
| *Osbeckia octandra* (L) DC | Known to have Hepatoprotective, antidiabetic and antimicrobial properties. The leaf curry of this herb is recommended as a treatment for jaundice. It is also used for hemorrhoids, ascites, hyperlipidemia and cancer | [18] |
| *Valeriana moonii* (Am.ex Clarke) | Whole plant is used to cure Alopecia, cancer, convulsion, cramp, debility, dysuria, epilepsy, Escherichia and snake bites | [19] |
| *Plectranthus zatarhendi* (Frossk.) E.A.Bruce var *tomentosa* (Benth.) Codd | Whole plant is used to cure fevers, dysentery, diarrhea, vomiting, excessive thirst, congestion of the liver, tarantula bites, abscess and heart diseases | [20] |

## Materials and Methodology

### Preparation of crude extract

Fresh plant materials of selected plants were collected from different region of the country. All plants were identified and authenticated by Uva Provincial Department of Ayurveda, Ayurveda Commissionaire's office, Diyatalawa, Badulla, Sri Lanka. The collected plant parts were cleaned and shade dried for 2-3 weeks at room temperature (30°C) to obtain complete dryness. Dried plant materials were ground well using mechanical blender [21]. The resulting coarse powders were sieved through 212 microne sieve to get fine powders and stored properly in labeled air tight dark glass bottles at $4^0$C until use. A 10 g of each powdered plant material was soaked in 100 ml of 80% methanol and they were placed in mechanical shaker at 350 rpm for overnight. It was filtered using whatman filter paper No.1 and filtrate was placed in the rotary evaporator at $40^0$C to obtain a crude extract by evaporating the excess methanol [21, 22].

### Qualitative screening

#### Alkaloids

Few drops of dilute HCl were added to 1ml of the plant extract and mixed well. One or two drops of freshly prepared Mayer's reagent was added to it. Appearance of white precipitate indicated the presence of alkaloids [5, 11].

#### Phenolic compounds

$FeCl_3$ test- Three to four drops of 5% $FeCl_3$ solution were added to the test tube containing 2ml of plant extract. Appearance of blue-black or blue-green colour indicated the presence of phenols in the extract [5, 25].

#### Flavonoids

Alkaline reagent's test- Two to three drops of NaOH (2N) were mixed with 1ml of plant extract. Appearance of intense yellow colour that disappears with the addition of diluted HCl and giving a clear solution was taken as a positive result for flavonoids [11, 25].

Lead acetate test: 1 ml of the plant extract was diluted with 2 ml of distilled water and 3 ml of 10% lead acetate was mixed with diluted plant extract. The presence of flavonoids was indicated as a bulky white precipitate [26].

#### Tannins

Wohler's test- 1 ml plant extract was diluted by adding 9 ml of distilled water. The diluted extract (1.6 ml) was mixed with a 5 – 6 drops of 1% lead acetate. A white precipitate showed the presence of tannins [5].

Ferric chloride test- 10ml of distilled water was added to 1ml of plant extract and was boiled well after mixing. A few drops of filtrated solution was taken in a separate test tube and mixed with 1% ferric chloride. Appearance of bluish black or bluish green colour showed the presence of tannins [1].

### Quantitative screening

#### Estimation of total tannin content

0.5ml plant extract was mixed with 0.25ml of Folin denis reagent, saturated $Na_2CO_3$ and 3.5ml of distilled water. The absorbance was taken within 30 minutes at 700 nm. Total tannin content in extracts were expressed as tannic acid equivalents (mg of TAE/g of extract) [34, 38].

#### Estimation of total Flavonoids

A 0.5ml of plant extract was mixed with 2ml of distilled water, 0.15ml of $NaNO_2$, 0.15ml of $AlCl_3$ and 2ml of NaOH. taken. After 15 minutes, the absorbance was recorded at 510 nm [34, 35]. Total flavonoid content in extracts were mentioned as rutin equivalents (mg of RE/g of extract) [21].

#### Estimation of total phenolic compounds

A 0.5ml of plant extract, 5ml of 10% Folin ciocalteu reagent and 4ml of 1M $Na_2CO_3$ were placed into a 50ml volumetric flask. The final volume was adjusted with distilled water. A 0.5 ml of mixture was withdrawn at the end of 30 minutes and its absorbance was measured at 760 nm using a blank solution without adding the plant extract [32]. Finally, the phenol concentration was determined by using the standard curve, then the total phenolic contents were stated as gallic acid equivalents (mg of GAE/g of extract) [29].

**Estimation of total alkaloids**

A 0.5 ml of plant extract, 5 ml of phosphate buffer (pH 4.7) and 5 ml of bromo cresol green were added in a separatory funnel and it was shaken vigorously with 5 ml of chloroform. The chloroform layer at bottom was carefully removed and its absorbance was measured at 470nm in the presence of blank without adding the plant extract [22, 29]. The alkaloid concentration was determined as atropine equivalents (mg of AE/g of extract) with reference to the standard curve of atropine.

**Statistical Analysis**

All experiments were carried out in three replicates and presented as mean± standard error (SE) using Excel. All graphs were plotted using Microsoft excel. One way analysis of variance (ANOVA) and statistically significant was considered at $P = 0.05$. Pearson correlation test was applied to determine the correlation coefficient value ($R^2$).

**Results and Discussion**

The present study reveals the presence of SMs such as alkaloids, phenolic compounds, flavonoids and tannins in variable amount in the selected endemic and native medicinal plants of the country.

All extractions were carried out using methanol extractions. It has proven that organic solvents such as ethanol and methanol or a mixture of organic solvents with water would give the best yield of phenolic compounds [33]. Many literature of similar studies have demonstrated the use of methanol as an extracting solvent/ medium [21, 34]. Similarly, due to the fact that flavonoids and tannins are also coming under the phenolic group, methanol was proven as the suitable solvent [39]. In the case of alkaloids, they are present in plants as salts of organic and inorganic acids together with other water soluble compounds such as proteins, minerals etc. Therefore, it is more preferable to use water and alcohol mixtures to extract alkaloids [40]. Moreover, it has been mentioned that most alkaloids in plants are soluble in methanol or ethanol [44]. The details about the chemical constituents of new plants which have not being subjected to a similar study are generally investigated by conducting a qualitative screening. In this study, qualitative analysis showed some notable results for all the plant extractions as shown in Table 2. Two standard tests for each metabolite were used to ensure the accuracy of the results. At least a single test gave positive result for a particular metabolite hence subsequently, that extraction was subjected to the quantitative estimation. O. *octandra* gave no reaction for Mayer's test but it gave moderate positive reaction for Dragendorff's test. Similarly, V. *moonii*, leaves and flowers of W. *antidysenterica* gave no reaction to Dragendorff's test but they gave weak positive reaction to Mayer's test. Both these reagents test the presence of alkaloids and they react with different types of alkaloids i.e., Dragendorff's reagent react mostly with tertiary amines and Mayer's reagent react with many alkaloids including tertiary amines [41]. It was clearly seen in the results of qualitative screening, that the selected medicinal plants are having all four SMs of interest.

**Table 2:** Qualitative test for alkaloids, phenolic compounds, flavonoids and tannin of crude extract

| Plant / Experiments | B.*ceylanica* | Leaves of W. *antidysenterica* | Flowers of W. *antidysenterica* | O. *octandra* | V.*moonii* | P.*zatarhendi* var *tomentosa* |
|---|---|---|---|---|---|---|
| Mayer's test | +++ | + | + | - | + | + |
| Dragendorff's test | + | - | - | ++ | - | ++ |
| Ferric chloride test | + | ++ | +++ | +++ | ++ | +++ |
| Alkaline reagent test | +++ | +++ | +++ | +++ | +++ | +++ |
| Lead acetate test | ++ | +++ | +++ | +++ | +++ | +++ |
| Wohler's test | ++ | +++ | +++ | +++ | +++ | +++ |
| Tannin Ferric chloride test | + | ++ | +++ | +++ | +++ | +++ |

(+++ strong positive reaction, ++ moderate positive reaction, + weak positive reaction, - no reaction).

The quantitative estimation of the SMs showed that they all exist in plants in different quantities. In general, phenols and flavonoids are omnipresent in the plant kingdom [11]. The phenolic compounds were found in highest quantity in all tested plants, followed by flavonoids, alkaloids and tannins respectively as shown in Table 3. Spectrophotometric determination of total alkaloids using BCG is a simple and a sensitive method. BCG forms a yellow colour complex with alkaloids and it is completely extractable in chloroform at pH 4.7 [22]. BCG reacts with the alkaloids that contain nitrogen atom within its cyclic structure. But it cannot react with amine and amide alkaloids which causes a limitation in this method. Therefore, the obtained results are giving an overall idea about the total alkaloid contents in the plants [22, 42]. The colour was allowed to develop for a constant time and the absorbance were measured at 700nm.

**Table 3:** Total contents of different SMs in the studied medicinal plants.

| Plant | Alkaloid mg AE/g dw | Phenol mg GAE/g dw | Flavonoid mg RE/g dw | Tannin mg TAE/g dw |
|---|---|---|---|---|
| B. *ceylanica* | 3.56 ± 0.08 | 7.75 ± 2.304 | 8.19 ± 1.029 | 0.69 ± 0.03 |
| Leaves of W. *antidysenterica* | 2.54 ± 0.30 | 15.39 ± 2.32 | 9.88 ± 0.56 | 0.72± 0.13 |
| Flowers of W. *antidysenterica* | 0.07 ± 0.04 | 31.40 ± 5.60 | 15.46 ± 1.88 | 0.51± 0.02 |
| O. *octandra* | 1.40 ± 0.21 | 80.28 ± 3.00 | 114.35 ± 11.61 | 30.81± 1.37 |
| V. *moonii* | 1.93 ± 0.56 | 15.16 ± 0.63 | 15.50 ± 1.09 | 0.64± 0.09 |
| P. *zatarhendi* var *tomentosa* | 1.02 ± 0.25 | 31.52 ± 1.71 | 147.37± 29.36 | 0.74± 0.05 |

The total tannin content in the medicinal plants extracts were expressed as mg tannic acid equivalent/g dry weight of the sample using tannic acid standard curve ($y=3.36x+0.04$, $R^2 = 0.96$). Usually, tannins are abundant in parts which are more vulnerable for herbivores i.e., young leaves, flowers etc. because tannins are good defensive chemicals that act as toxins and feeding repellents for most herbivores [47]. But flowers of W. *antidysenterica* showed very low tannin content and it had no significant difference with total tannin contents of B. *ceylanica*, V. *moonii*, P. *zatarhendi* var *tomentosa*. Similarly, Tannins are largely found in the bark (specifically, in the layer between cortex and epidermis) protecting the

plants by forming a barrier for microorganisms. The only stem extraction in this study gave less total tannin content. It might be because the bark of the stems used for this study were removed prior to drying. In leaf tissues tannins are mostly present in the upper epidermis of young leaves. But in the leaves of evergreen plants they are equally distributed [44]. As shown in figure 1(a), O. *octandra* was having the richest tannin content (30.81± 1.37).When compared to all other metabolites, tannin content was relatively low in selected parts of every selected medicinal plant.

To determine total flavonoid content Aluminum chloride spectrophotometric method was used. It is a simple, precise and a widely used method. Aluminum chloride undergoes a complexation reaction with flavonoids. For that $NaNO_2$ should be present in alkaline medium. At first, the aromatic ring having a catechol group (unsaturated six carbon ring with two hydroxyl groups attached to adjacent carbons) in its third or fourth position unsubstituted or not sterically blocked, undergoes nitration. $AlCl_3$ is added afterwards [4]. Then, $Al^{+3}$ reacts with hydroxyl groups of the flavonoid. It forms the yellow coloured complex which immediately turned to reddish colour with addition of NaOH. The intensity of colours are proportional to the flavonoid concentration in the extraction [33].

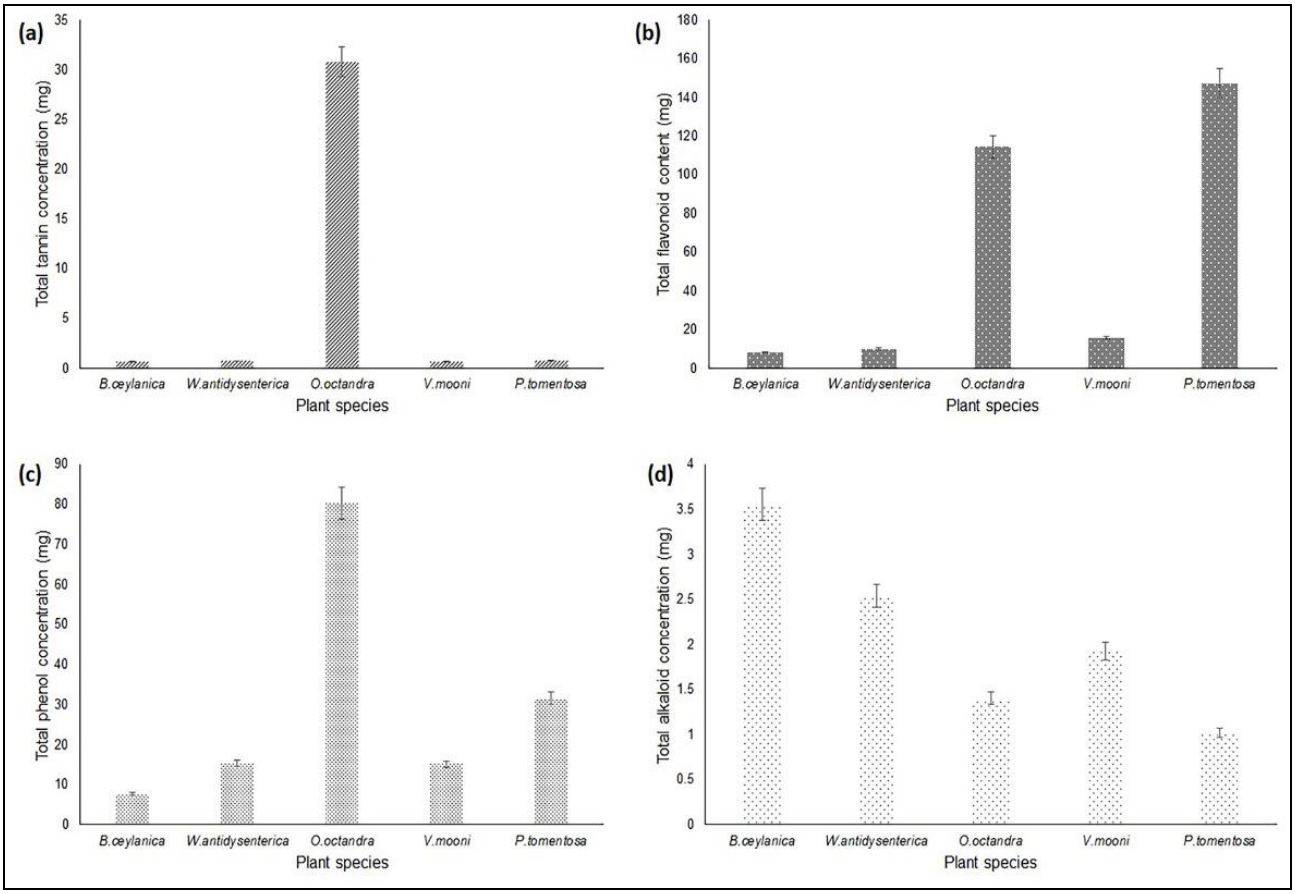


**Fig 1:** Total content of different secondary metabolites; tannin (1a), flavonoid (1b), phenol (1c) and alkaloid (1d) in tested medicinal plants.

The total flavonoid content in the medicinal plants extracts were expressed as mg rutin equivalent/g dry weight of the sample using rutin standard curve ($y=0.84x+0.00$, $R^2 = 0.97$). As shown in figure 1(b), P. *zatarhendi* var *tomentosa* was having the richest flavonoid content (147.37± 29.36). That may be the reason for its very pleasant aroma. Because flavonoids are responsible for the colour, aroma and flavor of plants [30]. O. *octandra* was having (114.35 ± 11.61) the second highest flavonoid content. It is recorded that the plants grow in tropical countries and in high altitudes are having higher flavonoid content than the plants growing in the temperate region. Because flavonoids accumulate in the plant leaves to protect them from UV-exposure [31]. Both these plants were collected from the mountain regions of the country, thus they get overexposure to the UV radiation. Based on the results of mean separation at 95% confidence level, there were no significant difference of flavonoid content in B. *ceylanica*, V.*moonii*, leaves and flowers of W. *antidysenterica* and they all contain low flavonoid content (Table 3).

UV-Vis spectroscopy has proven as one of the best and accurate procedures to quantify the phenolic content in plant extracts. Mainly because of the chemical structure of phenols i.e., phenol ring is capable to absorb UV light [44]. Folin-ciocalteu method was used to quantify the phenol content, ever since it was introduced by Otto Folin and Vintila ciocalteu in 1927 with little modifications [45]. Folin-ciocalteu reagent is a mixture of two acids; phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMO_{12}O_{40}$). They efficiently react with phenols in the plant extract and form phosphotungstic-phosphomolybdic complex which is blue in colour. It is quantified at 760nm [44]. In this method it is very important to follow the order of chemical addition to the extract. It helps to ensure that the reaction takes in alkaline medium ($Na_2CO_3$ is added after the addition of folin-ciocalteu reagent). The maximum absorption is depending on the alkaline nature of the medium and the phenol concentration in the extract. This reagent is decomposable in the alkaline solution. Therefore, excess amount of folin-ciocalteu reagent need to be added to allow the completion of the reaction. The reagent already contains lithium salts to prevent the buildup of turbidity or precipitates due to excess addition [46].

The total phenol content in the medicinal plant extracts were expressed as mg gallic acid equivalent/g dry weight of the sample using the gallic acid standard curve (y=6.48x+0.01, $R^2$=0.99). Phenols are extensively found in higher plants [47]. They protect the plants from UV radiation through their radical scavenging ability and from herbivores by acting as enzyme inhibitors and feeding deterrents [46]. The highest phenol content was measured in O. *octandra* (80.28 ± 3.00). Whereas, the lowest phenolic content was measured in B. *ceylanica* (7.75±2.30) as illustrated in figure 1(c). According to the results of mean separation at 95% confidence interval, there was no significant difference between the phenolic content in leaves of W. *antidysenterica* and V. *moonii*. Similarly, it showed that flowers of W. *antidysenterica* and P. *zatarhendi* var *tomentosa* had no significant difference in there phenolic content at 95% confidence interval.

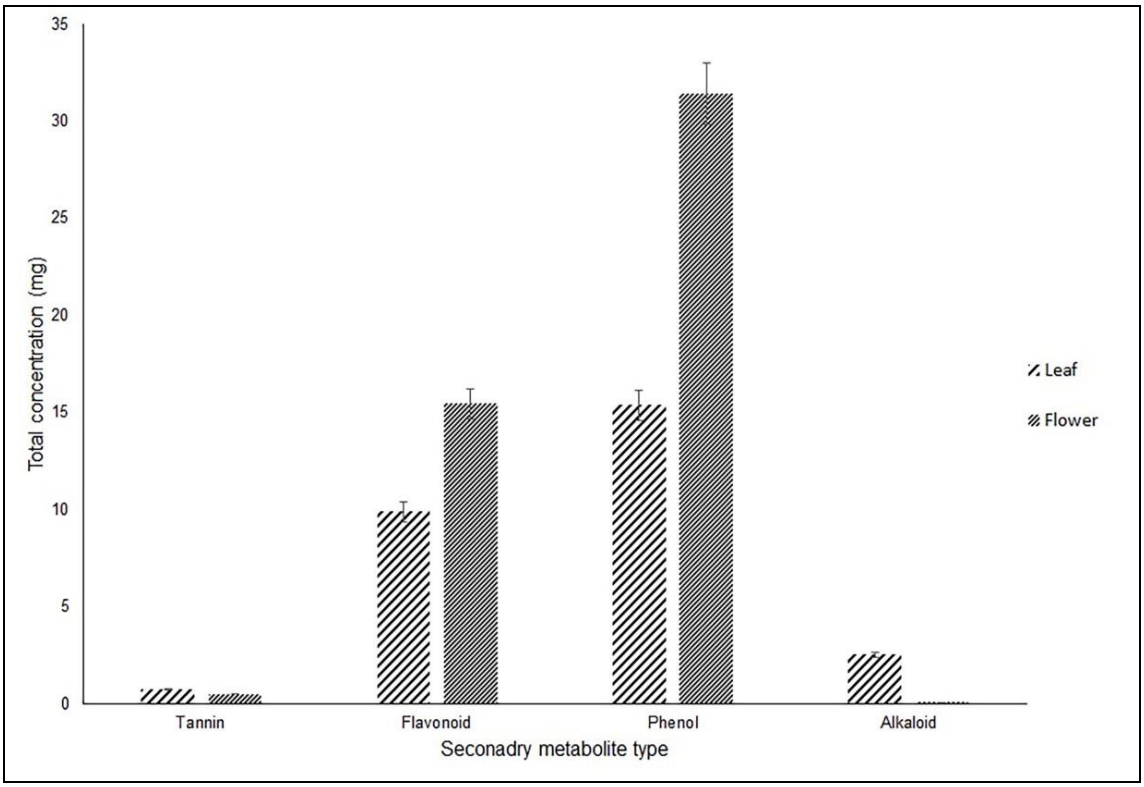


**Fig 2:** Total content of different secondary metabolites in both leaves and flowers of tested plants.

The alkaloid content in plant extracts were expressed as mg atropine equivalent/g dry weight of the sample using atropine standard curve (y=1.37x+0.03, $R^2$=0.98). As shown in the Figure 1(d), the highest alkaloid content was measured in the extraction obtained from the stems of *B. ceylanica* (3.56 ± 0.08). In angiosperms some plant families such as Paparvaceae, Fabaceae, Boraginaceae, Asclepiadaceae, Asteracea, Liliaceae, Gentaceae, Ranunculaceae, Rubiaceae, Solanaceae, Rutaceae, Apocynacea and Berberidaceae are known to have rich content of alkaloids [43]. Genus *Berberis* of family Berberidaceae is well known in the world for its high content of isoquinoline alkaloid berberin [16]. B. *ceylanica* is one among the three species of that genus that grow in Sri Lanka. The second highest amount of alkaloid was recorded from the leaves of W. *antidysenterica* (2.535±0.297) which belongs to the family Apocynaceae. The lowest was in the extraction of Flowers of W. *antidysenterica* (0.070 ± 0.039) (Table 3).

The distribution of SMs are vary among the parts of plants tested. Phenol is the major secondary metabolite in flowers of medicinal plants that used for this experiment and followed by the presence of flavonoid (fig. 2). However, phenol and flavonoid are dominantly prevailing substances in leaves compared to alkaloid and tannin. The figure 2 reveals that more alkaloid can be extracted from leaves.

Different SMs in plants have provided an outstanding contribution to the pharmaceutical industry [4, 45]. It shows the possible relationship of quantified SMs with the medicinal values of these plants proving that these endemic and native medicinal plants are rich reservoirs of therapeutically important SMs.

## Conclusions

The present study revealed that the stems of B. *ceylanica*, leaves of W. *antidysenterica*, flowers of W. *antidysenterica*, O. *octandra*, V. *moonii* and P. *zatarhendi var tomentosa* contains significant quantities of alkaloids, phenols, flavonoids and tannins. The highest alkaloid content was recorded in B. *ceyalnica* stem extraction. O. *octandra* was having the highest phenol and tannin content. The highest flavonoid content was recorded in P. *zatarhendi* var *tomentosa* plant extraction. The SMs found in these valuable medicinal plants should be further studied to isolate, identify, and elucidate the structures and to produce them chemically or biotechnologically to encourage their utilization in pharmaceutical and other possible industries. Due to the fact that this study contains four endemics and one native but a rare plant, scientists should urge to study them more as well as to distribute and conserve them for the future generations.

## 5. References

1. Khan FA, Hussain I, Farooq S. Phytochemical Screening of Some Pakistanian Medicinal Plants. 2011; 8(3):575-578.
2. Ullah M Zahoor N, Farhat A. A review on general introduction to medicinal plants its phytochemicals and role of heavy metal and inorganic constituents. J Life Sci. 2014; 11(7):520–527.

3. Ratha KK, Dixit AK, Hazra J, Rungsung W. Secondary metabolites of plants in drugs discovery. World J Pharm Res. 2015; 4(7):604-613.
4. Aiyelaagbe O, Osamudiamen P. Phytochemical screening for active compounds in Mangifera indica leaf from Ibadan Oyo State. Plants Sience Research. 2009; 2(1):11-14.
5. Ajuru MG, Williams LF, Ajuru G. Qualitative and Quantitative Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger Delta Region of Nigeria. J food Nutr Sci. 2017; 5(5):198-205. [doi: 10 11648/j jfns 20170505 16]
6. Mirocha A, Miller J. Medicinal Plants at Risk. Nature's Pharmacy, 2008, 1-15.
7. Goyal S, Merillon JM, Ramawat KG, Cluzet S, Lambert C. Secondary Metabolites and Plant Defence. J. Biolofical Control, 2014.
8. Ashraf MA, Riaz M, Arif MS, Iqbal M, Rasheed R, Iqbal H *et al*. Environmental Stress and Secondary Metabolites in Plants : An Overview, 2018, 153-167.
9. Koche D. An Overview of major Classes of Phytochemicals: Their Types and Role in Disease Prevention. J Hislopia. 2018, 9(1):1-11.
10. Ncube N, Van Staden J. Tilting Plant Metabolism for Improved Metabolite Biosynthesis and Enhanced Human Benefit. Molecules. 2015, 20:12698-12731. [doi: 10 3390/molecules200712698]
11. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011; 3(12):10-14.
12. Soni V, Jha A, Dwivedi J, Soni P. Qualitative and Quantitative Determination of Phytoconstituents in Some Antifertility Herbs. Indian J Pharm Sci. 2018; 80(1):79-84.
13. Ekor M. The growing use of herbal medicines : issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014; 4(1):1-10. [doi: 10 3389/fphar 2013 00177]
14. Jayasinghe B, Bandara BMR, Wickramasinghe A, Wijesundara DSA, Karunaratne V. The importance of harnessing the rich diversity of Sri Lankan flora for their medicinal value. Ceylon J Sci. 2017; 46(4):3-13.
15. Hewageegana HGS, Arawwawala LDAM. *Berberis ceylanica* schneider Stems : A Rich Source of Berberine with Antimicrobial Properties. J Pharm Pract Educ. 2018; 3(15):1-4.
16. Wickramaratne MN, Gunatilake LP, Anuradha NGD, Godavillathanna AN, Perera MGN, Nicholas I *et al*. Antioxidant Activity and Antibacterial Activity of *Walidda antidysenterica*. J Pharmacogn Phytochem. 2015; 4(3):121-126.
17. Perera R, Ekanayake S, Ranaweera K. *In vitro* study on antiglycation activity antioxidant activity and phenolic content of *Osbeckia octandra* L leaf decoction J Pharmacogn Phytochem. 2013; 2(4):158-161.
18. Pathirathna P, Paranagama P. Control of stored grain pest *Callosobruchus maculatus* (F) (Coleoptera : Bruchidae) using the essential oil isolated from *Plectranthus zeylanicus*. Nat Prod Res. 2012; 26(23):2219-22. [doi: 10 1080/14786419 2011 643883]
19. Baba SA, Malik SA. Determination of total phenolic and flavonoid content antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Sci Taibah Univ. 2015; 9(4):449-454. [doi: 10 1016/j jtusci 2014 11 001]
20. Ajanal M, Gundkalle MB, Nayak SU. Estimation of total alkaloid in Chitrakadivati by UV - Spectrophotometer. Anc Sci Life. 2018; 31(4):2-5. [doi: 10 4103/0257-7941 107361]
21. Udeozo IP, Akpaba ES, Ugwu OPC, Okoye NH, Umedum NL. Qualitative Alkaloidal Analyses of Some Selected Nigerian Medicinal Plants Used in Herbal. Int J Life Scieinces Biotechnol Pharma Res. 2013; 2(3):1-6.
22. Sasikala M, Sundaraganapathy R. Qualitative Analysis of Alkaloids Exist in the Hydroalcoholic Extract of *Ipomoea aquatica* for SSK in Tamil Nadu. Int J ChemTech Res. 2017; 10(7):446-454.
23. Dethe UL, Joshi SS, Desai SS, Aparadh VT. Screening of bioactive compounds of *Sesbania grandiflora* and *Pistia stratiotes*. Indian J Adv Plant Res. 2013; 1(1):27-30.
24. Krishnaveni G, Sailaja O, Kumar KK. Phytochemical screening and quantitative analysis of hexane acetone methanol & water extracts of *Salicornia virginica* by UV-spectrophotometry. Sch Res Libr. 2016; 8(16):52-56.
25. Vivek V, Raval K. First Order Dervative Method development and Validation For Simultaneous Estimation of Atropine Sulfate and Dexamethasone Eur J Pharm Med Res. 2016; 3(4):367-372.
26. Patel RK, Patel JB, Trivedi PD. Spectrophotometric Method for the Estimation of Total Alkaloids in the *Tinospora cordifolia* M and Its Herbal Formulations. Int J Pharm Pharm Sci. 2015; 7(10):7-9.
27. Dhumal JS, Chaudhari SR, Chavan MJ. Effect of Different Extraction Conditions on Total Alkaloids Total Phenolic and Total Flavonoid Content of *Vigna mungo* L hepper. J Drug Deliv Ther. 2019; 9(4):89-91.
28. Saputra A, Andayani S, Nursyam H. Total Quantity of Phenol and Isolation Methanol Tannin Extract of Red Betel Leaf (*Piper crocatum*) Int J Pharm Tech Res. 2016; 9(7):146-153.
29. Siddiqui N, Rauf A, Latif A, Mahmood Z. Spectrophotometric determination of the total phenolic content spectral and fluorescence study of the herbal Unani drug Gul-e- Zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sci. 2017; 12(4)360-363. [doi: 10 1016/j jtumed 2016 11 006]
30. Monteiro JM, De Souza JSN, Neto EMFL, Scopel K, Trindade EF. Does total tannin content explain the use value of spontaneous medicinal plants from the Brazilian semi-arid region?. Rev Bras Farmacogn. 2014; 24(2):116-123. [doi: 10 1016/j bjp 2014 02 001]
31. Amorim ELC, Nascimento JE, Monteiro JM, Sobrinho TJSP, Albuquerque UP. A Simple and Accurate Procedure for the Determination of Tannin and Flavonoid Levels and Some Applications in Ethnobotany and Ethnopharmacology. Funct Ecosyst Communities. 2008; 2(1):88-94.
32. Chakraborty K, Bhattacharjee S, Pal TK, Bhattacharyya S. Evaluation of *in vitro* antioxidant potential of Tea (*Camelia sinensis*) leaves obtained from different heights of Darjeeling Hill West Bengal J Appl Pharm Sci. 2015; 5(1):63-68. [doi: 10 7324/JAPS 2015 50112]
33. Zhao L *et al*. Determination of Total Flavonoids Contents and Antioxidant Activity of *Ginkgo biloba* Leaf by Near-Infrared Reflectance Method. Hindawi Int J Anal Chem, 2018, 1-8.
34. Padma R, Parvathy NG, Renjith V, Rahate KP. Quantitative estimation of tannins phenols and antioxidant activity of methanolic extract of *Imperata cylindrica*. Int J Res Pharm Sci. 2013; 4(1):73-77.
35. Kavitha CCI, Indira G. Quantitative estimation of total

phenolic flavonoids tannin and chlorophyll content of leaves of *Strobilanthes Kunthiana* (Neelakurinji). J Med Plants Stud. 2016; 4(4):282-286.
36. Pal S Bhattacharyya TK, Dey A. Evaluation of antioxidant activities of flower extract (fresh and dried) of *Saraca indica* grown in West Bengal. Int J Curr Microbiol Appl Sci. 2014; 3(4):251-259.
37. Garg P, Garg R. Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extract. Pharma Innov. 2019; 8(2):16-21.
38. Blainski A, Lopes G, Mello J. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. Molecules. 2013; 18:6852-6864. [doi: 10 3390/molecules18066852]
39. Pagare S, Bhatia M. Secondary metabolites of plants and their role: Overview. Curr Trends Biotechnol Pharm. 2016; 9(3):294-305.
40. Seca AML, Pinto DC. Biological Potential and Medical Use of Secondary Metabolites. Medicines. 2019; 6:1-6. [doi: 10 3390/medicines6020066]
41. Pekel A, Pyrzynska K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. Food Anal Methods. 2014; 7:1776-1782. [doi: 10 1007/s12161-014-9814-x]
42. Samanta A, Das G, Das SK. Roles of flavonoids in Plants. J Pharm Sci Tech. 2011; 6(1):12-35.
43. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. Int J Biol Sci. 2015; 11(8):982-991. [doi: 10 7150/ijbs 12096]
44. Galvao MAM, De Arruda AO, Bezerra, ICF, Ferreira MRA, Soares, LAL. Evaluation of the Folin Ciocalteu Method and Quantification of Total Tannins in Stem Barks and Pods from *Libidibia ferrea* (Mart ex Tul) L P Queiroz. Brazilian Arch Biol Technol. 2018; 61:1-20.
45. [45] Tempel AS. Tannin- Measuring Techniques: A Review. J Chem Ecol. 1982; 8(10):1289-1298.
46. Ashok PK, Upadhyaya K. Tannins are Astringent. J Pharmacogn Phytochem. 2012, 1(3):45-50.
47. Anulika NP, Ignatius EO, Raymond ES, Osasere O, Hilda, A. The Chemistry of Natural Product : Plant Secondary Metabolites. J Technol Enhanc Emerg Engineeering Res. 2016; 4(8):1-9.