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Pharmacognostical studies of *Eclipta prostrata* (Linn.) grown in Sri Lanka

[Estudios farmacognósticos de *Eclipta prostrate* (Linn.) cultivada en Sri Lanka]

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Abstract: *Eclipta prostrata* Linn. is an annual herbaceous plant used in traditional medicine, commonly known as "Trailing Eclipta" or False Daisy in English, '*Keekirindiya*' in Sinhala and '*Kayyantakara'* in Tamil. The aim of this study was to conduct a detailed pharmacognostical evaluation of *E. prostrata* found in Sri Lanka. Anatomical, physicochemical, phytochemical studies, and quantification of phyto-constituents were performed as per WHO guidelines. Whole plant was sequentially extracted into solvents with different polarities. Phytochemical screening and Thin Layer Chromatography (TLC) fingerprinting were carried out. Anatomical study and powder microscopy revealed useful diagnostic features. Physicochemical parameters such as moisture content, ash values (total, acid insoluble, water soluble) and extractable matter in water and ethanol were evaluated. Phytochemical screening and TLC fingerprinting revealed the presence of different types of phyto-constituents. Alkaloid, tannin, saponin, total flavonoid and total polyphenol contents were quantified. In conclusion, pharmacognostical study aids in establishing the standardization parameters of *E. prostrata* found in Sri Lanka.

Keywords: *Eclipta prostrata;* Pharmacognostical; Physico-Chemical; Phytochemical; Keekirindiya.

Resumen: *Eclipta prostrata* Linn. es una planta herbácea anual utilizada en la medicina tradicional, comúnmente conocida como "Trailing Eclipta" o False Daisy en inglés, 'Keekirindiya' en sinhala y 'Kayyantakara' en tamil. El objetivo de este estudio fue realizar una evaluación farmacognóstica detallada de *E. prostrata* encontrada en Sri Lanka. Se llevaron a cabo estudios anatómicos, fisicoquímicos, fitoquímicos y cuantificación de fitoconstituyentes según las directrices de la OMS. Se extrajo secuencialmente la planta entera en solventes con diferentes polaridades. Se realizaron pruebas de tamizaje fitoquímico y huellas dactilares de cromatografía en capa delgada (TLC). El estudio anatómico y la microscopía en polvo revelaron características diagnósticas útiles. Se evaluaron parámetros fisicoquímicos como el contenido de humedad, los valores de ceniza (total, insoluble en ácido, soluble en agua) y la materia extraíble en agua y etanol. Las pruebas de tamizaje fitoquímico y las huellas dactilares de TLC revelaron la presencia de diferentes tipos de fitoconstituyentes. Se cuantificaron los contenidos de alcaloides, taninos, saponinas, flavonoides totales y polifenoles totales. En conclusión, el estudio farmacognóstico ayuda a establecer los parámetros de estandarización de *E. prostrata* encontrada en Sri Lanka.

Palabras clave: *Eclipta prostrata*; Farmacognóstico; Fisicoquímico; Fitoquímico; Keekirindiya

INTRODUCTION

The use of herbal medicines has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Romano *et al*., 2021). Recently, it has been reported that many traditional Asian and African herbal remedies are used against diseases in a renewed interest in medicinal plants. Some of the herbs used to prepare medicines are considered as weeds by agriculturists. One such weed is *Eclipta prostrata* (Family Asteraceae) which is a dicotyledonous, semi-aquatic weed (Ediriweera, 2010). The plant is a small, branched annual herb which is native to the tropical and subtropical regions of the world with white flower heads (Khanna & Kannabiran, 2007). It is commonly known as "Trailing Eclipta" (False Daisy) in English (Rajakumar & Rahuman, 2011), "Keekirindiya" in Sinhala (Ediriweera, 2010) and "Kayyantakara" (Arunachalam *et al*., 2009) or "Karisilanganni" (Jayaweera, 1981) in Tamil. In Ayurveda, *E. prostrata* is used externally for hair growth, headache and skin diseases (Jayaweera, 1981).

It is believed to regulate blood circulation and, biliary and liver functions, as well as in proper digestion of nutrients. It has a protective role in ophthalmic health. The seed of *E. prostrata* is used to increase sexual vigor. The decoction is a remedy for tuberculosis and disease related to ear. The oil of this plant helps in alleviating respiratory distress and the juice helps to prevent abortion (Ayurveda, 1976). According to Ediriweera (2010), powdered equal parts of the entire plant of *E. prostrata* and pericarp of *Terminalia chebula* is a cure for gastritis.

Proper identification of the plant along with the identification of the crude drug and pharmacognostical studies help to identify the adulterants in the single drug as well as in the formulation. Despite the extensive use of the plant in traditional medical system in Sri Lanka (Ayurveda, 1976) existing reports reveal that no detailed pharmacognostical study had been done on *E. prostrata* grown in Sri Lanka. Hence, the aim of this study was to investigate *E. prostrata* by evaluating pharmacognostical, physico-chemical and phytochemical properties of different extracts of the whole plant grown in Sri Lanka.

MATERIAL AND METHODS

Chemicals

Distilled water was obtained from MANESTY L4 (England) distilled water plant. Hexane, dichloromethane, ethyl acetate, methanol, chloroform and glacial acetic acid were of analytical grade and all other chemicals were laboratory grade.

Instruments

Calibrated and well-maintained laboratory instruments were used in all procedures. Freeze drier (Alpha 1-2 Ld Plus, USA) and analytical balance (Precista, Swiss) were used.

Collection of plant materials

The whole plant of *E. prostrata* was collected from the rice fields of Batticaloa (Eastern province 7.7853° N, 81.4279° E), Kalutara (Western province 6.9016° N, 80.0088° E) and Matara (Southern province 6.2374° N, 80.5438° E) districts of Sri Lanka, from July to October in 2019. The herbarium specimen of the whole plant was authenticated at the National Herbarium, Peradeniya, Sri Lanka.

Preparation of plant powder

Whole plant of *E. prostrata* was washed thoroughly, air-dried and cut into small pieces. The dried plant was further dried in 40ºC in an oven until a constant weight was obtained and then grounded to a course powder. The plants collected from three different areas were combined (1:1:1 w/w) and used as a composite sample for the tests.

Organoleptic, macroscopic and microscopic analysis

Coarsely powdered sample of whole plant was evaluated for color, odor, taste, texture, etc. The organoleptic characters of the sample were evaluated based on the method described by Vinothaa *et al*., (2013). Morphology and anatomy of both vegetative and reproductive parts including the stem, leaf, flower, fruit and seed were examined microscopically (Dash *et al*., 2013).

Powder microscopy

Powdered samples of the whole plant of *E. prostrata* were examined under the microscope for their characteristic features following WHO guidelines (WHO, 2011).

Physico-chemical analysis

The parameters which were studied are the moisture content, water and ethanol extractable matters and total, acid insoluble and water-soluble ash values of the powder of whole plant of *E. prostrata* (WHO,

2011). Presence or absence of heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) were detected using an Inductively Coupled Plasma - Mass Spectrometer (ICP-MS).

Preliminary phytochemical screening

The coarse powder was extracted into hexane, dichloromethane, ethyl acetate, methanol and water using continuous Soxhlet extraction in accordance with their increasing polarity. The ratio of plant material to solvent was 1:5. Qualitative phytochemical screening of different extracts was carried out according to the methods described by Farnsworth (1966) and Evans (2009).

Thin Layer Chromatography (TLC)

Silica plates were used as the stationary phase. Different extracts of the whole plant of *E. prostrata* were spotted using glass capillaries on the same TLC plate. Methanol, cyclohexane, dichloromethane, ethyl acetate and diethyl amine in different ratios were used as the mobile phase. For polar solvent system the ratio of methanol, cyclohexane, dichloromethane, ethyl acetate and diethyl amine were 1:6:10:1:5 whereas for the non-polar solvent system the ratio was 1:10:10:1:0 respectively. TLC plate developed using above solvent systems were observed under UV at 254 nm and 366 nm (Zahiruddin *et al*., 2021). And retention factor (R_f) values were determined.

Quantification of phytochemicals

Total polyphenol content: Total polyphenol content (TPC) of the extract was determined by the Folin-Ciocalteu spectrophotometric method (Liu *et al*., 2021) using gallic acid as the standard phenolic compound.

Total flavonoid content: Total flavonoid content (TFC) was determined according to the method described by Zin *et al.* (2018), with slight modifications. Quercetin was used to prepare the standard curve and TFC was expressed as mg quercetin equivalents (QE)/g of the extract.

Alkaloid and saponin content: Quantitative determination of alkaloid and saponin were carried out according to the methodology by Koomson *et al*., (2018).

Tannin content: Tannin content was determined as described by Wangiyana *et al*. (2018).

Statistical analysis

Experiments were performed in triplicate (n=3) and the results were expressed as mean values with standard error of mean (SEM). The data were analyzed using Microsoft Excel and SPSS (version 25.0). A $p \leq 0.05$ was considered as significant.

RESULTS

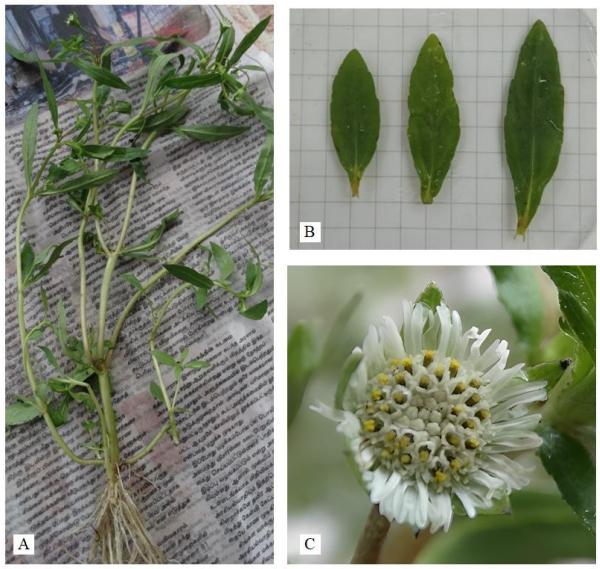
Morphological and anatomical characters of fresh plant material

Eclipta prostrata is an annual herbaceous plant (Figure No. 1A) with an average height of 20 - 50 cm. Stem is erect or prostrate and branched. Leaves are sessile, lanceolate, entire and with hairs (Figure No. 1B) on both surfaces. The type of the inflorescence is a Head bearing female ray florets and bisexual disc florets (Figure No. 1C). Corolla 3-5 toothed, stamens syngenesious and five in number, filaments epipetalous. Ovary is inferior, unilocular with one basal ovule. Brownish fruits are one seeded with a narrow wing and covered with warty excrescences. Seeds are non-endospermic.

Upper and lower epidermis of the leaves consist of polygonal cells (Figure No. 2A) and are covered with thin cuticle and trichomes. Multicellular trichomes are with unicellular or bicellular heads. Styloids (prismatic Ca-oxalate crystals) and druses are seen in the mesophyll cells of the lower surface. Anisocytic and anomocytic stomata (Figure No. 2B1 and No. 2B2) are present on both surfaces, but more abundant on lower surface. Stomatal index is 16.0-20.0 in upper and 21.5 -25.0 in lower surface. Palisade ratio is 3.8 - 4.5 and vein islet number is 3. The transverse section of the stem is circular in outline with a narrow ring of collateral vascular bundles surrounding the central parenchymatous pith containing prisms of Ca-oxalate crystals (Figure No. 2C and Figure No. 2D). The trichomes of the epidermis (Figure No. 2E) are similar to those observed in leaves. Phloem, a narrow strip composed of sieve elements and phloem parenchyma; Secondary phloem consists of sieve elements, phloem fibers. Xylem consists of numerous xylem vessels, tracheids, xylem fibers and xylem parenchyma in axial system and multiserate xylem rays in ray system.

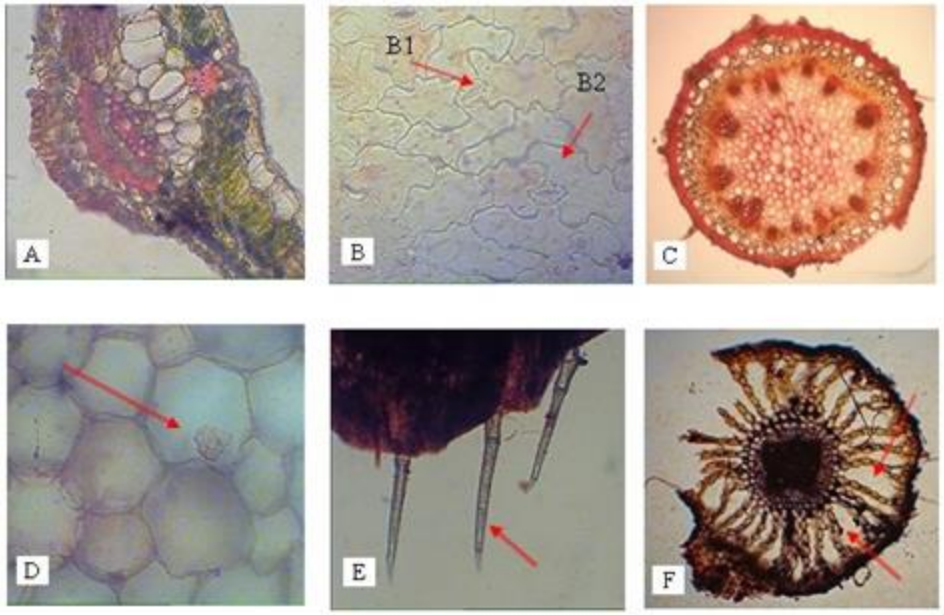
Well-developed tap root is observed, and main root gives rise to several secondary branches. Macroscopically the roots are cylindrical in shape, with rough external surface. Inner secondary cortex consists of comparatively bigger, irregular shaped parenchymatous cells with prominent air spaces (Figure No. 2F).

Figure No. 1



Morphology of *Eclipta prostrata*. A. A habit B. Diversity in size of leaves, C. Flower

Figure No. 2



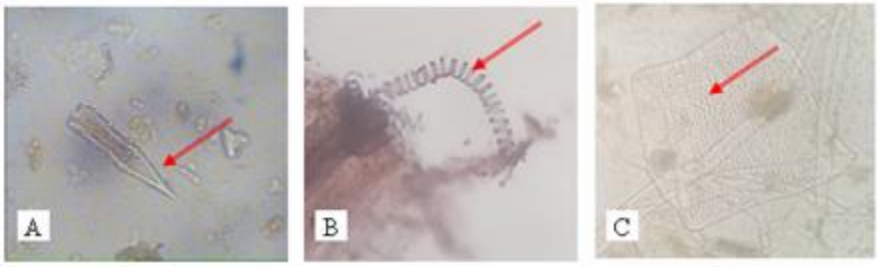
Anatomy of leaves, stem and root of *Eclipta prostrata*. A. Transverse section of leaf, B. Epidermal peel, B1. Anomocytic stomata, B2. Anisocytic stomata, C. Transverse section of stem, D. Crystal (druse) in cortex of the stem, E. Trichomes on stem surface, F. T.S of root with air spaces

Organoleptic evaluation and powder microscopy

Powder is dark green to brown, has a smooth texture, with no specific odor or taste. It is easily flowable with a fine to coarse texture. Diagnostic microscopic powder features include prismatic and crystals of calcium oxalate; starch granules are mostly simple, but rarely compound; Uniseriate trichome with pointed end (Figure No. 3A) and long lignified fibers; parts of epidermis in surface view showing straight-walled polygonal epidermal cells, paracytic stomata and spiral xylem vessels (Figure No. 3B). Pericyclic fibers along with phloem parenchyma, oval to

elongated, pitted stone cells and thickened xylem vessels (Figure No. 3C) were observed.

Figure No. 3



Powder microscopy pictures of *Eclipta prostrata*. A. Vessels with pitted thickening, B. Tracheid with a pointed end, C. Spiral thickening in vessels

Physico-chemical analysis

Physico-chemical parameters such as moisture content, ash values (total, acid insoluble, water soluble) and extractable matter in water and ethanol were obtained as in Table No. 1. The percentage of weight on loss or moisture content at 105ºC was found to be 9.9 ± 0.2%. The amount of the total, water soluble and acid insoluble ash values were 17.1 ± 0.3%, 9.5 ± 0.2% and 0.4 ± 0.0% respectively. Hot water (7.9 ± 0.1%) and cold water (6.0 ± 0.0%) extractable matter was significantly ($p \leq 0.05$) higher than that of hot ethanolic (1.8 ± 0.1%) and cold ethanolic (1.0 ± 0.0%) extractable matter respectively. Heavy metals such as Pb, Hg, Cd and As were not detected in the whole plant powder of *E. prostrata* grown in Sri Lanka.

Table No. 1
Physicochemical analysis of *Eclipta prostrata* Linn

Moisture content in *Eclipta prostrata*			**9.9 ± 0.2%**
Ash values	**Total Ash**		**17.1 ± 0.3%**
	Water soluble ash		**9.5 ± 0.2%**
	Acid insoluble ash		**0.4 ± 0.0%**
Extractable matter	**Water extractable**	**Hot**	**7.9 ± 0.1%**
		Cold	**6.0 ± 0.0%**
	Ethanol extractable	**Hot**	**1.8 ± 0.1%**
		Cold	**1.0 ± 0.0%**

Mean ± S.E.M; n=3

Thin Layer Chromatography

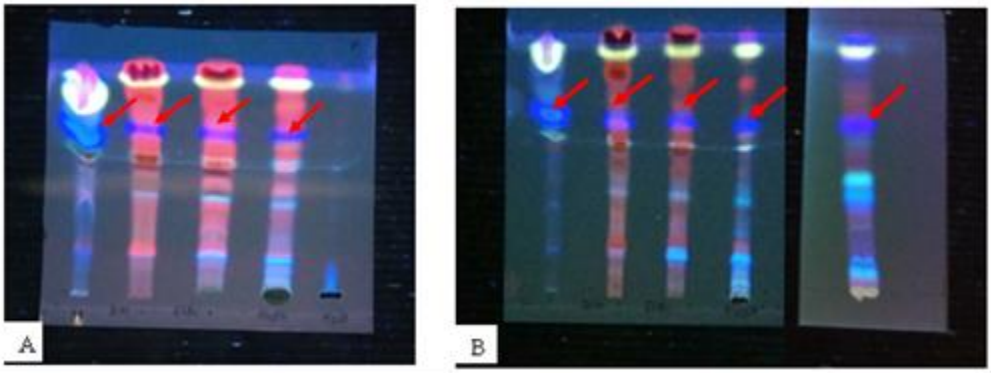
The TLC fingerprinting revealed the abundance of the compounds in different fractions of *E. prostrata*. The wide range of color produced indicates the presence of many secondary metabolites (Figure No. 4). A spot of violet color (R_f=0.56) was observed under UV light in both polar and nonpolar solvent systems (Figure No. 4A and Figure No. 4B). According to the literature, Wedelolactone gives a purple to violet color spot under UV light and its R_f value is recorded as 0.56 (Shanshol *et al*., 2013). Therefore, the spot observed in the present study could be likely the representation of Wedelolactone. However, commercially available reference standard of Wedelolactone should be used to confirm the aforesaid observation. Further the intensity of the spot and the diameter is bigger for the hexane extract which is eluted in the nonpolar solvent system than that of other extracts.

Phytochemical screening

The phytochemical profile of the plant revealed the presence of flavonoids, tannins, saponins, alkaloids, monoterpenes, quinones, steroids, coumarins, cardiac glycosides and phenolics in hot aqueous and methanol extracts (Table No. 2). In addition, sesquiterpenes, steroids, oil and fats were found in all hexane, dichloromethane and ethyl acetate extracts of the plant. Anthroquinones were not detected in any of the extracts.

Quantification of secondary metabolitesThe results obtained for the quantification of phytochemicals are shown in Table No. 3.

Figure No. 4



TLC fingerprinting of hexane, dichloromethane, ethyl acetate, methanol and hot water (From left to right) in, A. Nonpolar solvent system B. Polar solvent system

Table No. 2
Secondary metabolites present in *Eclipta prostrata*

Chemical Groups	Plant extracts				
	Hexane	DCM	Ethyl acetate	Methanol	Water
Alkaloids	+	+	+	+	+
Carbohydrates	-	-	+	+	+
Proteins and Amino Acids	-	-	-	-	-
Fixed Oils and Fats	+	-	+	+	-
Flavonoids	-	+	+	+	+
Phenolic compounds and tannins	+	+	+	+	+
Glycosides	+	+	+	+	+
Saponins	+	+	-	+	+
Steriods	+	+	-	-	-
Terepenes	+	+	-	-	+
Gums and mucilages	-	-	-	-	-
Anthroquinones	-	-	-	-	-

Table No. 3
Quantification results of hot water extract of *Eclipta prostrata* Linn

Secondary metabolite	Values
Total polyphenol content	22.7 ± 0.4 mg GAE/ g
Total flavonoid content	158.8 ± 0.9 mg QE/ g
Alkaloid content	2.0 ± 0.0%
Saponin content	2.1 ± 0.0%
Tannin content	15.3 ± 0.1%

Mean±S.E.M; n=3; GAE: gallic acid equivalents; QE: quercetin equivalents

DISCUSSION

It is difficult to standardize herbal medicines and Ayurvedic formulations. Proper identification of the plant microscopically as well as morphologically is necessary to overcome this problem and pharmacognostical studies also help to identify the adulterants in single drugs as well as in formulations (Sinha *et al*., 2021). To confirm the identity and degree of purity, pharmacognostical analysis should be carried out before any tests are done for

pharmacological activity. Diagnostic parameters are established by the morphological evaluations. The present macroscopic, microscopic plant anatomical studies and powder microscopy provide useful information for the quality control parameters of *E. prostrata* from its counterfeit.

Although most of the morphological characters observed in Sri Lankan plant are similar to that of other South Asian regions, presence of Ca-oxalate crystals has not been reported earlier (Indian Herbal Pharmacopoeia, 2002). However, the presence of acicular crystals was reported by Gopalkrishnan & Solomon, (1992). In the present study, the presence of Ca-oxalate crystals as styloids and druses were observed. These together with other characters reported in results could be used to identify the whole plant and the crude raw material of *E. prostrata*.

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form and it also indicates the presence of various impurities like carbonate, oxalates and silicates (Singh *et al*., 2014). The total ash value observed in the present study was 17.1%, whereas the reported value of Indian origin plant was 11.5% (Sharma *et al*., 2011). Moreover, the present study shows (Table No. 1) more water-soluble ash (9.5%) and acid-insoluble ash (0.4%). Very low percentage of acid insoluble ash content indicates the absence of earthy material in powdered plant sample. On the other hand, water soluble ash and acid insoluble ash contents of *E. prostrata* of Indian origin were 4.9% and 1.5% respectively (Sharma *et al*., 2011). However, *E. prostrata* grown in Sri Lankan has a comparatively higher total ash and water soluble ash contents compared to that of Indian origin.

WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (WHO, 2007) classified Cd, Pb, As, Hg and chromium (Cr) as a major toxic metal contaminant of herbs. According to current study, heavy metals such as Pb, As, Cd and Hg were not detected in the whole plant powder of *E. prostrata* grown in Sri Lanka. Having these heavy metals at undetectable levels is a definitive advantage. However, some preliminary work carried out in India on the elemental contents in *E. prostrata* has revealed that Mn, Pb and Cr (25.77 mg/kg, 13.33 mg/kg and 13.18 mg/kg dry weight respectively) are present at high concentrations (Arumugam 2012). Carrying out investigation on other heavy metals in the plant sample would help us comment on the profile of heavy metal contaminants.

Moisture content of initial raw material influences the quality and stability of the final product. Higher moisture content encourages microbial growth and undesirable enzymatic activity accelerating deterioration by hydrolysis of the herbal material. According to previous studies, the optimal initial moisture content of raw materials ranged from 8 to 38% (Zvicevičius *et al*., 2018). The moisture content was calculated through loss on drying method and was found to be 9.9 ± 0.2% in the present study. Thus, it can be concluded that the plant of our interest has a moisture content within the optimal range and not likely to deteriorate during the shelf life.

With the development of natural product chemistry, the potential use of chemotaxonomy has increased. The phytochemical research approach is considered as effective in discovering bioactive profile of plants of therapeutic importance (WHO, 2011). Flavanoids, tannins, saponins, monoterpenes, quinones, steroids, coumarins, cardiac glycosides and phenolics were found as secondary metabolites in hot aqueous and methanol extracts of the plant. In addition, sesquiterpenes, steroids, oil and fats were found in hexane, dichloromethane and ethyl acetate extracts (Table No. 2). In addition, proteins and amino acids were absent. In the present study, basic qualitative experiments were done to screen the proteins and amino acids in the extracts. However, more specific extraction technique/s for proteins and amino acids may give positive results. The difference observed in the presence of phytochemical constituents between different solvent extracts may be attributed to the nature of the solvent used in the extraction, nature of processing method and micro change in lab environment (Odeyemi *et al*., 2017).

According to the quantification studies carried out in Asia using *E. prostrata*, (Rana *et al*., 2019) the TPC was found to be less and the TFC was higher when compared to that of present study. Similarly, alkaloid and saponin contents were found to be less in Sri Lankan origin plant when compared to that of *E. prostrata* grown in India (Khanna & Kannabiran, 2007). The variability in these constituents due to genetic, environment or other factors like geographical sources, season and time of collection. Flavonoids and other polyphenols such as tannins are considered as health promoting and disease preventing secondary metabolites (Karak, 2019; Pizzi, 2021). Promising biological activities of flavonoids include cancer prevention, hormonal actions, cardioprotective actions and inhibitory effects on bone resorption (Hendrich, 2006). Similarly, tannins have remarkably diverse range of

pharmacological activity such as antimicrobial, anti-inflammatory, antioxidants, anticancer, virucides, antidiabetic, cardiovascular protection, wound repair, anti-diarrhoic and structural bone reparation (Pizzi, 2021). The aforementioned pharmacological activities are scientifically proven in studies conducted on *E. prostrata* (Feng *et al*., 2019). Therefore, it can be concluded that quantified phytochemicals that are abundantly present in *E. prostrata* might justify the traditional claim of this plant to be used medicinally.

The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development using the Sri Lanka origin of *E. prostrata*. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

CONCLUSION

Morphological and anatomical features reported in the present study could be used to identify *E. prostrata* plant and to authenticate raw material enabling the use of correct raw material in manufacturing medicine. Multiple phytochemical classes have been identified and the TLC fingerprinting revealed the abundance of the secondary metabolites in different fractions of *E. prostrata*. Even though flavonoids, tannins, saponins, alkaloids, and phenolics have been quantified further studies are warranted to isolate and characterize purified phytochemicals and elucidate their structure-relationships and possible synergetic effects. The information presented here could make people more aware of *E. prostrata* and can be beneficial for further research.

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