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Pharmacochemical Characterization, FT-IR and Antibacterial Activity of *Vernonia Cinerea* Less.

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ABSTRACT

The present study has been carried out to evaluate the pharmacochemical characterization and *in vitro* antibacterial activity of the whole plant extracts of *Vernonia cinerea.* Physicochemical parameters (Ash value and extractive value; fluorescence analysis) and phytochemical analysis were done by using the standard methods. FT-IR analysis of *V. cinerea* was carried out.The petroleum ether, chloroform, acetone, methanol and ethanol extracts were tested against *Pseudomonas aeruginosa*, *Streptococcus pyogenes, Streptococcus faecalis* and *Salmonella paratyphi* by the agar disc diffusion method. The total ash value of whole plant of *V. cinerea* is 11.34%. The extractive value of water is more than in the solvents investigated. Preliminary photochemical screening of whole plant showed the presence of alkaloid, anthraquinone, coumarin, phenol tannin, glycoside, xanthoprotein, and sugar. From the spectral data of FT-IR, presence of C=O, C-H, C=O, C-O, C-C, were identified. The whole plant extracts of *V. cinerea* showed potent antibacterial activity against both Gram positive and negative bacteria. The pharmacochemical characterization will be helpful to study the active principles using modern techniques in the later part of this work.

Keywords: *Vernonia cinerea, in vitro,* phytochemical screening, fluorescence analysis, *Streptococcus,* disc diffusion method.

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INTRODUCTION

Plants provide a variety of resources that contribute to the fundamental needs of human such as food, clothing and shelter. Among plants of economic importance, medicinal and aromatic plants have played a vital role in alleviating human sufferings [1]. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts [2]. Plants have the ability to synthesize secondary metabolites to defend them against their predators. Some of these compounds turn out to have beneficial effects towards human diseases [3]. Secondary metabolites are highly varied in structure, many are phenolic aromatic substances or oxygen substituted derivatives [4]. Many herbs and spices used by human yield useful medicinal compounds [5].

Vernonia cinerea Less. (Family: Asteraceae) is a terrestrial annual erect herb. It grows up to 80cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation. It is located especially in different Asian countries such as India, Bangladesh and Nepal. Stems are rounded solid hairy. Leaves are alternate spiral, elliptic and the length is more than 2 cm long/wide. Flowers are bisexual grouped together in a terminal head [6]. It has many therapeutic uses in different traditional medicine of the world. Different parts of the plants are of different therapeutic values to mention a few it could be used as antimalarial, astringent, anthelmintic, anti-diarrhoel and anti-viral activity. It is commonly known as sahdevi or little iron wood [7]. Root Decoction is used in the treatment of diabetes mellitus [8]. Stem is used in the treatment of human breast cancer [9].

Hence, the present investigation is an attempt of determination of pharmacochemical characterization, FT-IR analysis and antibacterial activity of different extracts of *V. cinerea* whole plant.

MATERIALS AND METHODS

Collection and processing

The whole plant of *Vernonia cinerea* Less. were collected from V. O. Chidambaram College campus, Thoothukudi, Tamil Nadu respectively. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Determination of physicochemical parameters

Determination of physicochemical parameters, such as ash and extractive values were done following the methods of Kalpanadevi *et al* [10], Mohan *et al* [11] .The behavior of the powdered leaf with different chemical reagents was studied and the fluorescence character was observed under UV light [12].

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Preparation of extracts for phytochemical screening and antimicrobial activity

Freshly collected whole plant of *V. cinerea* were dried in shade, and then coarsely powdered separately in a willey mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for phytochemical screening and antibacterial activity.

Phytochemical screening

All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures with little modification [13-15,].

Test for alkaloids

Mayer's test

To the powder, two ml of Mayer's reagent was added; a dull white precipitate reveals the presence of alkaloids.

Test for Terpenoids (Noller's Test)

To 1 ml extract with tin (one bit) and thionyl chloride (1 ml) were added. Appearance of pink colour indicates the presence of terpenoids.

Test for Steroids

Libermann-Burchard's test

The powder was dissolved in two ml of chloroform in a dry test tube. Ten drops of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green, indicates the presence of steroid.

Test for Coumarin

To 1 ml of extract, 1ml of 10% sodium hydroxide was added. The presence of coumarin is indicated by the formation of yellow colour.

Test for Tannin

The test solution was mixed with basic lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

Test for Saponin

The test solution was shaken with water. Copious lather formation indicates the presence of saponin.

Test for Flavones (Shinadow's Test)

To a few mg of the powder, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of red colour shows the presence of flavonoids.

Test for Anthraquinones (Borntrager's test)

The powder/extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet in the aqueous layer after shaking indicates the presence of anthraquinone.

Test for Phenols

To 1 ml of the extract, 2ml of distilled water was added followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Test for Protein

Millions test

Two drops of freshly prepared Millions reagent was added to the powder and heated. Formation of white precipitate indicates the presence of proteins.

Test for Sugar

Fehling's test

To the powder, equal quantity of Fehling's solution A and B were added and on heating, formation of brick red precipitate indicates the presence of sugar.

Test for fixed oil

The powder was gently scrubbed on filter paper formation of grease spot indicates the presence of fixed oil.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 − 400 cm^{-1}[16].

Antimicrobial activity

Antimicrobial study was carried out by disc diffusion method [17] against the pathogens viz *Pseudomonas aeruginosa, Staphylococcus pyogens, Streptococcus faecalis* and *Salmonella paratyphi.* A loopful of bacteria was taken from the stock culture and dissolved in 0.1 ml of saline. All the tests were done by placing the disc (^mm diameter) impregnated with (20 mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram Positive and Gram Negative bacteria.

Respective solvents without plant extract served as negative control. Standard antibiotic of tetracycline (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37° C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone sand antibacterial activity against the pathogenic bacteria was recorded. The experiments were repeated in triplicate and the results were documented.

RESULT AND DISCUSSION

Powder analysis of the drug

Physicochemical constants (Ash and Extractive values)

The result of the ash and extractive values of whole plant of *V. cinerea* are depicted in table 1. The total ash content of the powdered whole plant of *V. cinerea* is 11.34%. The extractive value of water is more than that in other solvents investigated in the present study.

These ash values are indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. A high value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. Samples have more water soluble ash than acid insoluble ash. The results of various types of ash and extractive values may provide a basis to identify the quality and purity of the drug.

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Table 1: Ash and extractive values of the powdered whole plant of *Vernonia cinerea*[a]

Ash values		
S.No	**Type of Ash**	**% of Ash**
1.	Total ash value of powder	11.34±0.46
2.	Water soluble ash	5.26±0.11
3.	Acid insoluble ash	2.14±0.07
4.	Sulphated ash	12.58±0.37
Extractive values		
S. No	**Name of the Extract**	**Extractive value (%)**
1.	Petroleum ether	7.67±0.13
2.	Benzene	8.34±0.08
3.	Chloroform	8.12±0.04
4.	Acetone	8.56±0.11
5.	Methanol	9.13±0.26
6.	Ethanol	9.26±0.13
7.	Water	10.04±0.56

[a] All values are mean of triplicate determination ± standard error.

Fluorescent analysis

The results of fluorescent analysis of whole plant of *V. cinerea are* shown in table 2. The powder from the whole plant of *V. cinerea* emitted dark green under day light and short UV and black in long UV light. The whole plant powder of *V. cinerea* shows the characteristic fluorescent green colour treated with aqueous 1N NaOH, 1N alcoholic NaOH, benzene and 50% HNO_3 under short UV light.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [18,19].

Table 2: Fluorescence analysis of whole plant of *Vernonia cinerea*

S.No	Group	Stretching Frequency (cm^{-1})
1	O-H	3321.19
2	C-H (Alkyl)	2923.88
3	C-H (Carbonyl compounds)	2854.45
4	C=C	1647.10 & 1637.45
5	C-CHO (aldehydes)	1400.22
6	C=O	1731.96
7	C-O	1112.85
8	C-Cl	609.45

Preliminary phytochemical analysis

The result of preliminary phytochemical screening of different extracts of *V. cinerea* whole plant is presented in table 4. The methanol and ethanol extracts of the whole plant of *V. cinerea* shows the presence of alkaloid, anthraquinone, coumarin, flavonoid, phenol, quinone steroid, tannin, glycoside, xanthoprotein and fixed oil.

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Different chemical compounds were detected whole plant of *V. cinerea* extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because of the pharmacological activity of any plant is usually traced to a particular compound [20].

Alkaloids act as antioxidant and immunomodulatory agent. Alkaloids are known to exhibit emetic amoebicides, expectorant, anaesthetics, antipyretics, analgesics, anthelmintic. Flavonoids elicit a wide range of therapeutic activities such as antihypertensive, antibiotic, antimicrobial, antitumor activities. They have wide pharmacological activities and have been used since past as tanning agents and they possess astringent, antiinflammatory, antidiarrhoeal, antioxidant and antimicrobial activities.

FT-IR analysis

The FT-IR spectral studies of *V. cinerea* whole plant gave the following characteristics absorption peaks as shown in Table 3 and Figure 1.

From the spectral data, presence of C=O, C-H, C=O, C-O, C-C, were identified. These bonding structures are responsible for the presence of alkyl group, methyl group, alcohol, ethers, esters, carboxylic acid and anhydrides. The more intense band occurring 3321.19 cm^{-1}, 2923 cm^{-1}, 2854 cm^{-1}, 1112 cm^{-1} and 609.45 cm^{-1} corresponding to OH/N-H, C-H, group C-O, and C-Cl/C-S stretching/ bending vibrations respectively indicate the presence of aminoacids, alkenes, ethers, organic halogen compounds and carbohydrates in plants. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcers, jaundice, head ache, stomatitis, hemicranias, fever, pain in liver, treatment of edema and rheumatic joint pains. Amines, amides and aminoacids are the main groups of protein synthesis and herbs serves as herb oil and hair tonic. Sulphur derivative compounds were used as disinfectant and dermal cream. Protein plays a vital role in physiology of living organisms.

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Table 3: FT-IR spectroscopic data of whole plant of *Vernonia cinerea*

Treatment	Colour		
	Under Ordinary Light	Under UV light	
		245nm	365mm
Powder as such	Dark Green	Dark Green	Black
Powder + 1NAqueous NaoH	Green	Fluorescent Green	Dark Green
Powder + 1NAlcoholic NaoH	Dark Green	Fluorescent Green	Fluorescent Green
Powder + 1 N HCl	Green	Dark Brown	Dark Brown
Powder + Con. HCL	Dark Green	Black	Green
Powder + Con.H_2So_4	Dark Green	Light Brown	Fluorescent Green
Powder+ 50% H_2So_4	Dark Green	Green	Fluorescent Green
Powder +Con. HNO_3	Brown	Red	Fluorescent Green
Powder +40% NaOH + 10% Lead Acetate	Light green	Green	Fluorescent Green
Powder + Acetic acid	Dark Green	Dark Green	Dark Green
Powder+ Ferric Chloride	Green	Dark Green	Fluorescent Green
Powder+ Chloroform	Dark Green	Brown	Brown
Powder + Benzene	Dark Green	Fluorescent green	Fluorescent green
Powder + Petroleum ether	Dark Green	Dark Green	Dark Green
Powder+ Methanol	Light Green	Dark Green	Dark Green
Powder+ Ethanol	Dark Green	Dark Green	Dark Green
Powder+ acetone	Dark Green	Dark Green	Dark Green
Powder+ NH_3	Dark Green	Dark Green	Fluorescent green
Powder+HNO_3+ NH_3	Brown	Brown	Brown
Powder+50% HNO_3	Brown	Fluorescent green	Brown

Antibacterial activity

Petroleum ether, chloroform, acetone, methanol and ethanol extracts of whole plant of *V. cinerea* were tested for their antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus pyogens, Streptococcus faecalis* and *Salmonella paratyphi.*

All the extracts of whole plant of *V. cinerea* exhibited antibacterial activity against all the tested pathogenic bacteria. Petroleum ether extract of whole plant of *V. cinerea* showed the maximum activity against *Streptococcus pyogens* (30mm) when compared with standard antibiotic tetracycline (23mm) (Figure 2).

Chloroform extract of whole plant of *V. cinerea* exhibited maximum antibacterial activity against *Salmonella paratyphi* (15mm) whereas acetone extract of whole plant of *V. cinerea* showed the maximum activity against *Streptococcus faecalis* (Figure 2).

Methanol and ethanol extracts of whole plant of *V. cinerea* showed the maximum activity against *Pseudomonas aeruginosa* (16mm and 21mm respectively) (Figure 2). Among the solvent tested, petroleum ether and ethanol extracts possessed higher antibacterial activity.

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P. aeruginosa cause skin and soft tissue infections, skeletol infections, endocarditis, pneumonia and eye infections. *S. pyogenes* is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. *S. faecalis* can cause endocarditis, as well as bladder, prostrate and epididymal infections; nervous system infections are less common. Paratyphoid fevers are a group of enteric illnesses caused by *S. paratyphi.* The results of the present study suggest that the whole plant of *V. cinerea* can be used to treat infections, pneumonia etc. Based on the present study, it is concluded that the whole plant of *V. cinerea* contains various bioactive compounds like alkaloid, phenol and flavonoid with high degree of antimicrobial activity against various pathogens.

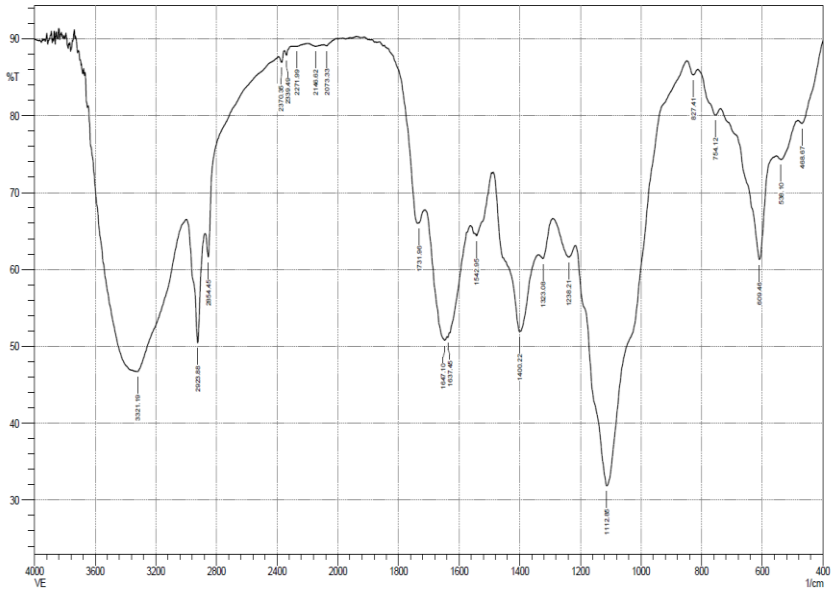

Figure 1: FT-IR Spectrum of *Vernonia cinerea* whole plant

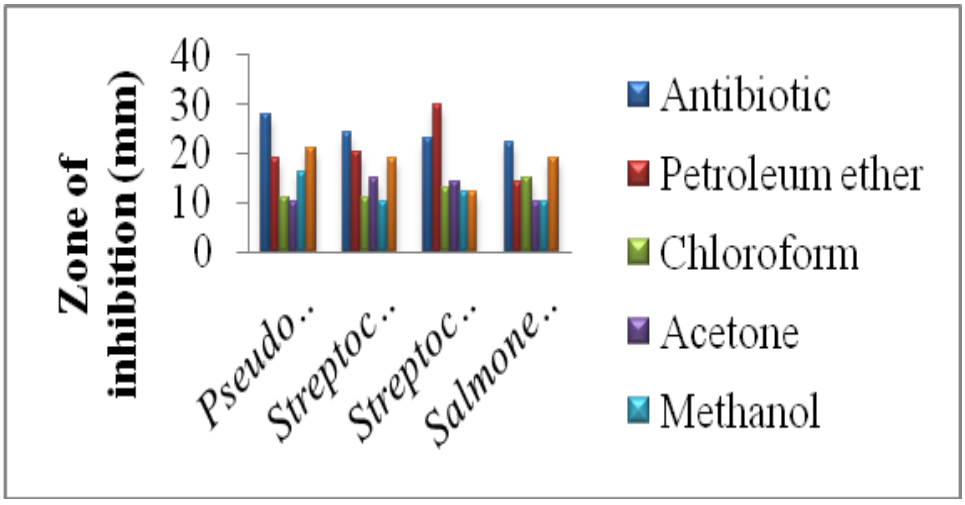

Figure 2: Antibacterial activity of different extracts of *Vernonia cinerea*

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Table 4: Preliminary phytochemical screening of different extracts of *Vernonia cinerea*

Bioactive components	Nature of extract				
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloids	+	+	+	+	+
Anthroquinones	+	+	+	+	+
Catechin	-	-	-	-	-
Coumarin	+	+	+	+	+
Flavonoids	-	-	-	+	+
Phenols	+	+	+	+	+
Quinones	-	-	-	+	+
Saponins	+	+	+	-	-
Steroids	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	-	-	-	-	-
Glycosides	+	+	+	+	+
Xanthoprotein	+	+	+	+	+
Sugar	+	+	+	+	+
Fixed oil	+	+	+	+	+

CONCLUSION

This study has demonstrated the pharmacochemical characterization and antibacterial activities of various extracts of whole plant of *V. cinerea.* Petroleum ether was better solvent for extraction of antibacterial substances compared to the other solvents. The study revealed that the whole plant of *V. cinerea* contain a considerable quantity of phenolic and flavonoid compounds that were found to be the major contributor for their antibacterial activities. Thus, the *V. cinerea* can be considered as an easily accessible source of natural antibacterial agents and may be considered in future to replace synthetic preservatives in food and pharmaceutical products. Further studies are needed to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress, cardiovascular arrests, inflammation, cancer and diarrhoea.

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