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Pharmacognostic Characterization and Antacid Activity of Aqueous Extract of *Desmodium triflorum* Linn and *Pogostemon heyneanus* Benth

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ABSTRACT

Present study was aimed to investigate the antacid potential of *Desmodium triflorum* Linn whole plant and *Pogostemon heyneanus* Benth leaves and to establish their diagnostic characteristics. Powder microscopy, detailed anatomical characteristics, analysis of phytochemicals and Ultra-Performance Liquid Chromatography were done. In addition, neutralizing effects on artificial gastric acid (AGA) by aqueous extracts (AE) of the plants, their ethyl acetate fractions (EAF) and residual aqueous fractions (RAF) were determined. Fordtran's model (a titration method) was used to evaluate the *In vitro* neutralization capacity. A modified model of Vatier's artificial stomach was used in the assessment of *In vitro* acid neutralization duration. Statistical analysis was performed using SPSS 25.0. Pharmacognostic study aids in establishing the standardization parameters. Treatments including AE, RAF of *D. triflorum* and *P. heyneanus* showed significant ($P<0.01$) acid neutralizing effects, duration for consistent neutralization and neutralization capacities. Findings of this study indicated that *D. triflorum* whole plant and *P. heyneanus* leaves possessed potent antacid effects.

Keywords: Fabaceae, Lamiaceae, Fordtran's model, Vatier's artificial stomach.

INTRODUCTION

An increase in the usage of herbal medicines in the developed world is observed in the last few years. The majority of the global population failed to afford medicines and medicinal products from Western Pharmaceutical Industry, which makes them rely solely or partly upon traditional medicines. Due to the notable reliance on medicinal plants in the treatment of ailments and their undetected potency in the process of drug discovery, it is timely to search for effective and safe plant medicines[1].

Desmodium triflorum Linn (Family Fabaceae) is a well-known medicinal plant in Sri Lanka and called as 'Heen-Undupiyaliya' in Sinhala. The plant is available in many other tropical countries including Java, Philippine, India, and Taiwan. In Sri Lanka, the plant is commonly found in the low country[2-3]. Different parts of *D. triflorum* such as the leaves, roots, and sometimes even the whole plant is used in Ayurvedic medicine for various treatment objectives[4-5]. The plant is used in the treatment of headaches, eye disease, dysentery, bone fractures, and in snake biting[2-3]. Antiproliferative, anthelmintic, anticonvulsant, analgesic, hypoglycaemic, and anti-inflammatory activities of *D. triflorum* have also been demonstrated recently in modern studies[6-9].

Pogostemon heyneanus Benth. (Family Lamiaceae), is an aromatic herb, commonly called as 'Kollan kola' or 'Gan kollan kola' in Sinhala. Shade-dried leaves of the plant yield a commercially important oil (Patchouli oil) which is comprised of a spicy, herbaceous fragrance[10]. The plant has been used widely in different traditional medicinal practices to treat many medical ailments. A decoction of the leaves is given for coughs and asthma, and poultices are applied for boils, headaches, jaundice and bilious fevers. It acts internally as an aromatic stomachic and carminative with astringent properties. Furthermore, the leaves are used for anorexia, chronic dyspepsia, and flatulence[11]. *P. heyneanus* is reported to possess antibacterial[12], antifungal[13], cytotoxic and anticancer[14], antioxidant[10], insecticidal[15] and wound healing[16] activities.

Pogostemon cablin, another species belonging to the genus of Pogostemon, is a well-known traditional Chinese medicinal herb for gastrointestinal diseases in South East Asia[17]. Moreover, the gastroprotective activity of *P. cablin* is well documented[18]. Despite the traditional use of *P. heyneanus* for gastric ailments, no studies were carried out to evaluate the antacid potency of *P. heyneanus* leaves so far.

Gastroprotective activity of *Desmodium gangeticum* has been reported[19]. However, *D. triflorum* has not been evaluated for its gastroprotective activity *In vitro*. Considering all the above facts, the present study aimed to assess the gastroprotective activity of aqueous extracts of *D. triflorum* and *P. heyneanus* and their fractions *In vitro*. Even though the study plants are of great medicinal importance yet there is no any study carried out for their pharmacognostic standardization as such data are required for authentication and quality control. Therefore, pharmacognostic characterization including macro and microscopic evaluation and the determination of phytochemical properties of *D. triflorum* whole plant and *P. heyneanus* leaves were also carried out.

MATERIALS AND METHODS

Collection, identification, and preparation of plant material

Fresh *P. heyneanus* leaves were obtained from the Western Province of Sri Lanka, and fresh *D. triflorum* were collected from the Southern Province and Western Province of the island during the months of January to April, 2020. Plants were authenticated from the National Botanical Herbarium, Peradeniya, Sri Lanka, and the voucher specimens were deposited under reference numbers, DT Pharm 001 (*Desmodium triflorum*) and PH Pharm 002 (*Pogostemon heyneanus*).

Extraction of plant materials

Collected fresh plants were washed thoroughly, dried under shade, and then dried in a hot air oven at 40°C until a constant weight was obtained and stored in air-tight polythene bags at a dry place. The dried plants were then ground to a coarse powder and this powder was stored in an air-tight and light-resistant container. The fresh leaves were used in the microscopic assessment. Small pieces of dried leaves (50 g) of *P. heyneanus* and dried *D. triflorum* whole plant (50 g) were weighed, respectively, and extracted with distilled water by refluxing. Crude aqueous extract (AE) of each plant was obtained and the extracts were fractionated using ethyl acetate. Fractions were concentrated and ethyl acetate fraction (EAF) and residual aqueous fraction (RAF) were obtained, respectively. Crude methanolic extracts of both plants were prepared and then used in the Ultra-performance liquid chromatography (UPLC) fingerprinting.

Pharmacognostic evaluations of *Desmodium triflorum* and *Pogostemon heyneanus*

Macroscopic evaluation

Macroscopic evaluations of the plants were done using 5 samples from each plant. Respective

taxonomical descriptions were made according to the data given in the literature[20].

Microscopic evaluation of stem and leaves of the plants

Microscopic evaluations of the stem and leaves of the plants were performed as per the methods described elsewhere[21]. Briefly, fresh cross-sections of the leaves were collected using a sharp blade. They were dehydrated in a graded alcohol series of 30%, 50%, and 70%, respectively, and transferred into a safranin solution (diluted in 70% alcohol). The anatomical observation was conducted under the microscope by mounting the selected cross-sectioned sample on a slide with 1-2 drops of chloral hydrate and then covering it with a cover slip. The prepared slides were examined under the light microscope.

Powder microscopy study

The powder microscopy study was performed according to the previously described method[22]. Shade dried leaves of *P. heyneanus* and leaves and stems of *D. triflorum* were finely powdered separately. Small quantities of powdered samples were placed on slides separately and each slide was mounted with 2-3 drops of chloral hydrate (75%) solution. Each slide was covered with a cover slip and examined under the light microscope.

Qualitative and quantitative analysis of phytochemicals

Phytochemical analysis was carried out on the AE of each plant sample and their fractions (EAF and RAF), following standard procedures[23]. Phytochemical screening of phenolics was carried out using vanillin and lead acetate tests. To detect flavonoids, ammonia sulfuric acid test was employed. Tannins were detected with vanillin, lead acetate, and ferric chloride tests. The frothing test was performed for the detection of saponins in the sample. The steroids and terpenoids were detected by Liebermann-Burchard test and Salkowski test respectively. Vanillin in ethanol solution and conc. H_2SO_4 were used for the presence of monoterpenes. The extract was treated with a few drops of conc. H_2SO_4 to test for the presence of sesquiterpenes. For the screening of alkaloids, the picric acid test was carried out and observed for the presence of a yellow crystalline precipitate. Cardiac glycosides were detected by Glacial acetic acid with $FeCl_3$ test. The Total phenolic content (TPC) of the AE of *D. triflorum* whole plant and *P. heyneanus* leaves respectively, were analysed by Folin-Ciocalteu colorimetric method[24]. Stock solutions (100 mg/mL) were prepared from the two extracts. Twenty microliters of three different concentrations of each extract were mixed with 110 µL of freshly prepared and ten times diluted Folin-Ciocalteu reagent. After that, 10% sodium carbonate (70 µL) was added to each and the mixtures were kept at room temperature (25±2°C) for 30 minutes. The absorbances of the mixtures were recorded at 765nm wavelength. Gallic acid was used as the standard. A calibration curve of gallic acid was prepared using different concentrations of gallic acid (mg/mL) in the same manner. The TPC was expressed as mg gallic acid equivalents (GAE)/g of the extract.

The Total flavonoid content (TFC) of the AE of *D. triflorum* whole plant and *P. heyneanus* leaves respectively, were analysed according to the aluminium chloride colorimetric method[24]. Stock solutions (100 mg/mL) were prepared from the two extracts. A volume of 100 µL of 2% aluminium chloride dissolved in methanol was mixed with three different concentrations of each extract (100 µL). The mixtures were allowed to stand at room temperature (25±2°C) for 10 min and then absorbance values were recorded at 415nm. Quercetin was used in the preparation of the standard curve. The TFC was expressed as mg quercetin equivalents (QE)/g of the extract.

Thin-layer chromatographic separation and Ultra-performance liquid chromatography analysis

The Thin layer chromatography (TLC) of AE of *D. triflorum* whole plant, *P. heyneanus* leaves and all fractions was done according to the standard procedure[25]. TLC analyses were carried out using ALUGRAM Xtra SIL G/UV254 aluminium sheets (Sigma-Aldrich). The solvent system was made from ethyl acetate, dichloromethane, and cyclohexane in 3:2:1 (v/v/v). The bands were observed under UV light at 254nm and 365nm wavelengths. The vanillin solution (1 g vanillin+100 mL conc. H_2SO_4) was used as the visualizing agent and the R_f values were determined.

Ultra-performance liquid chromatography (UPLC) fingerprint analyses were performed on Waters Acquity UPLC H class (Waters

Corporation, USA) Ultra-performance liquid chromatography machine with Waters Acquity UPLC BEH C18 column (2.1mm×100mm, 1.7 µm). The mobile phase used in the analyses of both plants consisted of aqueous 0.2% formic acid (solvent A) and acetonitrile (solvent B). Gradient elution conditions in the analysis of *P. heyneanus* were: 0–5 min, 30%–33% B; 5–8 min, 33% B; 8–14 min, 33%–41% B; 14–25 min, 41% B; 25–29 min, 41%–54% B; 29–35 min, 54%–100% B; 35–40 min, 100% B. The flow rate was 0.3 mL/minute. The injection volume was 0.5 µL. Analysis was done at 286nm. Gradient elution conditions in the analysis of *D. triflorum* were: 0–4 min, 5%–14% B; 4–7 min, 14% B; 7–12 min, 14%–28% B; 12–18 min, 28%–55% B; 18–19 min, 55%–5% B. The flow rate was 0.3 mL/minute. The injection volume was 0.5 µL. Analysis was done at 272 nm.

Determination of antacid activity

Neutralizing effect on artificial gastric acid

Neutralizing effects of AE and the fractions (EAF and RAF) of *D. triflorum* whole plant and *P. heyneanus* leaves were evaluated by previously reported *In vitro* method[26-27]. In brief, 90 mL of test samples and distilled water each, and the standard, ENO, a dispersible antacid powder (90 mL) were added to the artificial gastric acid (100 mL) separately. The pH values of the test samples were recorded to determine the neutralizing effects. For the preparation of artificial gastric acid, 2.0 g of sodium chloride and 3.2 mg of pepsin enzyme (SIGMA) were dissolved in 500 mL of distilled water. HCl at a volume of 7.0 mL and an adequate amount of water were added to make up the solution to 1000 mL in volume. Then the pH of the solution was adjusted to 1.2.

Neutralizing capacity using the titration method of Fordtran's model

Neutralizing capacity of AE and the fractions (EAF and RAF) of *D. triflorum* whole plant and *P. heyneanus* leaves was evaluated using Fordtran's titration method[27]. The test samples and distilled water each, and the standard drug, ENO (90 mL) were put separately in a 250 mL beaker and this was maintained at 37°C. A magnetic stirrer was placed in it to run continuously at 30 rpm while imitating the gastric movements. Each test sample, distilled water, and the reference drug were titrated with 0.1N HCl to the end point of pH 3. The consumed volume of the HCl was measured as the parameter of acid-neutralising capacity.

Duration of consistent neutralization on artificial gastric acid using the modified model of Vatier's artificial stomach

Duration of consistent neutralization on artificial gastric acid by AE and the fractions (EAF and RAF) of *D. triflorum* whole plant and *P. heyneanus* leaves was determined using the Vatier's artificial stomach, a modified model[26-27]. The test samples and distilled water at 90 mL of each, and the standard drug, ENO (90 mL) were mixed separately to 100 mL of artificial gastric juice in the artificial stomach at 37°C. Contents in the artificial stomach reservoir were continuously stirred at 30 rpm using a magnetic stirring apparatus. Artificial gastric juice at pH 1.2 was pumped in and out at 3 mL/min at the same time. A pH meter was connected to measure the changes in pH continuously in the artificial stomach. The duration of the neutralization effect was evaluated when the pH was returned to pH 1.2, the initial pH value.

Statistical analysis

All results were expressed as mean±standard error of the mean (SEM) and, where applicable, p-value <0.05 was considered as significant. Statistical analysis of the results was performed by One-way ANOVA, using SPSS 25.0.

RESULTS AND DISCUSSION

Desmodium triflorum and *Pogostemon heyneanus* are important medicinal plant materials extensively used in traditional medicine in Sri Lanka. In the present study, the *In vitro* gastroprotective activities of AE of *D. triflorum* whole plant and *P. heyneanus* leaves and their fractions were evaluated. Pharmacognostic characterization of *D. triflorum* whole plant and *P. heyneanus* leaves was also carried out as pharmacognostic standardization is necessary for the identification and quality standardization of the plants.

The percentage yield for extraction from *D. triflorum* whole plant (50 g) was found to be: AE-8.2% w/w; EAF-3.8% w/w; RAF-4.6% w/w. Powder of *P. heyneanus* leaves (50 g) yielded AE-7.8% w/w; EAF- 4.5% w/w; RAF-3.3% w/w.

Macroscopic and microscopic description of a plant is considered as the first step in identifying and examining its degree of purity[28]. In the macroscopic study, different parts of the plant are examined by the naked eye. It gives a morphological explanation of the relevant plant and helps to differentiate features among the species within a single genus[29].

According to the macroscopic evaluation, *D. triflorum* is a very small, terrestrial prostate herb up to 50 cm long with rooting at nodes. Leaves of the plant are small, stipulate, alternate, and trifoliate. Terminal leaflets are 4-7.5mm in length and 3-10mm in width. Flowers are very small and bright purple in colour. *P. heyneanus* is an undershrub aromatic herb. The four-angled leaves of the plant are lanceolate, simple, opposite, without stipules, and slightly hairy on both sides. About 5-12.5 cm in length and petioles are 2.5-7.5 cm long.

The leaf anatomy of *D. triflorum* and *P. heyneanus* was evaluated microscopically. Fig. 1a shows the transverse sections (T.S) of the leaf of *D. triflorum*. The cuticle was observed as the outermost layer. Just below the cuticle, epidermal cells were observed. Single layered upper epidermis contained few or no chloroplasts. Parenchymatous cells were compactly arranged and the outer walls of the cells were thick. Stomata could not be observed in the upper epidermis. Palisade parenchyma lies under the upper epidermis. There were 2-3 rows of long parenchymatous cells with plenty of chlorophyll and they were compactly packed without any intercellular spaces. Vascular bundles were centrally located and consisted of the xylem and phloem. Different cell arrangements and their anatomy were observed in the T.S of the leaf of *P. heyneanus* (Fig. 1b). The outermost waxy cuticle layer called the epidermis consisted of trichomes. Just after the epidermis, cells called collenchyma consisting of thick deposits of cellulose in their cell walls were observed in a circular or oval shape. Lower epidermis was observed on the lower side of the leaf. Vascular bundles, consisting of the xylem and phloem were centrally located.

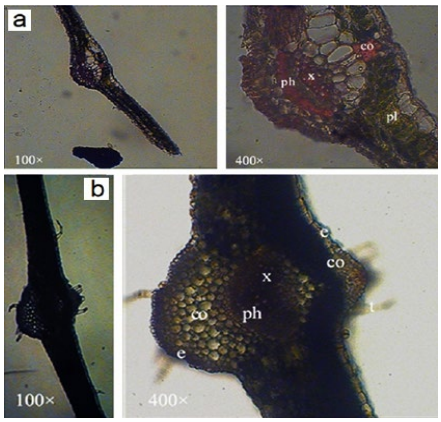

Fig. 1. Transverse sections of the leaf of *Desmodium triflorum* and *Pogostemon heyneanus* a. Transverse sections of the leaf of *Desmodium triflorum*; b. Transverse sections of the leaf of *Pogostemon heyneanus*; co, collenchyma; e, epidermis; ph, phloem; pl, palisade tissue; x, xylem; c, cortex; pi, pith; t, trichomes; vb, vascular bundles; b, bundle sheath; ca, cambium

Stem anatomy of *D. triflorum* and *P. heyneanus* was observed microscopically. Fig. 2a shows the T.S of the stem of *D. triflorum*. The T.S of the stem showed the cellular anatomy and the shape of the different cells. The outermost cell layer, the epidermis was observed as a compactly arranged single-cell layer without intercellular spaces. Below the epidermis, the endodermis was observed with compactly arranged cells. Vascular bundles were centrally located and arranged in a ring around the pith. Each bundle had a patch of xylem towards the centre, a patch of phloem towards the periphery, and a cambium in between them. The pith was the central portion of the stem. It was composed of thin-walled, rounded or polygonal parenchymatous cells with intercellular spaces. Xylem lies towards the pith of the vascular bundles. Fig. 2b shows the T.S of the stem of *P. heyneanus*. The outermost layer, the epidermis consisted of trichomes. Just after the epidermis, the cortex was observed with collenchyma cells consisting of irregular thick cell walls that provide support to the structure. The cortex and pith together called ground tissue and it mainly consisted of parenchyma cells. Vascular bundles were observed and the vascular cambium could be identified in between the xylem and phloem in the vascular bundles. They were connected to form a continuous cylinder confirming the dicotyledon cell organization.

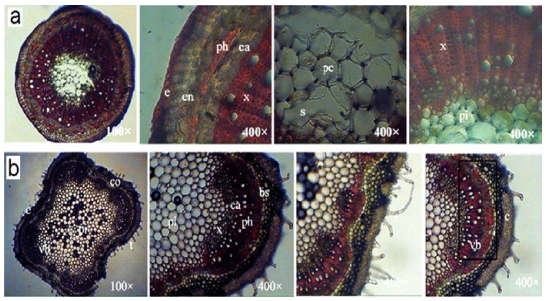

Fig. 2. Transverse sections of the stem of *Desmodium triflorum* and *Pogostemon heyneanus* a. Transverse sections of the stem of *Desmodium triflorum*; b. Transverse sections of the stem of *Pogostemon heyneanus*; co, collenchyma; pc, parenchyma; e, epidermis; en, endodermis; ph, phloem; pl, palisade tissue; x, xylem; c, cortex; pi, pith; s, intracellular space; t, trichomes; vb, vascular bundles; b, bundle sheath; ca, cambium; gt, ground tissue system

Transverse section examination of both plants showed special features of the cellular organization of the stem and leaf. Therefore, macroscopical and microscopical studies help to identify the diagnostic characters of both plants which can be used in the proper identification of the plants[29].

In the identification of crude drugs, powder analysis plays a crucial role and would assist in the identification of the right variety and adulterants as well. Furthermore, powder microscopic evaluation can be used as the simplest and cheapest method to establish the correct identity of the source materials and an important approach in the standardization of the plant material and a path of pharmacological and therapeutic evaluation[30].

The prominent diagnostic features of the powder microscopy of *D. triflorum* revealed the presence of pitted vessels, calcium oxalate crystals, epidermal cells, simple fibres and starch granules (Figure 3).

The powder microscopy of *D. triflorum* stem powders indicated the presence of cork cells, epidermal cells, fibres, trichomes, calcium oxalate crystals, sclereids, pitted vessels and starch granules (Fig. 4). Powder microscopical evaluation of dried leaf powder of *P. heyneanus* revealed the presence of trichomes, sclereids, calcium oxalate crystals, irregular shaped starch granules, lignified vessels, fibres etc. (Fig. 3). A relatively high abundance of fibres was present in the samples collected from dry climatic geographical areas (Fig. 3). Therefore, these powder microscopical features along with the observed anatomical features of the plants will aid in establishing the diagnostic characters that facilitate the correct identification of the species and provide useful parameters in quality control. These can be used as standards in authenticating the respective crude drugs.

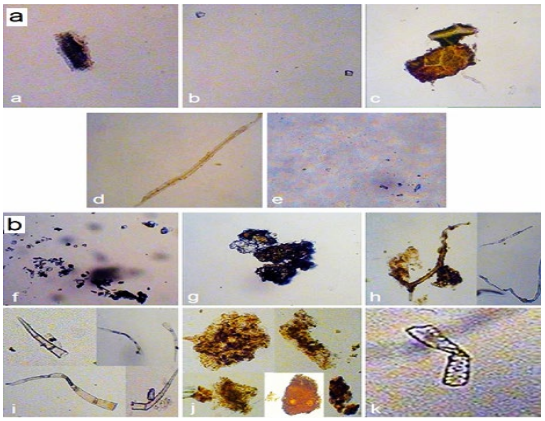

Fig. 3. Powder microscopy of leaves of *Desmodium triflorum* and *Pogostemon heyneanus* a. Powder microscopy of leaves of *Desmodium triflorum*; (a) pitted vessels (b) calcium oxalate crystals (c) epidermal cells (d) simple fibres (e) starch granules b. powder microscopy of leaves of *Pogostemon heyneanus*; (f) starch granules (g) trichomes (h) calcium oxalate crystals (i) fibers (j) sclereids (k) pitted vessels

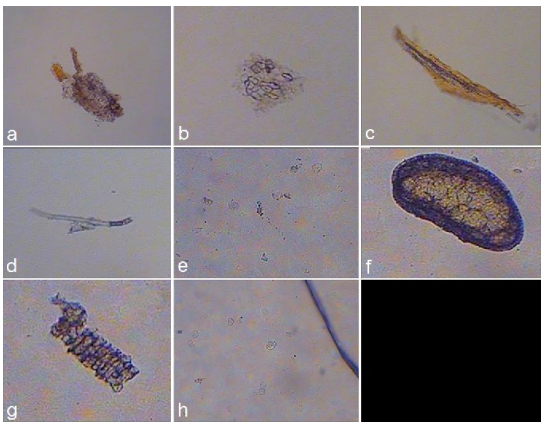

Fig. 4. Powder microscopy of the stem of *Desmodium triflorum*; (a) cork cells (b) epidermal cells and crystals (c) fibres (d) trichomes (e) calcium oxalate crystals (f) sclereids (g) pitted vessels (h) starch granules

The results of the preliminary phytochemical screening of *D. triflorum* whole plant and *P. heyneanus* leaves are represented in Table 1. Presence of tannins, phenolics, flavonoids, saponins, as well as cardiac glycosides in the AE of *D. triflorum* whole plant was observed. Phenolics, flavonoids, tannins,

monoterpenes, and sesquiterpenes were present in both EAF and RAF. In addition, alkaloids and saponins were observed in RAF. Phytochemical screening of *P. heyneanus* leaves revealed the presence of tannins, phenolics, flavonoids, terpenoids, saponins, as well as cardiac glycosides in the AE. Phenolics, tannins, terpenoids, and cardiac glycosides were observed in both EAF and RAF. Flavonoids were only found in EAF. Alkaloids and saponins were only observed in RAF.

Table 1: Qualitative phytochemical analysis of aqueous extracts of *Desmodium triflorum* whole plant and *Pogostemon heyneanus* leaves and their fractions

Phytochemicals	*D. triflorum* whole plant			*P. heyneanus* leaves		
	Aqueous extract	Ethyl acetate fraction	Residual aqueous fraction	Aqueous extract	Ethyl acetate fraction	Residual aqueous fraction
Phenolics	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	-
Tannins	+	+	+	+	+	+
Saponins	+	-	+	+	-	+
Terpenoids	-	+	-	+	+	+
Cardiac glycosides	+	-	-	+	+	+
Alkaloids	-	-	+	+	-	+
Steroids	-	-	-	-	-	-

+ Presence of the compound
- Absence of the compound

Presence of various phytochemicals in AE of *D. triflorum* and other organic extracts were reported[6,31]. Similar to the present study, Singh *et al.,* 2016, have not identified terpenoids, alkaloids, and steroids in the AE of *D. triflorum*. Medicinal effects of plants are widely varied with the region of cultivation. Therefore, it is essential to analyse the type of phytoconstituents present in *D. triflorum* grown in Sri Lanka. Similar to the present study, Dharmadasa *et al.,* 2014, have reported the presence of saponins, alkaloids, tannins, and flavonoids in AE of Sri Lankan grown *P. heyneanus* leaves[10]. Moreover, the secondary metabolites observed in *D. triflorum* and *P. heyneanus* may be responsible for various pharmacological effects[32-33]. Biomarker compounds are considered as key compounds in the identification and determining the quality of plant materials. Phytochemical screening of plant materials will lead to establish such biomarker compounds[34].

In the present study, TPC and TFC contents of AE of *D. triflorum* whole plant and *P. heyneanus* leaves were analysed. The TPC of AE of *D. triflorum* whole plant and *P. heyneanus* leaves were 0.69±0.16 mg GAE/g and 0.45±0.00 mg GAE/g respectively. The TFC of AE of *D. triflorum* whole plant and *P. heyneanus* leaves were 0.96±0.08 mg QE/g and 1.34±0.00 mg QE/g respectively. The TPC value of *P. heyneanus* leaves of the present study was lower than the reported data (0.83 ± 0.01 mg GAE/ g)[10]. The TPC and TFC values of different extracts of *D. triflorum* were reported[35-36]. However, the amount of secondary metabolites will depend on the season and the maturity of the plants.

The results of TLC of both plant extracts when viewed under UV 254nm and UV 365nm were shown in Fig. 5. The solvent system was ethyl acetate, dichloromethane, and cyclohexane 3:2:1(v/v). When a TLC was carried out for AE, RAF, and EAF in the same TLC plate showed that there were no significant separations in AE and RAF compared to the EAF. The coloured bands were observed on the spraying detecting agent, 1% vanillin indicating the presence of alcoholic and carbonyl compounds. Proving the presence of different groups of compounds both EAFs of *D. triflorum* and *P. heyneanus* separated in upto 8 bands, at varying R_f values depicted in Table 2 and Fig. 5. TLC of EAF depicts that the fraction contains a number of active constituents. Moreover, the characteristics patterns of TLC represented by EAF may be served as idiosyncratic fingerprints for qualitative evaluation. However, for the AE and RAF need to be separated by developing a suitable mobile phase in future studies.

Table 2: Thin layer chromatographic studies of ethyl acetate fractions of *Desmodium triflorum* whole plant and *Pogostemon heyneanus* leaves

Fractions	Solvents	Ratio	R_f values
EAF of *D. triflorum*	Ethyl acetate, dichloromethane and cyclohexane	3:2:1(v/v)	0.10,0.32,0.50,0.61,0.70,0.77,0.87,0.95
EAF of *P. heyneanus*			0.11,0.20,0.31,0.47,0.56,0.65,0.73,0.80

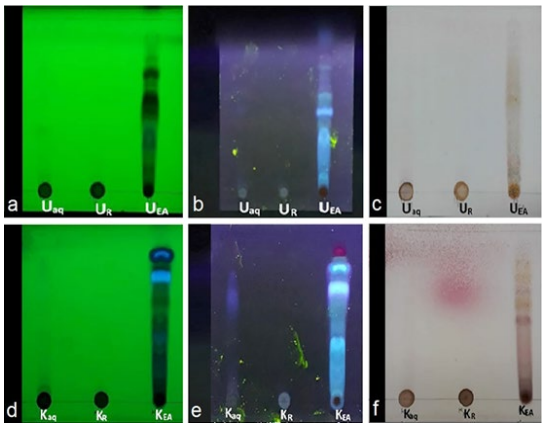

Fig. 5. Thin layer chromatography of different extracts of *Desmodium triflorum* and *Pogostemon heyneanus*. U_{aq}, AE of *D. triflorum*; U_R, RAF of *D. triflorum*; U_{EA}, EAF of *D. triflorum*; K_{aq}, AE of *P. heyneanus*; K_R, RAF of *P. heyneanus*, K_{EA}, EAF of *P. heyneanus* a: Different extracts of *D. triflorum* viewed under UV 254nm, b: Different extracts of *D. triflorum* viewed under UV 365nm, c: *D. triflorum* viewed after staining with vanillin spray reagent, d: Different extracts of *P. heyneanus* viewed under UV 254nm, e: Different extracts of *P. heyneanus* viewed under UV 365nm, f: *P. heyneanus* viewed after staining with vanillin spray reagent

UPLC analysis of methanolic extracts of *D. triflorum* and *P. heyneanus* are shown in Fig. 6a and Fig. 6b respectively. The chromatogram of *D. triflorum* comprises three prominent peaks, which were observed between 0 to 20 min of the analysis. In the UPLC analysis of *P. heyneanus*, the chromatogram resulted in a greater number of prominent peaks between 0 to 42 min of analysis. The results of UPLC analysis comply with the HPLC observations of previous studies that the HPLC fingerprint of *D. triflorum* consists of less number of prominent peaks (due to the less number of compounds extracted to methanol in higher amounts) compared to *Pogostemon* spp[36-37].

Peptic ulcer disease (PUD) has become one of the common causes of morbidity and mortality worldwide and can be characterized by erosions in the gastric and duodenal mucosal linings[38]. The imbalance between defensive factors (prostaglandin, mucin, nitric oxide, bicarbonate, and growth factors) and offensive factors (pepsin, acid, and *Helicobacter pylori*) causes this disease[39]. PUD is usually treated with proton pump inhibitors, H2-blockers, antacids, and anticholinergics. However, most of these therapeutic agents have limited efficacy, drug interactions, and side effects[38-39]. Therefore, increasing attention towards botanical drug products can be seen to satisfy the requirement of novel therapeutic agents for gastric ulcers with favourable effectiveness, relatively low cost, and fewer adverse effects[40]. Consequently, *D. triflorum* and *P. heyneanus* are used traditionally for the treatment of gastrointestinal problems in Asian countries[11]. Therefore, the present study attempted to evaluate the *In vitro* antacid activity of AE of *D. triflorum*, *P. heyneanus*, and its fractions comparatively.

The neutralizing effects of different concentrations of AE of each plant and their fractions were studied (Table 3).

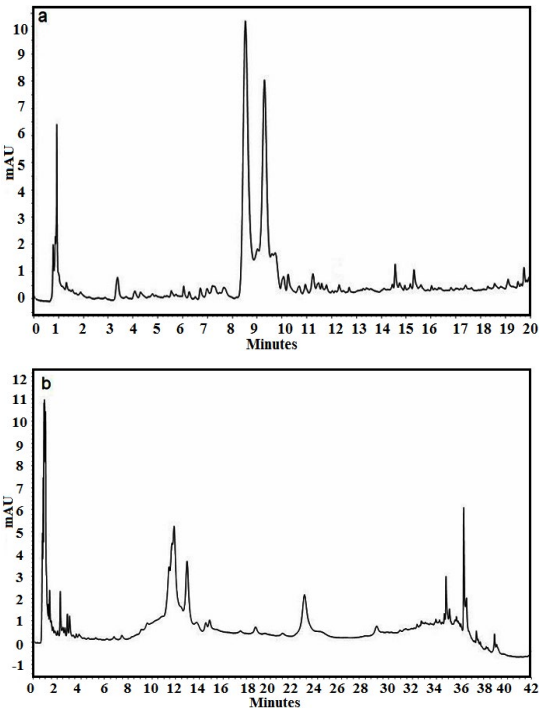

Fig. 6. Ultra-performance liquid chromatogram of crude methanolic extract of *Desmodium triflorum* and *Pogostemon heyneanus* a. Ultra-performance liquid chromatogram of crude methanolic extract of *D. triflorum*; b. Ultra-performance liquid chromatogram of crude methanolic extract of *P. heyneanus*

Table 3: *In vitro* antacid activity of *Desmodium triflorum* whole plant and *Pogostemon heyneanus* leaves using aqueous extracts and their fractions

Sample	Neutralizing effect		Neutralizing capacity	Duration of consistent
	Initial pH	End pH	(Volume, mL)	neutralization (seconds)
Distilled water	6.36±0.26	1.49±0.01	0.33±0.03	13.72± 3.33
Reference Drug (ENO)	6.70±0.04	4.58±0.07†	18.63±0.07†	453.01±21.00†
AE.D–58.00 mg/mL	4.70±0.00	4.17±0.00†	41.82±0.02†	201.69± 0.34†
AE.D–29.00 mg/mL	4.72±0.00	3.55±0.01†	18.33±0.09†	105.05± 5.53†
AE.D–14.50 mg/mL	4.75±0.01	2.82±0.00†	-	82.72± 2.35*
AE.P–37.20 mg/mL	5.05±0.00	4.02±0.01†	33.90±0.06†	196.37±12.47†
AE.P–18.60 mg/mL	5.08±0.01	3.49±0.01†	17.87±0.07†	114.41± 2.85†
AE.P–9.30 mg/mL	5.17±0.01	2.73±0.01†	-	87.06±13.03 *
RAF.D–42.00 mg/mL	5.13±0.01	4.16±0.00†	32.75±0.06†	184.70± 1.46†
RAF.D–21.00 mg/mL	5.23±0.00	3.57±0.00†	16.60±0.04†	120.36± 4.33†
RAF.D–10.50 mg/mL	5.29±0.01	2.69±0.00†	-	91.03± 4.49*
RAF.P–43.00 mg/mL	5.37±0.00	4.11±0.01†	35.77±0.03†	258.06± 4.61†
RAF.P–21.50 mg/mL	5.31±0.00	3.62±0.00†	17.70±0.06†	125.73±18.35†
RAF.P–10.75 mg/mL	5.19±0.00	2.76±0.00†	-	90.03± 0.58 *
EAF.D–2.00 mg/mL	4.94±0.16	1.31±0.01	0.33±0.04	49.39±17.84
EAF.P–2.00 mg/mL	4.76±0.22	1.31±0.01	0.39±0.33	108.68± 8.35†

*$p < 0.01$ and †$p < 0.001$ compared to negative control (distilled water), ANOVA

Observed final pH values of plants at each concentration of AE, RAF, and the standard drug were significantly higher than negative control, demonstrating a significantly better neutralizing effect than distilled water ($p<0.001$). AE of *D. triflorum* (58 mg/mL) and AE of *P. heyneanus* (37.20 mg/mL) exhibited the highest neutralizing effects among the tested concentrations of AE of both plants. The concentrations of 42.00 mg/mL and 43.00 mg/mL possessed the highest neutralizing effects among the tested concentrations of RAF of *D. triflorum* and *P. heyneanus*, respectively. However, the standard drug expressed the highest neutralizing effect among the tested samples. However, EAF was not capable in neutralizing gastric acid when compared with distilled water. Moreover, the extracts which exhibited a final pH>3 possessed excellent neutralizing effects when compared with distilled water ($p<0.001$), thereby increasing the pH of the artificial gastric juice from pH 1.2 to a pH value more than 3. These results were comparable with the neutralizing effect expressed by the standard drug which showed the final pH 4.58±0.07. Furthermore, it can be observed that for all plant samples, the AE and RAF had consistently higher antacid potential than the EAF.

The neutralizing capacity of different concentrations of AE of *D. triflorum* and *P. heyneanus* and their fractions were performed by Fordtran's *In vitro* titration model and compared with that of the standard and control. The volume of 0.1N HCl consumed to reach pH 3 was recorded for the test samples. According to Table 3, neutralizing capacity of tested concentrations of AE and RAF of each plant were significant ($p<0.001$) when compared to distilled water. It was noted that AE of *D. triflorum* (58.00 mg/mL), AE of *P. heyneanus* (37.20 mg/mL), RAF of *D. triflorum* (42.00 mg/mL) and RAF of *P. heyneanus* (43.00 mg/mL) have exhibited the potent neutralization capacity ($p<0.001$) among the tested samples, and were able to consume 41.82±0.02 mL, 33.90±0.06 mL, 32.75±0.06 mL and 35.77±0.03 mL of 0.1N HCl respectively. The neutralization capacity of the standard was 18.63±0.07 mL and it was found to be less ($p< 0.001$) when compared to the above-mentioned samples. Moreover, AE of *D. triflorum* at 29.00 mg/mL and AE of *P. heyneanus* at 18.60 mg/mL showed good neutralizing capacities which were comparable with the neutralizing capacity of the standard drug. EAF of both plants at 2 mg/mL did not exhibit significant neutralizing capacity (Table 3).

According to the results represented in Table 3, the duration of neutralization was highest for RAF of *P. heyneanus* at 43.00 mg/mL which was found to be 258.06±4.61 seconds. AE of *D. triflorum* at 58.00 mg/mL concentration also demostrated a relatively similar duration of neutralizing effect of 201.69±0.34 seconds, whereas the standard

drug ENO showed the best activity with a duration of neutralization of 453.01±21.00 seconds. The duration of consistent neutralization effect of tested AE and RAF doses of both plants and EAF of *P. heyneanus* were lesser than the reference drug but significantly ($p<0.01$) better than the negative control, distilled water. EAF of *D. triflorum* did not exhibit significant effect when compared to the distilled water. Hence, it can be suggested that the possibility of a relapse in acidity will be delayed with AE and RAF of both plants and EAF of *P. heyneanus*.

Fordtran's titration model and Vatier's artificial stomach model, which mimic some of the regular physiological functioning of a human stomach, were mainly used in this study to determine the *In vitro* antacid activity of the selected plants. These models are commonly adapted in research studies to discover the gastroprotective potential of herbal remedies or plants[41-42]. Fordtran's model mimics the regular physiological conditions of the stomach by maintaining the temperature at 37°C and inducing stomach movements by stirring at 30 rpm[26,41]. The artificial stomach model consists of three parts which include a a pH recording system, stomach and a peristaltic pump. The stomach is devised with three parts as the reservoir, secretory flux and gastric emptying flux. The secretory flux corresponds to acid secretion and emptying flux corresponds to gastric emptying. It mimics gastric secretion and empties with a 3 mL/min rate. Furthermore, the reservoir of the artificial stomach is maintained at 37°C and stirred continuously (30 rpm) for providing the physiological situations[43].

In the present study, the highest concentrations of AE and RAF of each plant exhibited the highest neutralizing capacities and were higher than that of the positive control, ENO. Incorporation of AE and RAF of both plants and EAF of *P. heyneanus* to the gastric reservoir caused an increase of pH and significantly longer lag times for initial pH recovery than the negative control, indicating consistent antacid effects. Therefore, AE and RAF of both plants were found to have potent antacid activity *In vitro*. The positive control, ENO demonstrated the highest duration of consistent neutralization when compared to that of AE and RAF doses of both plants and EAF of *P. heyneanus* in the present study. This may be due to the crude nature of the plant extracts. A similar type of finding was observed in the study conducted by Panda and Shinde, 2016. The standard drug, ENO comprised of $NaHCO_3$, citric acid, and sodium carbonate. Owing to the dispersible nature, powder exhibits a fast dissolution in gastric juice and high alkaline nature has brought a quick rise in pH and a longer antacid activity.

Moreover, the investigation showed that the polar solvent extracts and fractions of both plants have more antacid activity than nonpolar solvent fractions. It can be attributed that bioactive compounds which are responsible for antacid activity may present in the polar extracts than in the nonpolar extract.

Antacids act either by neutralizing the gastric HCl content through releasing anions into the medium or chemically reacting with buffer quantities in the gastric content without any direct effect on the ultimate output and relief of the hyperacidity condition in the stomach by reducing acid concentration[44]. Antacids are known to express some side effects such as constipation, nephrolithiasis, diarrhoea, hypercalcemia, milk alkali syndrome, and intestinal blocking of phosphorus absorption. Drug interactions of antacids are also a major clinical issue[45]. Considering the interactions and side effects of antacids, *D. triflorum*, *P. heyneanus* are suitable alternatives for the treatment of PUD.

In conclusion, the diagnostic characters discussed may be of potential application in the identification of the *D. triflorum* whole plant and *P. heyneanus* leaves, and to establish standardization parameters. *D. triflorum* whole plant and *P. heyneanus* leaves exhibited potent antacid activity and the plants further needed to be explored for accountable bioactive constituents. Evaluation of the antacid potential of both plants using a suitable *In vivo* model and elucidation of its mechanism of action is recommended in future studies.

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