A pharmacognostical study of *Vernonia cinerea* Less (Asteraceae) and evaluation of anti-inflammatory and antibacterial activities of stem

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Aim
This study aimed to establish the pharmacognostical characteristics of leaf, stem and root of *Vernonia cinerea*, Asteraceae (ash-coloured fleabane), and to verify the anti-inflammatory and antibacterial activities of various extracts of the stem.

Background
V. cinerea (Asteraceae) is traditionally used to treat inflammation, diarrhoea, cough, smoking cessation, asthma, Parkinson's disease and leprosy.

Materials and methods
Leaf, stem and root and their powders were examined microscopically, and pharmacognostic standardization parameters were determined according to WHO guidelines. Extracts of different organs of the plant in petroleum ether, chloroform, ethanol, ethanol (50%) and water were prepared and examined by thin-layer chromatography. An antibacterial assay of the stem extracts for *Staphylococcus aureus* was performed. The anti-inflammatory activity of the same extracts was studied using a carrageenan-induced paw oedema model in Wistar rats.

Results and conclusion
Microscopic characterization of the different organs of the plant indicated the presence of trichomes, arrangement of vascular bundles (stem: radial, root: scattered), anomocytic and diacytic stomata, and wavy epidermal cells in stomata. The antibacterial assay indicated a zone of inhibition of 20 ± 0 and 19.33 ± 0.33 mm with alcoholic and chloroform extracts of *V. cinerea* leaf, respectively (extracts of stem showed a zone of inhibition of 21.00 ± 0.57 and 21.00 ± 0.57 mm, respectively). Diclofenac sodium and chloroform extract showed 11.11% inhibition of inflammation, whereas 16.66 and 13.33% inhibition were observed with alcoholic and hydroalcoholic extracts, respectively. Microscopic and pharmacognostic parameters aid in the identification and characterization of different organs of the plant. Traditional claims of antimicrobial and anti-inflammatory activities of the stem have been verified. Various extracts showed significant results for anti-inflammatory and antimicrobial action.

Keywords:
anti-inflammatory, antimicrobial, extraction, microscopy, phytochemical; *Vernonia cinerea* Less

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Introduction

Vernonia cinerea Less (Asteraceae), commonly known as purple fleabane, ash-coloured fleabane and *Sahadevi* [1], is used for the treatment of inflammation [2], diarrhoea, cough, smoking cessation [3,4], asthma [5], Parkinson's disease [6] and leprosy [7]. The plant also has immunmodulatory [8] and nephroprotective [9] actions. The leaves are useful in the treatment of conjunctivitis and tumours [10], whereas the seeds are useful in alleviation of worm infestation, psoriasis and leucoderma. The roots are used as an antipyretic [11].

V. cinerea contains vernolide-A and vernolide-B (two novel sesquiterpene lactones) [12]; β-amyrin, lupeol and their acetates; and β-sitosterol, stigmasterol, α-spinasterol and phenolic resin in the whole plant [13]. In addition, the leaves contain urticifolene (new polyene), lutein (carotenoid) and sitosterol (triterpenoid) [14,15]. The stem, bark and leaves contain lupeol, 12-oleanen-3-ol-3β-acetate and stigmasterol [14,15]. The roots contain δ-amyrin acetate, α-amyrin acetate, β-amyrin acetate, β-amyrin and α-amyrin [13].

The anti-inflammatory potential of alcoholic extract of *V. cinerea* flower was studied by adjuvant-induced arthritis in rats [16]. The methanolic extract of *V. cinerea* leaf showed potent anti-inflammatory activity when studied in acute (carrageenan-induced, histamine-induced and serotonin-induced rat paw oedema) and a chronic model (cotton pouch-induced granuloma) [2]. The analgesic, antipyretic and anti-inflammatory actions of various extracts of *V. cinerea* leaf in methanol, chloroform and ether have been

proven [17]. The antibacterial potential of leaf extracts [1,7,18] and flower extracts of *V. cinerea* has been explored [1].

The current literature indicates that pharmacognostic parameters have not been established for *V. cinerea*, and that antibacterial and anti-inflammatory activities have not been explored for the stem.

This study aimed to establish pharmacognostical parameters and to explore the antibacterial action of chloroform and petroleum ether extracts of stem of *V. cinerea*. *Staphylococcus aureus* (MTCC-80) was used as the test bacteria for screening of the antibacterial activity of prepared extracts of *V. cinerea* and ciprofloxacin was used as a standard antibacterial drug. The anti-inflammatory potential of aqueous, ethanolic, hydroalcoholic and chloroform extracts of the stem of *V. cinerea* was studied by carrageenan-induced paw oedema in rats and diclofenac sodium as a positive control.

Materials and methods

Plant material

The whole plant, *V. cinerea*, was collected from the medicinal garden of Jodhpur National University (Jodhpur, India) and authenticated by Dr R.P. Pandey at the Botanical Survey of India (Jodhpur, India). A voucher specimen, JNU/PH/2011/V_cV_1, was deposited in the herbarium of Jodhpur National University (Jodhpur, India). The roots, stems and leaves were dried in the shade for 1 month, ground using an electric mixer-grinder and the powders were used for analysis, fluorescence studies and preparation of various extracts.

Microscopic investigation

Transverse sections of fresh root, stem and leaf were treated with chloral hydrate to remove chlorophyll, and used for microscopic characterization to observe stomatal number, stomatal index, vein-islet number and vein termination number. Safranin, phloroglucinol HCl, iodine, ruthenium, Sudan red, sulphuric acid, etc. were used to stain different sections for different observations.

Constants

Total ash, acid-insoluble ash, sulphated ash, water-soluble ash, loss on drying, and petroleum ether, chloroform, ethyl acetate, ethanol, hydroalcoholic (1 : 1) and water extractives were estimated [19].

Preparation of extracts

The powder (10 g) was extracted in a Soxhlet extractor with petroleum ether, chloroform, ethanol, ethanol (50%) and water, separately, to extract nonpolar and polar compounds. The extracts were filtered, concentrated and dried by evaporating the solvent in a waterbath. The residual moisture in the extract was removed by drying in an oven, followed by placing the powdered extract in a desiccator.

Phytochemical screening

Different plant organs were subjected to a preliminary phytochemical analysis for different constituents, viz., alkaloids, glycosides, terpenes, coumarins, flavonoids, carbohydrates, proteins, volatile oils, saponins, etc., using standard reagents and solutions [20]. Chromatographic profiles were obtained for different phytoconstituents using several solvent systems [21,22].

Antimicrobial activity

Preparation of solutions of extracts and standard reference

Extract (10 mg) was dissolved in 10 ml dimethylformamide and diluted to 100 ml with distilled water. This stock solution was used to prepare different dilutions in distilled water, viz., 50, 100 and 150 μg/ml. Ciprofloxacin solutions (50, 100 and 150 μg/ml) were prepared in distilled water and used as standards.

Microorganism, growth conditions and preparation of inoculum

S. aureus (MTCC-80) was obtained from the Institute of Microbial Technology (IMTECH, Chandigarh, India), cultured in nutrient broth and used as the test bacteria. A vial containing lactose dilution of *S. aureus* was broken using a sterile scalpel knife under aseptic conditions in a flask containing 100 ml of nutrient broth. This flask was incubated for 24 h in a biological oxygen demand incubator maintained at 37°C. After 24 h, turbidity was observed in the flask. The cell suspension was adjusted with nutrient broth to obtain turbidity equivalent to 6×10^9 CFU/ml.

Antimicrobial assay

All the prepared extracts were screened for antibacterial activity using the cup–plate agar diffusion method by measuring the zone of inhibition. The laminar airflow work bench was swabbed with 70% alcohol and UV was switched on for 30 min. All the reagents, media, inocula and glassware were transferred aseptically to the laminar airflow work bench. About 20–25 ml of sterilized nutrient agar medium was poured while hot into each sterilized Petri plate. The Petri plates were left for cooling and solidification of the medium. Inoculum (0.1 ml) was added on the surface of solidified agar media using a sterile pipette and spread

over the entire surface using a sterilized L-shaped glass rod. Quadrate wells on the surface of agar media were created with a stainless-steel borer of 8 mm diameter. The Petri dishes were suitably marked with the name of the microorganism, date of inoculation, name of extract and drug concentration. Two wells were filled with the same concentration of each extract and two other wells with the same concentration of a standard drug (ciprofloxacin) in each of the plates; three such plates were prepared for each test. After diffusion of drug, the Petri plates were incubated at 37°C for 24 h. A simultaneous negative control (medium without drug and without inoculums) and a positive control (medium without drug, but with inoculums) were prepared and placed in an incubator at 37°C for 24 h and then observed for bacterial growth and zone of inhibition [7,18].

Anti-inflammatory activity

Carrageenan-induced rat paw oedema anti-inflammatory model

Oedema was induced in rats to evaluate the anti-inflammatory potential of the prepared extracts. Wistar rats of either sex weighing between 100 and 150 g were used. The animals were starved overnight, with water provided *ad libitum*. The extracts and standard were dissolved in water (or dispersed in a 1% agar solution) and administered orally. Thirty minutes later, the rats were challenged by a 0.1 ml of 1% w/v solution of carrageenan on the planter surface of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed into the water column of a plethysmometer to measure the paw volume. The paw volume was measured immediately after the carrageenan injection and then at intervals of 30 min, 1, 2, 3 and 4 h. Rats were divided into six groups of five rats in each group and treated as follows:

Group 1: Control group, which received normal saline at an oral dose of 5 ml/kg.

Group 2: Standard group, which received diclofenac sodium at a dose of 50 mg/kg.

Group 3: Test group, which received an aqueous extract at a dose of 500 mg/kg.

Group 4: Test group, which received an alcoholic extract at a dose of 500 mg/kg.

Group 5: Test group, which received a hydroalcoholic (50%) extract at a dose of 500 mg/kg.

Group 6: Test group, which received a chloroform extract at a dose of 500 mg/kg.

The increase in paw volume at 4 h was calculated as percentage compared with the volume measured immediately after the injection of carrageenan for each animal. The difference in the average volume between the treated animals and the control groups was calculated for each treatment [23].

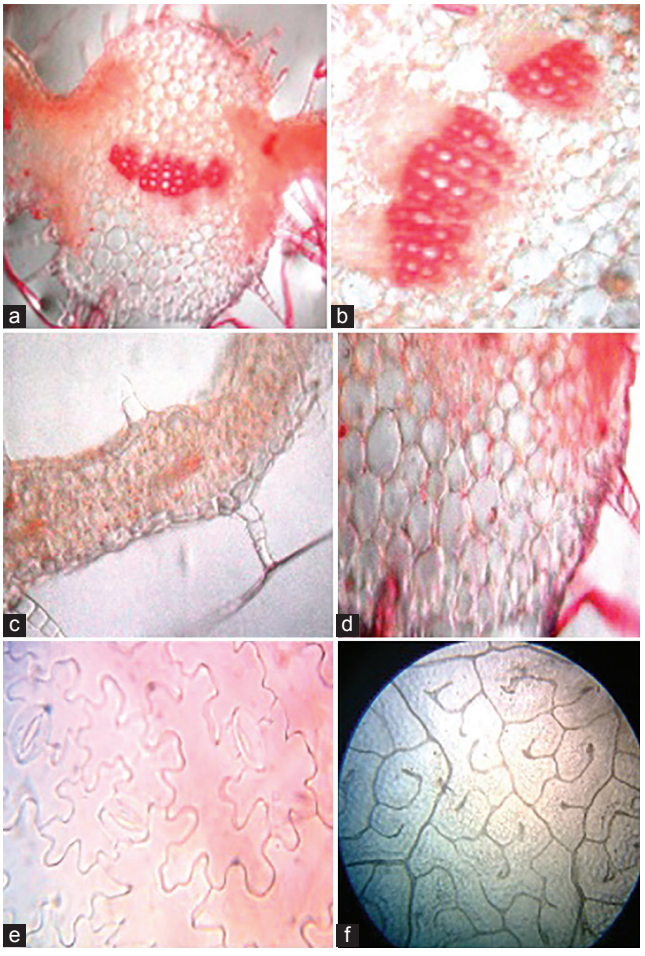
Results and discussion

Microscopy

Leaves

The transverse section of the leaf showed the epidermis, on both surfaces, covered with cuticle, glandular and covering trichomes. The epidermal layer was followed by collenchymatous cell layers at both the upper and the lower side (Fig. 1a and d). The midrib was composed of a large central vascular bundle and two lateral ones (Fig. 1b). The lamina had single-layered palisade cells and spongy

Figure 1



Microscopic characteristics of *Vernonia cinerea* leaf. (a) Petiole section showing vascular bundles, collenchymatous cells and trichomes. (b) Magnified view of the vascular bundle. (c) Leaf lamina showing the epidermis, palisade cells and multicellular trichomes. (d) Collenchymatous region with multicellular trichomes, (e) anisocytic stomata, (f) vein-islet and vein terminations.

parenchyma below palisade cells, which indicates the dorsiventral nature of a leaf (Fig. 1c). Anomocytic stomata (Fig. 1e) were present on both surfaces of leaves; vein islets and vein terminations are shown in Fig. 1f. The powder of leaves showed the presence of parenchymatous cells, epidermal cells, vessels, trichomes and fibres (Fig. 2a and b).

Stem

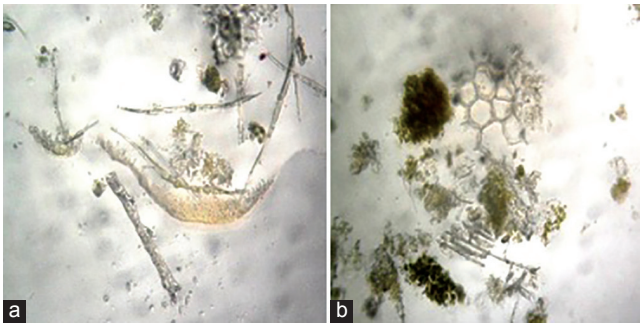
The transverse section showed a single-layered epidermis, covered with cuticle and multicellular trichomes (Fig. 3b). The cortex contained thin-walled parenchymatous cells, followed by few layers of collenchymatous cells between the epidermis and the parenchymatous cortex, radial arrangement of vascular bundles and a single-layered endodermis (Fig. 3b–d). The pith was composed of hexagonal to polygonal, thin-walled parenchymatous cells (Fig. 3a). A few parenchymatous cells were observed containing calcium oxalate crystals. The powdered stem showed the presence of cork cells (Fig. 4c), parenchymatous cells (Fig. 4d), trichomes, fibres and vessel (Fig. 4a and b).

Root

The transverse section of the root showed cork, consisting of tangentially elongated, thick-walled cells filled with reddish-brown contents. The secondary cortex was composed of thin-walled, parenchymatous cells (Fig. 5a). The secondary phloem contained scattered vascular bundle and medullary rays (Fig. 5b). Root powder was found to contain vessels, both simple and spiral (Fig. 6a), fibres (Fig. 6b), starch grains and aleurone grains (Fig. 6c and d).

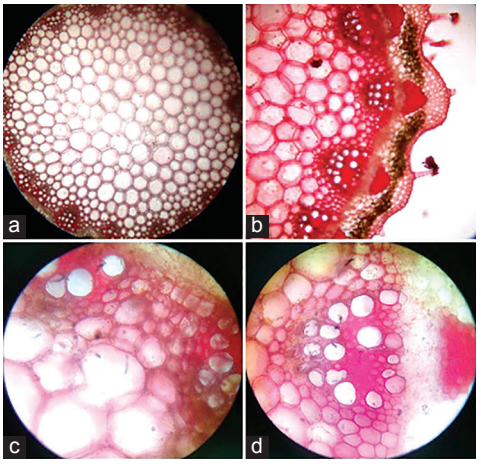
Staining with reagents indicated the presence of starch, lignified tissues and absence of calcium oxalates

Figure 2



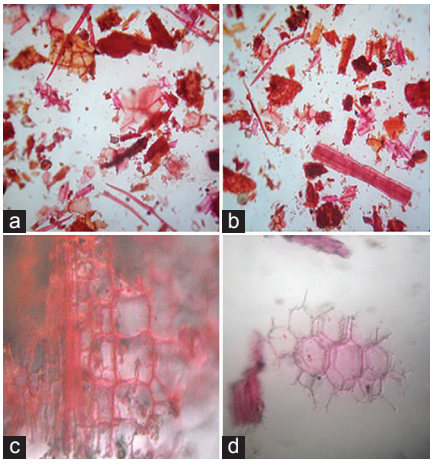
Powder characteristics of *Vernonia cinerea* leaf: (a) vessels, trichomes and fibres; (b) parenchymatous cells, epidermal cells, fibres and trichomes.

Figure 3



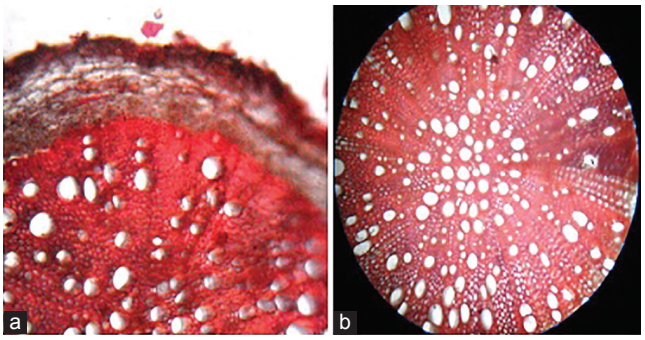
Microscopic characteristics of *Vernonia cinerea* stem: (a) radial arrangement of the vascular bundle; (b) parenchymatous cells, vasular bundles, trichomes and cork layer; (c) parenchymatous cells between vascular bundles; (d) vascular bundles.

Figure 4



Powder characteristics of *Vernonia cinerea* stem: (a) trichome and parenchymatous cells; (b) fibres and vessels; (c) cork cells; (d) parenchymatous cells.

Figure 5



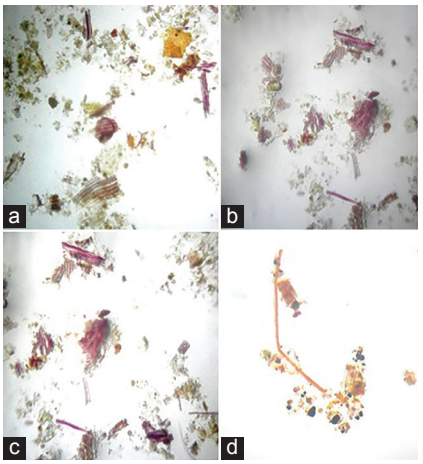
Microscopic characteristics of *Vernonia cinerea* root: (a) cork and cortex layers; (b) scattered vascular bundle and medullary rays.

Table 1 Standardization parameters as per World Health Organization guidelines

Parameters	% w/w		
	Leaf	Stem	Root
LOD	13.33 ± 0.89	10.33 ± 1.29	09.27 ± 0.44
Total ash values	13.83 ± 0.99	06.08 ± 1.00	03.39 ± 0.16
Acid-insoluble ash	01.44 ± 0.27	0.44 ± 0.08	0.33 ± 0.11
Water-soluble ash	06.23 ± 0.26	03.05 ± 0.70	02.22 ± 0.18
Sulphated ash	07.33 ± 0.22	01.95 ± 0.18	01.55 ± 0.30
Extractive value in water	15.73 ± 0.06	15.48 ± 0.26	11.10 ± 0.23
Extractive value in hydroalcoholic solvent	17.03 ± 0.11	08.03 ± 0.65	–
Extractive value in alcohol	18.76 ± 0.11	03.52 ± 0.17	06.25 ± 0.38
Extractive value in chloroform	03.21 ± 0.22	0.99 ± 0.16	0.41 ± 0.04
Extractive value in petroleum ether	01.43 ± 0.04	0.59 ± 0.07	0.94 ± 0.02

LOD, Loss on drying.

Figure 6



Powder characteristics of *Vernonia cinerea* root: (a) vessels, both simple and spiral; (b) fibres and vessels; (c) aleurone grains and vessels; (d) fibres and starch grains.

Table 2 Phytochemical testing of *Vernonia cinerea* extracts

Phytoconstituents	Chemical tests performed	Plant part	Tests found positive				
			Petroleum ether extract	Chloroform extract	Ethanol extract	Hydroalcoholic extract	Water extract
Carbohydrates	MSH, FHG, IDN	Leaf	MSH	MSH, FHG, IDN	MSH, FHG	MSH, FHG	MSH, FHG
		Stem	MSH	MSH, FHG, IDN	MSH	MSH	MSH, IDN
		Root	MSH	MSH, FHG, IDN	MSH	[a]	MSH
Alkaloids	DGF, MYR, MQI	Leaf	–	DGF, MYR, MQI	DGF, MQI	DGF, MQI	DGF
		Stem	MYR, MQI	–	–	–	–
		Root	–	–	DGF, MYR	[a]	DGF, MYR
Steroids	SKK	Leaf	–	SKK	SKK	SKK	SKK
		Stem	SKK	SKK	SKK	SKK	SKK
		Root	SKK	SKK	–	[a]	–
Proteins	BUT, NHD, MLN	Leaf	–	NHD	BUT, NHD	BUT, NHD	BUT, NHD
		Stem	–	–	NHD	–	BUT, NHD, MLN
		Root	–	–	–	[a]	–
Saponins	FOM	Leaf	–	–	–	FOM	FOM
		Stem	FOM	–	–	–	–
		Root	–	–	–	[a]	–
Volatile oils	SDN	Leaf	–	–	–	–	–
		Stem	–	–	–	–	–
		Root	SDN	–	SDN	[a]	–
Glycosides	BTG, MBTG, KKL, LGL	Leaf	MBTG, KKL, LGL	MBTG, KKL, LGL	–	MBTG, KKL, LGL	MBTG, KKL, LGL
		Stem	MBTG, KKL, LGL	MBTG, KKL	MBTG, KKL, LGL	LGL	LGL
		Root	–	–	–	[a]	–
Tannins and phenolic compounds	FRC, LAT, GTN	Leaf	–	–	FRC, LAT	FRC, LAT	FRC, LAT
		Stem	LAT, GTN	LAT, GTN	LAT, GTN	LAT, GTN	FRC, LAT, GTN
		Root	–	–	–	[a]	–
Flavonoids	SND	Leaf	–	SND	SND	SND	SND
		Stem	–	–	–	–	–
		Root	–	–	–	[a]	–
Coumarins	FAN, SAV	Leaf	FAN, SAV	–	–	–	–
		Stem	FAN, SAV	FAN, SAV	FAN, SAV	FAN	–
		Root	–	–	FAN	[a]	

—, absent; BTG, Borntrager; BUT, Biuret; DGF, Dragendorff's; FAN, $FeCl_3$+HNO_3; FHG, Fehling; FOM, foam; FRC, ferric chloride; GTN, gelatin; IDN, iodine; KKL, Killer Killani; LAT, lead acetate; LGL, Legal; MBTG, mod. Borntrager; MLN, Millon's; MQI, Marqui's; MSH, Molish's; MYR, Mayer's; NHD, ninhydrin; SAV, NaOH+UV; SDN, Sudan III; SKK, Salkowski; SND, Shinoda; [a]Study not performed.

in stem, leaf and root of *V. cinerea*, but tannins were present in the stem and absent in the leaf and root.

World Health Organization standardization parameters

Total ash, acid-insoluble ash, water-soluble ash and sulphated ash were estimated and the results are presented in Table 1.

Fluorescence studies with different solvents and powder were carried out and no fluorescence was observed in them [24].

Standardization parameters and extractive values by petroleum ether, chloroform, ethanol, ethanol (50%) and water were determined (Table 1). Phytochemical screening and chromatographic profiles of the extracts of leaf, stem and root in different solvents were carried out (Tables 2–4).

The presence of glycosides, tannins, proteins and coumarins was found in both the stem and the leaf, whereas the root and the leaf showed the presence of alkaloids and terpenes. Flavonoids and resins were found only in the leaf. Steroids were also observed in the whole plant.

Table 3 Chromatographic profile of *Vernonia cinerea* stem and root extract in various solvents

Phytoconstituents	Solvent system	Detecting reagent	Plant part	Water extract		Alcohol extract		Hydroalcoholic extract		Petroleum ether extract		Chloroform extract	
				Spots	Colour	Spots	Colour	Spots	Colour	Spots	Colour	Spots	Colour
Alkaloids	Methanol : ammonia (200 : 3)	UV	Stem	–	–	–	–	–	–	0.33, 0.38	Yellow	–	–
			Root	0.82, 0.94	Yellow	0.74	Yellow	[a]	[a]	0.63	Yellow	–	–
		Hager's reagent	Stem	–	–	–	–	–	–	–	–	–	–
			Root	–	–	–	–	[a]	[a]	–	–	–	–
Glycosides	Ethyl acetate : chloroform (90 : 10)	UV	Stem	0.76	Yellow	0.76	Yellow	–	–	0.77	Yellow	0.76	Yellow
			Root	–	–	–	–	[a]	[a]	–	–	–	–
Tannins	Acetic acid : chloroform (1 : 9)	UV	Stem	0.52	Yellow	0.19	Yellow	–	–	0.76, 0.89	Yellow	0.88	Yellow
			Root	–	–	–	–	[a]	[a]	–	–	–	–
		Vanillin + sulphuric acid	Stem	–	–	0.19	Light yellow	0.09, 0.79	Light yellow	–	–	–	–
			Root	–	–	–	–	[a]	[a]	–	–	–	–
Proteins	Butanol : acetic acid : water (4 : 1 : 5)	UV	Stem	–	–	–	–	–	–	0.91	Pink	0.93	Pink
			Root	–	–	–	–	[a]	[a]	–	–	–	–
		Ninhydrin reagent	Stem	–	–	0.15	Pink	0.16	Pink	–	–	–	–
			Root	–	–	–	–	[a]	[a]	–	–	–	–
Coumarins	Butanol : acetic acid : water (4 : 1 : 5)	UV	Stem	–	–	0.56, 0.69	Pink	–	–	–	–	–	–
			Root	–	–	–	–	[a]	[a]	–	–	–	–
Terpenes	Chloroform : benzene (1 : 1)	UV	Stem	–	–	–	–	–	–	–	–	–	–
			Root	–	–	–	–	[a]	[a]	–	–	–	–
		Alcoholic ferric chloride	Stem	–	–	–	–	–	–	0.4, 0.58	Blue	0.59	Blue
			Root	0.35	Blue	0.31	Blue	[a]	[a]	0.12, 0.64, 0.80	Blue	0.64	Blue

–, absent; UV, ultraviolet; [a]Study not performed.

Table 4 Chromatographic profile of *Vernonia cinerea* leaf extract in different solvents

Phytoconstituents	Solvent system	Detecting reagent	Water		Hydroalcoholic		Alcohol		Petroleum ether		Chloroform	
			Spot	Colour	Spot	Colour	Spot	Colour	Spot	Colour	Spot	Colour
Alkaloids	Chloroform : methanol (95 : 5)	UV	0.29	Blue	0.31, 0.46	Green	0.31, 0.46	Green	–	–	0.27, 0.40	Red
		Hager's reagent	0.29	Brown	0.31, 0.46	Brown	0.31, 0.46	Brown	–	–	0.27, 0.40	Brown
Glycosides	Ethyl acetate : methanol : water (81 : 11 : 8)	UV	0.51, 0.66	Blue	0.51, 0.94	Green	0.51, 0.94	Green	0.51	Blue	–	–
Tannins	Alcohol : chloroform (1 : 9)	UV	0.38, 0.69	Green	–	–	0.72, 0.92	Light green	–	–	0.52	Green
		Vanillin and sulphuric acid	0.38, 0.69	Green	0.72, 0.92	Light green	0.58, 0.31	Green, blue	–	–	0.40, 0.45, 0.72, 0.92	Dark green, light green
Steroids	Chloroform : acetic acid : ethanol (65 : 30 : 05)	UV	0.58	Blue	0.58	Green	0.47	Red	–	–	–	–
		UV and ammonia	0.29	Blue	0.31	Blue	0.61	Blue	–	–	0.38, 0.77	Blue
Proteins	Butanol : acetic acid : water (4 : 1 : 5)	UV	–	–	–	–	–	–	–	–	–	–
		Ninhydrin reagent	0.41	Red	0.47	Red	0.47	Red	–	–	0.88	Green
Flavonoids	Ethyl acetate : formic acid : glacial acetic acid : water (100 : 11 : 11 : 26)	UV	0.57	Blue	0.61	Blue	0.31, 0.46	Green	–	–	0.76	Blue
Resins	Petroleum ether : diethyl ether (75 : 25)	UV	–	–	–	–	0.31, 0.46	Brown	–	–	–	–
Terpenes	Butanol : acetic acid : water (4 : 1 : 5)	UV	–	–	–	–	0.51, 0.94	Green	–	–	–	–
		Bromine water	0.43	Green	0.47	Red	–	–	–	–	–	–

–, absent; UV, ultraviolet.

Antimicrobial assay

S. aureus was used to perform an antimicrobial assay on *V. cinerea* extracts. *V. cinerea* extracts were comparatively more active against Gram-positive bacteria than Gram-negative bacteria [25]. Alcoholic and chloroform leaf extracts of *V. cinerea* showed a zone of inhibition measuring 20.00 ± 0.00 and 19.33 ± 0.33 mm, respectively. Alcoholic and chloroform stem extracts of *V. cinerea* showed a zone of inhibition of 21.00 ± 0.57 and 21.00 ± 0.57 mm, respectively, whereas the standard drug, ciprofloxacin, showed a zone of inhibition measuring 26.66 ± 1.20 mm. Chloroform and alcoholic extracts of *V. cinerea* leaves and stem possess antibacterial activity against *S. aureus*, but their potency seemed to be less than that of the standard drug ciprofloxacin (Table 5).

Table 5 Antibacterial activity of *Vernonia cinerea* chloroform and alcoholic extracts

Plant parts	Tested extract	Zone of inhibition (mm) (n = 6)
Leaf	Chloroform	19.33 ± 0.33
	Alcoholic	20.00 ± 0.00
Stem	Chloroform	21.00 ± 0.57
	Alcoholic	21.00 ± 0.57
Standard drug	Ciprofloxacin	26.66 ± 1.20

Anti-inflammatory activity

Inflammation is a process that is accompanied by the local release of chemical mediators that include histamine, 5-HT, bradykinin and eicosanoids. The inflammatory response in rheumatoid arthritis is manifested by an acute inflammatory exudate of neutrophils, leucocytes in the synovial space and chronic inflammation of the synovial tissues [26]. It is evident that carrageenan-induced paw oedema in rats is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT in the early stage, followed by kinin release and then prostaglandin in the later phase [27]. Thus, it may be suggested that its anti-inflammatory activity in extract is possibly backed by its anti-5-HT activity, which is responsible for the same. Carrageenan-induced paw oedema in rats was used to study the anti-inflammatory activity. The standard drug, diclofenac sodium, and chloroform extract showed 11.11% inhibition of inflammation, whereas alcoholic

Table 6 Mean ± SEM and inhibition of inflammation measured after oral administration of various extracts of *Vernonia cinerea*

Groups	Treatment of dose	Paw volume (before carrageenan injection) (ml)	Paw volume (0 min) (after carrageenan injection) (ml)	Inflammation (ml) [inflammation inhibition (%)]				
				30 min	1 h	2 h	3 h	4 h
1	Negative control	0.88 ± 0.04	0.90 ± 0.04	1.18 ± 0.04	1.36 ± 0.07	1.16 ± 0.04	0.95 ± 0.04	0.90 ± 0.04
2	Diclofenac sodium	0.69 ± 0.05	0.71 ± 0.05	0.86 ± 0.02 (27.11)**	1.08 ± 0.06 (20.58)*	0.91 ± 0.01 (21.55)*	0.84 ± 0.02 (11.57)**	0.80 ± 0.02 (11.11)**
3	Aqueous extract	0.79 ± 0.08	0.81 ± 0.08	1.06 ± 0.14 (10.16)	1.16 ± 0.16 (14.7)	0.97 ± 0.07 (16.37)	0.86 ± 0.07 (9.47)*	0.83 ± 0.06 (7.77)*
4	Alcoholic extract	0.73 ± 0.18	0.75 ± 0.06	0.91 ± 0.03 (22.88)*	0.96 ± 0.132 (29.41)**	0.86 ± 0.09 (25.86)	0.78 ± 0.07 (17.89)	0.75 ± 0.06 (16.66)
5	Hydroalcoholic extract	0.77 ± 0.18	0.78 ± 0.18	1.14 ± 0.18 (3.38)	1.10 ± 0.14 (19.11)	0.90 ± 0.07 (22.41)**	0.82 ± 0.13 (13.68)	0.78 ± 0.13 (13.33)
6	Chloroform extract	0.79 ± 0.03	0.80 ± 0.04	0.92 ± 0.04 (22.03)*	1.14 ± 0.09 (16.17)	0.95 ± 0.06 (18.10)	0.84 ± 0.03 (11.57)	0.80 ± 0.04 (11.11)**

*Significant difference at $P < 0.01$ versus the control; no significant difference at $P < 0.001$ versus the control; one-way ANOVA, followed by Dunnett's *t*-test; **Significant difference at $P < 0.01$ versus the control and $P < 0.001$ versus the control; one-way ANOVA, followed by Dunnett's *t*-test.

and hydroalcoholic extracts inhibited inflammation by 16.66 and 13.33% after 4 h, respectively (Table 6).

Many sesquiterpenes were found to have anti-inflammatory activity [28]. Thus, the anti-inflammatory activity may be because of the terpenoids that are present in the extract [29]. Further, the phenolic constituents of *V. cinerea* were effective inhibitors of the oxidative burst of activated polymorphonuclear leucocytes and may therefore also contribute towards the anti-inflammatory activity [30]. The results confirmed the traditional use of *V. cinerea* as an anti-inflammatory drug.

Conclusion

The present study provides us information on the microscopic features, chemical constituents, inorganic content, soluble fraction of drug in different solvents and anti-inflammatory activity of *V. cinerea*. Results from microscopic studies with different plant parts may aid in the correct identification and standardization of a plant or its parts. Furthermore, adulteration and differentiation between species may also be feasible by characterization of different plant parts. Phytochemical tests and thin-layer chromatography aid identification of different phytoconstituents present in different extracts. Phytochemical screening and the chromatographic profile of the stem and leaf showed the presence of glycosides, tannins, proteins and coumarins, whereas the root and leaf showed the presence of alkaloids and terpenes. In addition, steroids were also observed in the whole plant. Flavonoids and resins were found only in the leaf. Good results have been obtained for anti-inflammatory activity and antimicrobial assay when tests were performed with *V. cinerea* extracts. This indicated that some potent anti-inflammatory phytoconstituents may be identified and isolated from *V. cinerea*.

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Conflicts of interest

There are no conflicts of interest.

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