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CHEMICAL PROFILE AND ANTIOXIDANTS PROPERTY OF *ERYNGIUM FOETIDUM* L. LEAVES – USE OF HPLC METHODS TO OPTIMIZE EXTRACTING SOLVENTS TO BE USED IN FUNCTIONAL FOOD DEVELOPMENT

Shanthirasekaram Kokilananthan, Vajira P. Bulugahapitiya✉, Harshi Manawadu, Chinthaka Sanath Gangabadage

Department of Chemistry, Faculty of Science
University of Ruhuna, Matara, **Sri Lanka**

ABSTRACT

Background. *Eryngium foetidum* L. grows in the wet zone of Sri Lanka. Despite the fact that this plant is widely used for healthcare purposes and as a culinary spice in tropical regions, limited scientific research has been available. This study aimed to investigate phytoconstituents, essential oil (EO), antioxidative potential (AP), and proximate composition, as well as find a suitable extracting solvent to extract potential constituents using the HPLC-PDA application in order to transform leaves into functional foods as an extended study.
Materials and methods. The chemical constituents of the EO in the leaves were identified using the GC-MS analysis after extraction via hydro-distillation. Phytoconstituents of leaves were extracted into five different solvents via maceration with methanol, ethanol, water, acetone, and acetonitrile. Analytical HPLC was applied to compare the phytochemical profile under each solvent. Chemical analysis was used to reveal phytoconstituents in methanolic extract using standard methods. DPPH and FRAP assays were employed to determine AP of EO and the methanolic extract.
Results. About 43 compounds were identified in the EO, showing 2-dodecenal (13.7%) and 2,4,5-trimethylbenzaldehyde (13.1%) as major ones. Cyclopropane, nonyl-, butylated hydroxytoluene, octadecane, hexacosane, and tetracosane were found in the EO of *E. foetidum* leaves for the first time. The HPLC chemical profiling revealed that methanol is a better solvent for extracting optimum phytochemicals compared to other solvents. The AP of the EO has been proven to be 118.16 ±1.32 μL Trolox Eq/L for FRAP, whereas the value of 95.74 ±3.47 mg Trolox Eq/g was shown for methanolic extract of leaves. The saponin level was greater (387.40 ±0.41 mg SE/g) in the solvent extract out of all other phytochemicals, and the leaves showed acceptable proximate compositions.
Conclusion. To conclude, the solvent used has an influence on extractable phytochemicals and the technique of HPLC showed a promising method for phytochemical profiling. This is the first detailed study of EO and solvent extract of leaves of *E. foetidum*, and the finding of this study would be helpful pharmacological applications and be beneficial for functional food development.

Keywords: antioxidants, chemical compositions, *Eryngium foetidum*, essential oil, high-performance liquid chromatography, proximate compositions

✉vajira@chem.ruh.ac.lk, https://orcid.org/0000-0003-1178-1052

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INTRODUCTION

Eryngium foetidum L. (Apiaceae) is a biennial, pungently scented tropical herb that is also referred to as long coriander, spiny coriander, and Andu in Sinhala (Dalukdeniya and Rathnayaka, 2017; Dawilai et al., 2013; Garcia et al., 1999; Okon et al., 2013). It is found in moist and humid environments and on open banks and grasslands (Dalukdeniya and Rathnayaka, 2017). It is grown in the wet zone of Sri Lanka. *E. foetidum* leaves is utilized as a healthy food due to its high levels of vitamins A, B, and C, riboflavin, iron, calcium, carotene, and proteins (Kouitcheu Mabeku et al., 2016). This plant is utilized in traditional medicine to cure various illnesses including malaria, hypertension, fevers, vomiting, chills, headache, burns, earache, asthma, stomach ache, scorpion stings, snake bites, arthritis, epilepsy, and diarrhoea (Dawilai et al., 2013; Kouitcheu Mabeku et al., 2016; Promkum et al., 2012). In traditional medicine, a decoction of the leaves is used as a vulnerary, hypotensive, and digestive aid. Crushed leaves are put in the ear to relieve discomfort and are used to treat arthritic conditions locally (Devi et al., 2021; Garcia et al., 1999).

Some of the few previous studies have provided information about the phytochemicals' availability in *E. foetidum* extracts (Aly, 2010; Dawilai et al., 2013; Forbes et al., 2014; Garcia et al., 1999). They have pharmacological properties, such as antioxidant, antibacterial, antidiabetic, anti-inflammatory (Anusha et al.; Dalukdeniya and Rathnayaka, 2017; Dawilai et al., 2013; Garcia et al., 1999; Malik et al., 2016; Okon et al., 2013; Thomas et al., 2017), due to the presence of those important phytochemicals.

Essential oil has recently seen a surge of interest in the biological functions of EOs (Majeed et al., 2015). These essential oils are used for several purposes, such as food preservation, aromatherapy, fragrance industries, and several pharmacological properties, such as anticancer, antioxidant, and antibacterial due to the present of active compounds present into them (Irshad et al., 2020). Despite the fact that *E. foetidum* leaves have been used to extract essential oil in the past from various countries (Chowdhury et al., 2007; Leclercq et al., 1992; Martins et al., 2003; Wong et al., 1994), no study has been carried out in Sri Lanka. Because chemicals and nutritional values may change with geographical distribution, investigation of essential oil extraction and chemical analysis of *E. foetidum* grown in Sri Lanka are beneficial. Furthermore, as chemical profiling via high-performance liquid chromatography (HPLC) is a sophisticated analyzing technique, no research has been conducted yet based on *E. foetidum* leaves extracts. As the primary goal is to use *E. foetidium* leaves in the preparation of functional foods, there is insufficient scientific information available concerning *E. foetidium* leaves. Therefore, this study aimed to investigate phytoconstituents, essential oil, antioxidative potential, and proximate composition, as well as find a suitable extracting solvent to extract active constituents using the HPLC-PDA application, as this information is very beneficial for transforming them into functional foods.

MATERIALS AND METHODS

Plant materials and chemicals

Fresh *E. foetidum* leaves were collected from the southern province of Sri Lanka (Tangalle, latitude 6.0289ºN and longitude 80.7856ºE), and plant material was validated and lodged with the voucher No AHEAD/DOR-05/C1 in Peradeniya Botanical Garden, Sri Lanka.

Absolute ethanol, acetic anhydride, acetone, acetonitrile, aluminium chloride anhydrous, ammonia, bromocresol green, n-butanol, chloroform, copper acetate, copper sulphate, 2,2'-diphenyl-1-picrylhydrazyl, dichloromethane, dimethyl sulfoxide, Folin-Ciocalteu reagent, ferric chloride hexahydrate (× $6H_2O$), gallic acid monohydrate, glacial acetic acid, hydrochloric acid, lead acetate, linalool, magnesium ribbon, nitric acid, phosphomolybdic acid, potassium hydroxide, pyridine, olive oil, sodium chloride, sodium carbonate monohydrate, sodium hydroxide, sodium nitroprusside, sulfuric acid, sodium sulphate anhydrous, Trolox, 2,4,6-tripyridyl-S-triazine, and tannic acid are classified as analytical grade (AR). Diethyl ether, benzene, methanol (MeOH), and hexane are classified as gas chromatographic grade (GC grade), whereas quercetin is classified as HPLC grade from Merck, Sigma-Aldrich.

Isolation of essential oil

The EO was isolated from the leaves using the hydro-distillation technique by using the Clevenger

apparatus (fresh leaves; 200.00 g, for three hours, 1:5 w/v; Arain et al., 2019). The collected EO was dehydrated, and the moisture-free oil was kept in a sealed, amber-colored vial in the refrigerator at 4ºC until it was needed to perform the GC-MS analysis. The yield percentage was calculated on a weight basis (Kokilananthan et al., 2022b; Weli et al., 2019).

Characterization of EO by GC-MS analysis

The amount of 10.0 mL of GC grade n-Hexane was used to dissolve the EO (0.5 ml). The dehydrated sample was filtered using membrane filter paper (PTFE, 0.45 μm of pore size). The sample (1.0 μL) was then injected into GC-MS (Agilent 7890A series) by autosampler which was interfaced with the mass selective detector (Agilent 5975C series MSD version, Agilent Technologies, USA). HP-5MS 5% Phenyl Methyl Silox (30 m × 250 μm × 0.25 μm) column was utilized. The chromatographic conditions were: oven temperature 70°C for 4 minutes, then 8°C/min to 270°C, then stable for 10 minutes, run time was 39 minutes, and helium (flow rate 1 ml/min) was used as a carrier gas. The mass unit settings were as follows: ion source temperature of 230°C, mass spectrum collected at 1624 ionization voltage with a mass scan range of 33–550 m/z. A single GC-MS analysis was carried out for the isolated essential oil. GC-MS mass spectra were used to identify individual components in the recovered EO. The chemical compositions of EO were evaluated by comparing mass spectral data obtained with the NIST 08 and NIST Chemistry WebBook (NIST Standard Reference Database Number 69), as well as by referencing the literature (Adams, 2007).

Extraction of constituents from the leaves

The washed, air-dried, and crushed leaves (100.00 g) were macerated with methanol, ethanol, acetone, water, or acetonitrile for 48 hours at room temperature. The extraction was performed until optimal quantities were extracted into solvent. The extracts were filtered using cotton plugs followed by Whatman No-01 filter paper and subjected to rotary evaporation (Model No: HS-2005S). Freeze drying (S/No: FD 2020062222 and Model: FE-10-MR) was used to get powdered extracts and once these were obtained, they were stored at –30ºC in an amber color glass container until needed for further usage (Mtewa et al., 2018).

Samples preparation for HPLC applications

All five different solvent extracts (methanol, ethanol, acetone, water, acetonitrile) mentioned in this study were compared using chemical profiling/identification with analytical HPLC (SHIMADZU; Sathyanarayanan et al., 2017). To remove impurities including nonpolar compounds, all five solvent extracts of *E. foetidum* leaf were defatted with diethyl ether and dichloromethane (tripled). Diethyl ether and dichloromethane filtrates were discarded, and the remaining residues were concentrated with a rotary evaporator and a freeze dryer. All five solvent extracts' crude powders were used to make 500 ppm solutions for HPLC applications.

HPLC-PDA application for comparison of five different solvent extracts

The chemical profiles of all five solvent extracts were compared using analytical HPLC (Al-Rimawi et al., 2017; 2018; Sathyanarayanan et al., 2017). HPLC analyses were performed with SHIMADZU LC-20AP liquid chromatograph (Japan) with four solvent delivery system quaternary pumps (FCV-200AL) including a photodiode array detector (SPD-M40). The LabSolutions (SHIMADZU) software was used as a data processor. The compounds identification was analyzed by analytical HPLC technique with the analytical column: Shim-pack GIST C18-AQ μm, 4.6 I.D. × 150 mm. 10.0 μL of the sample was injected into the column by the auto sampler. The samples were eluted through the column with a gradient mobile phase consisting of ultra-pure water (A) and methanol (B) with a flow rate of 1 ml/min, for sixty minutes. Prior to injection into the column, the solvent gradient was programmed by gradually changing the polarity from pure solvent A to pure solvent B. The samples were monitored by a PDA director with a wavelength range from 190–800 nm at 35ºC of column oven temperature.

Chemical analysis of phytochemicals

Qualitative tests for bioactive compounds such as polyphenolic, tannin, flavonoid, saponin, alkaloid, terpenoid, coumarin, glycoside, anthocyanin, betacyanin, chalcones, and quinones of methanolic extract were carried out in triplicates using standard procedures described in the literature (Abubakar and Haque, 2020; Gayathri and Kiruba, 2014; Kokilananthan et al., 2022c; Wadood et al., 2013).

Quantification of some phytochemicals

0.10 g of methanolic extract was dissolved in 0.25 ml of DMSO and diluted with 100.0 ml of methanol to make a 1000 ppm concentration. The prepared solution was then utilized for phytochemicals spectrophotometric measurements such as total phenolics (TPC), total tannins (TTC), total flavonoids (TFC), terpenoids (TC), saponins (SC), and alkaloids contents (AC) as described in the literature (Kokilananthan et al., 2020; 2021; 2022a; 2022c; Shanthirasekaram et al., 2021).

The TPC and TTC were determined using the Folin-Ciocalteu method. TPC was determined in mg of gallic acid equivalents (mg GAE/g) using a gallic acid standard curve and the tannic acid equivalents (mg TAE/g) were used to quantify the TTC of methanolic extract using a tannic acid standard curve. Aluminium chloride spectrophotometric technique was used to determine TFC. The TFC of methanolic extract was estimated using a quercetin standard curve and expressed in mg QE/g plant extract. Phosphomolybdic acid spectrophotometric analysis was used to estimate the TC. A linalool standard curve was created to assess TC, and the TC of methanolic extract was represented in linalool equivalents (mg LE/g). Vanillin-sulfuric acid spectrophotometric approach was used to assess the SC, and it was determined as saponin equivalents (mg SE/g). Bromocresol green spectrophotometric method was used to evaluate the AC, and it was given as atropine equivalents (mg AE/g).

Antioxidant analysis of *E. foetidum* leaves extracts

DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) assay were applied to determine the antioxidative potential of methanolic extract and the EO of *E. foetidum* leaves (Kokilananthan et al., 2020; 2021; 2022a; 2022c; Shanthirasekaram et al., 2021). The IC_{50} value for free radical scavenging activity of methanolic extract was determined using a percentage of radical scavenging effect vs. concentration plot where ascorbic acid and Trolox were used as standards of antioxidants. The total antioxidative potential of methanolic extract and EO was estimated through FRAP assay using a Trolox standard curve and expressed in mg Trolox Eq/g plant extract.

Proximate compositions analysis of *E. foetidum* plant leaves

The proximate composition, such as moisture, crude fiber, ash, crude fat, carbohydrate, total solids, crude protein, and energy of air-dried *E. foetidum* leaf, was determined using the standard approaches – AOAC methods (Busuttil-Griffin et al., 2015; Ilodibia et al., 2014; Kumari et al., 2017; Maisarah et al., 2014; Nielsen, 1998; 2021).

Statistical analysis

Non-parametric statistics (Cochran's Q-test), analysis of variance (ANOVA), and the T-test (LSD; Least Significant Difference) were used to analyze and compare the data. R-studio and SAS OnDemand for Academics: Studio (SAS 9.4) software were used for the statistical analysis. The data were presented in the form of means and standard deviations.

RESULTS

Chemical compositions of essential oil

On a fresh leaves weight basis, the hydro-distillation process yields 0.15% ±0.02 (v/w percent) EO of *E. foetidum* leaves. 43 chemical components have been discovered in the EO of *E. foetidum* leaves, with ten compounds having a matching value greater than 90%, which are decanal (1.2%), 2,4,5-trimethylbenzaldehyde (13.1%), tridecanal (3.2%), cyclopropane, nonyl- (6.0%), butylated hydroxytoluene (6.0%), dodecanoic acid (3.2%), nerolidol (2.2%), octadecane (1.6%), hexacosane (2.9%), and tetracosane (2.1%). The chemical compositions such as cyclopropane, nonyl-, butylated hydroxytoluene, octadecane, hexacosane, and tetracosane were found in the EO of *E. foetidum* leaves for the first time. All 43 components were tabulated in Table 1. The antioxidative potential of EOs was found to be 118.16 ±1.32 mg Trolox Eq/g in FRAP assay.

HPLC-PDA chemical profiling of five different solvent extracts

The HPLC method was used to compare the five different solvent extracts of *E. foetidum* leaves (methanol, ethanol, water, acetone, and acetonitrile) to determine the best extracting solvent for *E. foetidum* leaves phytochemicals. As a result, an HPLC method was

Table 1. Chemical constituents of isolated EO from *E. foetidum* leaves

Chemical name	MF	MW	MV	RT	RP
Decanal	$C_{10}H_{20}O$	156.27	91	10.188	1.2
2,4,6-trimethyl-1,3,6-heptatriene	$C_{10}H_{16}$	136.23	50	11.224	0.7
Cyclohexanone, 2,5-dimethyl-2-(1-methylethenyl)-	$C_{11}H_{18}O$	166.26	43	11.693	1.4
Pyrrole-2-carbonitrile, 5-formyl-3,4-dimethyl-	$C_8H_8N_2O$	148.16	80	11.996	0.9
Endo-1,5,6,7-tetramethylbicyclo[3.2.0]hept-6-en-3-ol	$C_{11}H_{18}O$	166.26	43	12.511	1.4
2,4,5-trimethylbenzaldehyde	$C_{10}H_{12}O$	148.20	94	12.585	13.1
3-methyl-2,4-pentadien-1-ol	$C_6H_{10}O$	98.14	38	13.009	0.6
3-penten-2-one	C_5H_8O	84.12	46	13.046	1.1
alpha-durenol	$C_{10}H_{14}O$	150.22	58	13.134	0.9
Tridecanal	$C_{13}H_{26}O$	198.34	91	13.183	3.2
2-methylene cyclopentanol	$C_6H_{10}O$	98.14	35	13.749	0.6
2-dodecenal	$C_{12}H_{22}O$	182.30	86	13.947	13.7
Cyclopropane, nonyl-	$C_{12}H_{24}$	168.32	95	14.019	6.0
Butylated hydroxytoluene	$C_{15}H_{24}O$	220.35	94	14.599	6.0
Dodecanoic acid	$C_{12}H_{24}O_2$	200.32	95	15.086	3.6
Nerolidol	$C_{15}H_{26}O$	222.37	91	15.182	2.2
(E)-2,2-dimethyl-3-hexene	C_8H_{16}	112.21	64	15.453	1.4
Anozol	$C_{12}H_{14}O_4$	222.24	43	15.583	1.7
1-(3-hydroxy-1-adamantyl)ethanone	$C_{12}H_{18}O_2$	194.27	37	16.23	0.8
N-methyladrenaline, tri-TMS	$C_{19}H_{39}NO_3Si_3$	413.80	20	16.321	0.7
(Z)-14-methylhexadec-8-enal	$C_{17}H_{32}O$	252.40	72	16.417	2.0
Isocitronellol	$C_{10}H_{20}O$	156.26	35	16.557	0.7
1,3-benzodioxol-5-yloxy acetonitrile	$C_9H_7NO_3$	177.16	38	17.077	0.5
Tolazoline	$C_{10}H_{12}N_2$	160.22	25	17.792	2.1
2-methylnonan-3-one	$C_{10}H_{20}O$	156.26	27	18.794	2.3
1,1,1,5,7,7,7-heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{13}H_{39}O_5Si_6$	443.96	40	22.315	0.7
2-methylheptadecane	$C_{18}H_{38}$	254.50	83	22.491	1.1
Octadecane	$C_{18}H_{38}$	254.50	90	23.319	1.6
Tetrakis(trimethylsiloxy)silane	$C_{12}H_{36}O_4Si_5$	384.84	38	23.504	1.1
Benzamide, 3-(4-isopropylbenzylidenamino)-	$C_{17}H_{18}N_2O$	266.34	25	23.591	0.2
4-thiazoleacetic acid, 2-(4-chlorophenyl)-, methyl ester	$C_{12}H_{10}ClNO_2S$	267.73	9	23.835	0.3
1-benzyl-4-(4-methylphenyl)piperidine	$C_{19}H_{23}N$	265.40	9	23.904	0.5
Pentadecane	$C_{15}H_{32}$	212.41	89	24.115	2.3
Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester	$C_{26}H_{42}O_4$	418.60	72	24.545	3.2
Phthalic acid, cyclohexyl neopentyl ester	$C_{19}H_{26}O_4$	318.40	47	24.592	3.7
Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.07	53	24.655	1.5
Hexacosane	$C_{26}H_{54}$	366.70	95	24.963	2.9
Tetracosane	$C_{24}H_{50}$	338.70	91	25.948	2.1
Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.40	59	26.026	1.4
Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	458.99	58	27.779	2.0
Octadecane	$C_{18}H_{38}$	254.50	60	28.556	1.6
Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	533.10	42	30.083	1.6
Heneicosane	$C_{21}H_{44}$	296.60	60	30.324	1.1

MF – molecular formula, MW – molecular weight, MP – matching value, RT – retention time, RP – relative percentage.

Table 2. HPLC-PDA spectra peaks data with different applications

	Methods	Extracting solvents				
		A	B	C	D	E
No of the peaks of compounds detected	wavelength 254 nm, 4 nm	147	136	171	207	173
	wavelength 204 nm, 4 nm	145	150	174	208	212
	wavelength 654 nm, 4 nm	145	133	180	205	158
	wavelength 734 nm, 4 nm	147	133	197	205	157
	wavelength 224 nm, 4 nm	151	145	177	214	176

A – acetone, B – acetonitrile, C – ethanol, D – methanol, E – water.

developed for *E. foetidum* plant extracts, and the results were compared. The extracted substances in all five extracts were first directly examined on HPLC-PDA at various wavelengths, including 204 nm, 224 nm, 254 nm, 654 nm, and 734 nm; results are shown in Table 2. The number of compound peaks varies depending on the extraction solvents and wavelength.

Chemical analysis of phytochemicals

The macerated methanolic extract from the leaves of *E. foetidum* was utilized for chemical analysis. The yielded percentage of the methanolic extraction was found to be 10.07 ±0.03% dried powdered leaves basis. In order to establish the existence and lack of availability of phytochemicals in the methanolic extract of *E. fetidum* leaves, Cochran's Q test was used – a non-parametric approach. The availability of important metabolites such as polyphenols, tannins, flavonoids, terpenoids, glycosides, saponins, alkaloids, coumarins, and betacyanin was found in the methanolic extract. The quinones, chalcones, and anthocyanins were found to be lacking in *E. foetidum* leaves. Detailed information of the phytochemicals qualitative analysis was tabulated in Table 3.

A quantitative study of methanolic extract of *E. foetidum* leaves showed various amounts of polyphenolics, saponins, terpenoids, flavonoids, alkaloids, and tannins, as shown in Table 4. Out of all the phytochemicals examined in this study, saponins contained more than the other phytochemicals (387.40 ±0.41 mg SE/g), and statistical analysis confirmed that the proportion of saponins contained higher and there is a significant difference with others at a 5% significant level.

Table 3. Phytochemicals qualitative study of *E. foetidum* leaves methanolic crude extracts

Phytochemicals	Method	Availability
Alkaloids	Mayer's Test	+
	Wagner's Test	+
	Dragendroff's Test	+
Glycosides	Keller-kilani Test	+
	Modified Borntrager's Test	+
	Legal's Test	+
Flavonoids	Alkaline reagent Test	+
	Shinoda Test / Mg turning Test	+
	Lead acetate Test	+
	$AlCl_3$ Test	+
	NH_4OH Test	+
Saponins	Froth Test	+
	Olive Oil Test	+
Tannins	Bramer's Test	+
	Lead Acetate Test	+
Terpenoids	Liebermann- Burchardt Test	+
	Copper Acetate Test	+
Polyphenols	Ferric Chloride Test	+
Coumarins	UV light Test	–
	NaOH Test	+
Anthocyanins	HCl & NH3 Test	–
Chalcones	NaOH Test	–
Betacyanin	NaOH Test	+
Quinones	H_2SO_4 Test	–

+ – present, – – absent.

Table 4. Phytochemicals quantitative study of *E. foetidum* leaves' methanolic crude extracts (values represent the mean ±standard deviation of the triplicate samples)

Phytochemicals	Quantity
Total phenolic content (TPC)	66.78 ±2.50 mg GAE/g
Total flavonoid content (TFC)	28.41 ±0.10 mg QE/g
Total tannin content (TTC)	61.70 ±1.31 mg TAE/g
Terpenoids content (TC)	14.51 ±0.14 mM LE/g
Saponins content (SC)	387.40 ±0.41 mg SE/g
Alkaloids content (AC)	5.32 ±0.02 mg AE/g

Antioxidant analysis of plant extract

The antioxidative assay results revealed that the *E. foetidum* leaves show less antioxidant capacity than the standards used. The DPPH radical scavenging test evidenced that it possesses scavenging effect (IC_{50} value: 11.36 ±0.01 mg/ml; standards such as ascorbic acid and Trolox: 0.14 and 0.12 mg/ ml, respectively). In addition, the total antioxidant capacity by FRAP assay also confirmed that methanolic extract of *E. foetidum* leaves has better antioxidant potential (95.74 ±3.47 mg Trolox Eq/g of extract).

Proximate composition analysis

Proximate compositions which include moisture, fat, fiber, protein, ash, etc., are extremely important variables in the food industry that can determine the quality of the produced food products. Therefore, it is important to have an understanding of the proximate compositions of the materials when preparing functional foods or nutraceuticals from them. The proximate compositions of *E. foetidum*'s leaves on dry matter were investigated, and the results were displayed in Table 5.

Table 5. Proximate compositions of *E. foetidum* leaves on dry matter (values represent the mean ±standard deviation of the triplicate sample)

Proximate compositions	Quantity
Moisture content	17.56 ±0.15%
Total solid content	82.44 ±0.15%
Ash content	9.49 ±0.15%
Crude fat content	2.34 ±0.22%
Crude fiber content	18.94 ±2.66%
Crude protein content	11.51 ±0.14%
Carbohydrate content	59.11 ±0.17%
Energy content	303.54 ±1.39 kcal/100 g

DISCUSSION

The obtained yield is similar to the previously reported research (Thomas et al., 2017). Since the EO of *E. foetidum* leaves has good antioxidative potential, which can be utilized for the purpose of preparing interesting products and in pharmacological applications or as a part of dietary supplement. The highest number of compounds/peaks was found in the methanolic extract, compared to other extractants. Other authors also discovered that methanol is superior to other extraction solvents (Chigayo et al., 2016; Dhawan and Gupta, 2017). The solvents that have the greatest potential for extracting phytochemicals from *E. foetidum* leaves were methanol, ethanol, water, acetone, and acetonitrile, according to the number phytoconstituents detected by the HPLC-PDA analysis. In another study (Bhavana et al., 2013), there were more polyphenols and tannins content in the methanolic extract of *E. foetidum* identified; however, the flavonoids content appears to be similar. According to the authors Dalukdeniya and Rathnayaka (2017) and Anusha et al. (2013), the polyphenols and flavonoids contents in the methanolic extract of *E. foetidum* were much lower than in the current study. Differentiation in the obtained results might be related to regional variation, as Bhavana et al. (2013) conducted their investigation in India. The findings presented in Table 4 demonstrate that the methanolic extract has a greater amount of key plant chemical compounds, such as polyphenols and flavonoids, terpenoids, tannins, alkaloids, and saponins.

When considering the antioxidant potential of *E. foetidum* leaves' methanolic extract, our study revealed that it has acceptable antioxidant potent and Malik et al. (2016) also revealed the same. Thi et al. (2020) have compared the radical scavenging activity of aqueous and ethanolic extracts of *E. foetidum,* and both extracts have also been shown to have the radical scavenging

effect in varying amounts. Therefore, based on the previous research reports and our current studies, it was proven that the leaves of *E. foetidum* has radical scavenging activity. Moreover, the antioxidant potential of *E. foetidum* leaves was supported by results in the FRAP assay.

As the medicinal plant *E. foetidum,* which is used in functional foods and nutraceuticals products, is of scientific interest, this work is concerned with a detailed examination of the presence of some phytochemicals in crude extracts, as well as the essential oil and showed antioxidant capacities of the *E. foetidum*'s leaves to be used in the applications. As compared with the proximate composition of *E. foetidum*, the results of the present study are similar to those reported by Khan et al. (2006) in which the protein content was found to be 18.34%. Lepcha et al. (2018) found that the protein content of *E. foetidum* was 2.63%. Based on the scientific investigation of Lepcha et al. (2018), the fat content of *E. foetidum* varied from 0.23–2.63% based on the geographical distribution and which is comparable with the present study. Crude fiber content and fat content were also the same as Lepcha et al. (2018) found (16.83–31.50%) based on the geographical distribution. Importantly, our study mainly focused on phytochemicals and antioxidant potential of methanolic extract for further study and which is supported by HPLC applications with different solvent extracts.

As previously stated, geographical distribution plays an important role in phytochemical presence and quantity, the antioxidative potential, and the nutritional value of many plants. As a result, research into these parameters should be strengthened in at least each country for future research based on this plant; for example, these parameters are important factors in the designing of functional foods and nutraceuticals, as well as probably in pharmacological applications. Since no study has been conducted yet on these factors in Sri Lanka, and the preparation of functional food and nutraceuticals is a current trend, *E. foetidum* leaves were shown to have important phytochemicals in this study. The study is a detailed chemical investigation based on the crude extract and EO of the leaves, and it will serve as a beacon for future researchers in Sri Lanka who are looking for *E. foetidum* to be used in the preparation of new functional foods or nutraceuticals.

CONCLUSIONS

As a summary, the EO of *E. foetidum* leaves revealed 43 chemical components, and the most prominent compounds were 2-dodecenal and 2,4,5-trimethylbenzaldehyde. Cyclopropane, nonyl-, butylated hydroxytoluene, octadecane, hexacosane, and tetracosane were found in the EO of *E. foetidum* leaves for the first time. Analysis with HPLC-PDA has shown that methanol has the greatest potential for extracting phytochemicals from *E. foetidum* leaves. The methanolic extract of leaves confirms that there is a variety of phytoconstituents in varying amounts, with a high concentration of saponins. Antioxidant investigations on herbal extracts and the EO revealed the substantial potency of antioxidant properties. This is the first detailed study of EO and extracts of leaves of *E. foetidum*, and the finding of this study would be beneficial for interesting pharmacological applications with novel fortified products.

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