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Phytochemical Analysis and Evaluation of in Vitro Antioxidant Potential of Vernonia Cinerea

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Vernonia cinerea, a medicinal plant widely used in traditional medicine, possesses a rich phytochemical profile and potential antioxidant properties. This study aims to analyze the phytochemical composition and evaluate the in vitro antioxidant potential of different extracts of Vernonia cinerea.

Phytochemical screening of successive extracts revealed the presence of alkaloids, carbohydrates, phytosterols, flavonoids, phenolic compounds, tannins, and proteins in varying concentrations. The methanolic and aqueous extracts exhibited a higher abundance of bioactive compounds compared to petroleum ether, benzene, chloroform, and acetone extracts.

The in vitro antioxidant activity was assessed using DPPH, ABTS, and nitric oxide scavenging assays. The results demonstrated that both methanolic and aqueous extracts exhibited dose-dependent free radical scavenging activity. The aqueous extract showed the highest scavenging potential in DPPH (61.34% at 200 µg/ml), ABTS (56.47% at 200 µg/ml), and nitric oxide assays (50.96% at 200 µg/ml), indicating a significant antioxidant capacity. Furthermore, the total antioxidant activity showed a linear correlation with concentration, comparable to the standard ascorbic acid.

These findings suggest that Vernonia cinerea contains bioactive phytochemicals with potent antioxidant potential, making it a promising candidate for further pharmacological and nutraceutical applications.

Keywords: Antioxidant activity, Vernonia cinerea

1. Introduction

Medicinal plants have been used for centuries as sources of therapeutic agents, providing valuable compounds for the treatment of various ailments. Among these, Vernonia cinerea, commonly known as the little ironweed, has gained significant attention due to its diverse pharmacological properties. This plant belongs to the Asteraceae family and is widely distributed in tropical and subtropical regions. Traditional medicine has long recognized the potential of Vernonia cinerea in treating inflammatory conditions, fever, infections, and other health issues. Recently, scientific interest in this plant has increased, particularly regarding its phytochemical composition and antioxidant potential [1-2].

An imbalance between the generation of ROS and the body's capacity to use antioxidants to mitigate their negative effects leads to oxidative stress. Superoxide radicals, hydrogen

peroxide, and hydroxyl radicals are examples of ROS that can harm lipids, proteins, and DNA in cells, resulting in a number of degenerative diseases. By scavenging free radicals and strengthening the body's defenses, antioxidants—both enzymatic and non-enzymatic—play a critical role in shielding cells from oxidative damage. [3-4]

Natural antioxidants derived from plants have gained considerable attention due to their ability to mitigate oxidative stress with minimal side effects. Vernonia cinerea has been reported to possess strong antioxidant activity attributed to its high flavonoid and phenolic content. Evaluating the in vitro antioxidant potential of Vernonia cinerea extracts is essential for validating its traditional uses and exploring its potential for pharmaceutical and nutraceutical applications [5-6].

The primary objective of this study is to elucidate the phytochemical composition and evaluate the antioxidant potential of Vernonia cinerea extracts. By identifying key bioactive constituents and assessing their ability to neutralize oxidative stress, this research aims to validate the traditional use of Vernonia cinerea in herbal medicine. Additionally, the findings may provide a foundation for further pharmacological investigations, leading to the development of plant-derived antioxidants as potential therapeutic agents [7-8].

2. Pharmacognostical Study

- Materials

Leica DMLS microscope attached with Leitz MPS 32 camera, stoppered conical flask, stage micrometer, alcohol (95%), grinding mixer, petroleum ether, watch glass, chloral hydrate solution, sonicator (Lequitron), silica crucible, chloroform water, digital electronic balance (Dhaus Corp), hydrochloric acid, UV apparatus, sudan red-III, ashless filter paper (Whatman no.44), acetone, compound microscope, phloroglucinol, benzene, glass slide, magnetic stirrer, chloroform, cover slip, hot air oven (Osworld), water, petridish, ruthenium red, sodium hydroxide, and glycerin.

- Source of plant

The plant materials were collected from in and around Bhopal, Madhya Pradesh, India during the month of August. The plant was authenticated and sample submitted [10].

- Anatomical study

To remove the coloring material, free-hand pieces of Vernonia cinerea leaves were cleaned with chloral hydrate, stained with phloroglucinol and HCl, placed on a glycerin-sanitized glass slide, and covered with a cover slip (Johansen, 1940). The sections were first seen at low power (10 X) and then at high power (40 X).

- Photomicrography

A Leica DMLS microscope with a Leitz MPS 32 camera attached was used to take the microphotographs [10].

- Powder analysis

The microscopic characteristics of Vernonia cinerea leaf powder were investigated. After passing through filter number 60 and being cleaned with chloral hydrate to get rid of coloring material, the powder was examined under a microscope to look for calcium oxalate crystals and other characteristics. For the lignified structures, such as stone cells, the cleared powder was subsequently stained with phloroglucinol in the presence of hydrochloric acid and examined under a microscope as previously mentioned [11-12]. (Brain andTurner, 1975b; Kokate, 1986a; Trease and Evans, 2005)

- Physicochemical constants (The Indian Pharmacopoeia, 1996; Quality Control Methods for Medicinal Plant Materials, WHO, 1998).

A) Ash values

The residue left over after burning is typically regarded as the crude drug's ash content [13]. In addition to inorganic materials added for the aim of adulteration, it represents the inorganic salts that are naturally present in the medicine and adhere to it.

The residue left over after burning is known as total ash. The portion of total ash that is insoluble in diluted hydrochloric acid is known as acid insoluble ash [14]. The portion of total ash that dissolves in hot water is known as water-soluble ash.

- Total ash

In a tarred silica crucible, around 2g of the powdered medication was precisely weighed. A thin layer of the drug powder was applied to the crucible's bottom. Until the crucible was carbon-free, it was burned at a temperature of no more than 450°C. After cooling, the crucible was weighed. Until a consistent weight was noted, the process was repeated [15]. With reference to the air-dried medication, the percentage of the total ash was computed three times.

- Acid insoluble ash

For five minutes, the ash that was collected according to the procedure for calculating total ash was heated with 25 milliliters of hydrochloric acid. Filtration was used to gather the insoluble ash on ashless filter paper, which was then cleaned with hot water. After being moved into a silica crucible that had been tarred, the insoluble ash was fired, allowed to cool, and then weighed. Until a consistent weight was noted, the process was repeated. The air-dried medication was used to calculate the proportion of acid-insoluble ash [16-17].

- Water soluble ash

25 milliliters of water were used to boil the ash that was produced as specified in the total ash determination for five minutes. After being collected on ashless filter paper, the insoluble material was cleaned with hot water [18]. After being moved into a silica crucible that had been tarred, the insoluble ash was fired at a temperature of no more than 450°C. Until a consistent weight was noted, the process was repeated [19]. The weight of the whole amount of ash was deducted from the weight of the insoluble substance. Water-soluble ash was defined as the weight difference. Using the air-dried medication as a reference, the proportion of water-soluble ash was determined.

B) Extractive values

The amount of the medicine that is extracted by solvents is measured by its extractive value [20]. Water-soluble, ethanol-soluble, and ether-soluble extractives are examples of extractive value. Unless otherwise instructed, maceration is used to extract value.

- Water soluble extractive

100 ml of chloroform water (1:99) was used to macerate 4 g of previously weighed air-dried powdered material in a glass stoppered flask. After six hours of vigorous shaking, it was left to stand for eighteen hours [21]. In order to prevent solvent loss, it was filtered quickly. In a tared, flat-bottomed petri dish, 25 milliliters of filtrate were evaporated till dry, dried at 105°C, cooled in a desiccator, and weighed. Using the air-dried medication as a reference, the percentage of water-soluble extractive was determined.

- Ethanol soluble extractive

With the exception of replacing the solvent (water) with ethanol, the identical process for the water-soluble extractive was used [22].

- Ether soluble extractive

With the exception of replacing the solvent (water) with ether, the identical process for the water-soluble extractive was used [23].

C) Determination of moisture content

A surplus of water in medicinal plant materials will promote the growth of microorganisms, the presence of insects or fungi, and degradation after hydrolysis [24]. As a result, water content limits ought to be established for each type of plant. This is particularly crucial for materials that readily absorb moisture or degrade when exposed to water.

D) Loss on drying (LOD)

A flat weighing bottle that had been previously dried and tarred was used to precisely weigh 2–5 grams of the produced air-dried material. After being equally spread, the sample was put in the oven to dry [25]. The bottle was taken out of the oven, quickly closed, and allowed to cool to room temperature before being weighed. The drying process involved heating the bottle to between 100 and 105°C. Unless the test technique specified otherwise, the experiment was repeated until two consecutive weigh-ins did not deviate by more than 5 mg. After drying, the weight reduction was computed.

E) Foaming index

When an aqueous decoction is agitated, saponins included in many therapeutic plant materials can produce persistent froth [26]. A foaming index was created to gauge how well plant materials and their extracts foamed when aqueously decocted.

Procedure

After precisely weighing one gram of the coarse powder, it was put into a 500 ml conical flask with 100 ml of boiling water and kept there for 30 minutes. After cooling and filtering the mixture, enough water was added to a 100 ml volumetric flask to reach the desired volume.

Ten stoppered test tubes were filled with the aforementioned decoction in sequential quantities of 1, 2, 3, and up to 10 ml. The volume of the liquid was then adjusted in each tube with water to reach 10 ml. After stopping the tubes and shaking them lengthwise for 15 seconds at two frequencies per second, they were left to stand for 15 minutes, and the height of the foam was measured [27-28].

The following is how the results were evaluated:

- The foaming index was less than 100 if the foam height in each tube was less than 1 cm.
- The index was calculated using the volume of the plant material decoction in tube (a) if a height of 1 cm of foam was measured in any tube. For a more accurate result, an intermediate dilution was made if this tube was the first or second in a series.
- The foaming index exceeded 1000 when the foam height in each tube exceeded 1 cm. In this instance, a new set of dilutions of the decoction had to be used to determine the outcome.

Foaming Index = 1000/a

Where a, is the volume in ml of the decoction used for preparing the dilution in the tube where foaming was observed.

F) Tannin Content

By interacting with proteins to generate water-soluble molecules that are resistant to proteolytic enzymes, tannins are chemicals that can transform animal hide into leather [29]. This technique is employed as a medicinal agent because it is known to be astringent when administered to living tissue.

Procedure

A conical flask was filled with roughly 2 g of the drug powder after it had been weighed. After adding 150 ml of water, it boiled for 30 minutes. After cooling, it was moved to a 250 ml volumetric flask and filled with water to reach the desired volume. After filtering the solution, 50 milliliters of the extract were evaporated until dry, and the drying process was continued at 105 degrees Celsius until a consistent weight was achieved, as shown in T1 [30-31]. This allowed for the determination of the total amount of material extractable into water. It was established how much plant material was extractable into water and how much remained unbound to the hide powder after its inclusion. About 80 cc of the aforementioned extract was mixed with about 6 g of hide powder. After 60 minutes of shaking, the mixture was filtered. A consistent weight (T2) was achieved by drying 50 milliliters of the filtrate at 105 degrees Celsius. Six grams of hide powder were mixed with eighty milliliters of water, agitated for sixty minutes, and then filtered to test the solubility of the powder. As previously said, 50 milliliters of clear filtrate were evaporated until they were dry, and this was recorded (T0). Thus, the following formula was used to determine the amount of total tannins.

Quantity of tannins (%) = $[T_1 – (T_2 + T_0)] \times 500 / w$

Where w, is the weight of leaf powder in grams

G) Determination of swelling index

A 25 ml glass stoppered measuring cylinder was filled with 1 g of the powdered material. 25 ml of water was then added, and the mixture was shaken well every 10 minutes for one hour. It was shaken and then left to stand at room temperature for three hours [32]. It was measured how many milliliters (ml) the plant material, including sticky stuff, occupied.

Phytochemical study

Materials, instruments and chemicals

TLC chamber, sprayer, desiccators, distillation equipment, test tubes, soxhlet apparatus, and digital electronic weighing scale. All of the chemicals and reagents utilized came from Central Drug House Pvt. Ltd [33]. (CDH), New Delhi; Ranbaxy Fine Chemicals Ltd., Punjab; Fischer Inorganics & Aromatics Ltd., Madras; and NICE Chemicals Ltd., Cochin.

Preliminary phytochemical screening (Kokate, 1986b; Harborne, 1988).

Preparation of extracts

A) Petroleum ether extract

50 g of coarsely dried Vernonia cinerea leaf powder was extracted using petroleum ether at 60–80°C for 12 hours using a hot extraction method (soxhlet). Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum [34].

B) Benzene extract

After the drug's extract from petroleum ether was dried, the marc was extracted using benzene using a heat extraction method (soxhlet) for 12 hours [35]. Following the extraction process, the solvent was extracted by distillation and then concentrated in a vacuum.

C) Chloroform extract

Following benzene extraction, the drug's remaining extract marc was dried and extracted using chloroform using a hot extraction method (soxhlet) for 12 hours. Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum [36].

D) Acetone extract

Following the chloroform extraction, the drug's remaining marc was dried and extracted using acetone using a hot extraction method (soxhlet) for 12 hours [37]. Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum.

E) Ethanolic extract

After the acetone extraction was dried, the drug's extract marc was removed using 95% ethanol using a hot extraction method (soxhlet) for 12 hours. Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum.

F) Aqueous extract (Chloroform: water – 1:99)

After the ethanolic extraction was dried and macerated with chloroform water, the drug's extract marc remained. Following the extraction process, the solvent was extracted using distillation and concentrated in a vacuum.

Studies on phytochemistry were conducted using the extract mentioned above. Each extract's

extractive values were determined and noted.

Phytochemical analysis of extract of Vernonia cinerea (Harborne, 1998; Khandelwal, 2001)

To determine the presence of different phytochemical ingredients, the following chemical tests were performed on several Vernonia cinerea extracts [38].

- Test for alkaloids

Two N HCl was used to dissolve the Vernonia cinerea extracts. After the mixture was filtered, three equal amounts of the filtrate were separated [39]. A few drops of Mayer's reagent were applied to one section, an equivalent amount of Dragendroff's reagent was applied to another, and an equal amount of Wagner's reagent was applied to the third.

- Test for saponins

The Frothing test was used to identify the presence of saponins [40]. After giving the Vernonia cinerea extracts a good shake with distilled water and letting them stand for ten minutes, the saponin content was categorized as follows: When there is no froth, saponins are absent; when there is stable foam that is greater than 1.5 cm, saponins are present.

- Test for carbohydrates

Molisch’s test, Fehling’s test, Benedict’s test

- Test for glycosides

Borntrager’s test, Modified Borntrager’s test

- Test for steroids

The presence of steroids was checked using the Liebermann-Burchard response. A few drops of concentrated H2SO4 were dropped down the test tube's sides after an acetic anhydride treatment was performed on a chloroform solution containing Vernonia cinerea extracts [41].

- Test for fixed oils and fats

Spot test, Saponification test

- Test for tannins and phenolic compounds

The extracts of Vernonia cinerea were treated with alcoholic ferric chloride ($FeCl_3$) reagent.

- Test for proteins and amino acids

Biuret test, Ninhydrin test

- Test for gums and mucilage

Precipitation with absolute alcohol, Molisch’s test

- Test for flavonoids

Shinoda test: The Shinoda test was used to estimate the content of flavonoids. Magnesium turnings and a few drops of strong HCl were added to the Vernonia cinerea extracts [42].

Analysis of extracts

Fluorescence analysis was performed on the petroleum ether, benzene, chloroform, acetone, alcohol, and chloroform water extracts under daytime and UV light (254 and 366 nm).

IN VITRO ANTIOXIDANT STUDY:

All life on Earth depends on oxygen to survive. About 5% of oxygen is univalently converted to oxygen-derived free radicals, such as superoxide, hydrogen peroxide, hydroxyl, and nitric oxide radicals, during the oxygen consumption process in a typical physiological and metabolic process [43]. Each human cell experiences over 10,000 oxidative hits each second as a result of the oxidative stress caused by all of these radicals, also referred to as reactive oxygen species (ROS). (Mondal et al, 2006).

An atom or molecule having one or more unpaired electrons, such as a superoxide anion radical, hydroxyl radical, nitric oxide, peroxyl radical, or alkoxyl radical, can be classified as a free radical [44]. Free radicals can exist on their own and destroy tissue through oxidative stress. Because they don't have unpaired electrons, non-radical oxidants like hydrogen peroxide and hypoclorous acid can also cause oxidative tissue damage. Lipid peroxidation is brought on by oxidative stress, which speeds up the generation of free radicals. As a result, various in vitro antioxidant models were applied to Vernonia cinerea extracts [45].

Nice Chemicals in Mumbai provided all of the analytical-grade chemicals and solvents. Gallic acid was purchased from Nice Chemicals in Mumbai, while 1,1-Diphenyl-2-picryl Hydrazine (DPPH), 2, 2-Azino bis (3-ethyl Benzo Thiazoline – 6-Sulphonic acid (ABTS), and quercetin were acquired from Sigma Chemicals in the United States. Sulfanilic acid, sodium nitroprusside, and naphthylethylene diamine hydrochloride were the additional compounds utilized [46]. Throughout the entire study, ascorbic acid (Ranbaxy Fine Chemicals Ltd.) was utilized as the standard.

Plant extracts

- Methanolic extract: Vernonia cinerea leaves were shade-dried, ground into a powder, and then roughly 100 g of the powder was extracted using methanol using a hot extraction method (soxhlet) for 72 hours. Following extraction, the solvent was extracted, distilled, and then concentrated in a vacuum [47].

- Aqueous extract (Chloroform: water – 1:99): Vernonia cinerea leaves were macerated with chloroform water for seven days after being shade-dried and ground into powder [48]. After filtering and solvent removal, it was vacuo-concentrated.

DPPH radical scavenging activity (Peihong et al, 2010):

Principle

The antioxidant produces 1, 1-Diphenyl-2-picryl Hydrazine by a reaction with the stable free radical DPPH. The absorbance at 517 nm was used to measure the capacity to scavenge the free radical, DPPH [49].

(DPPH: 2,2-diphenyl-1-picryl hydrazyl)

R=-OH or -NO2

1(4-Hydroxyphenyl)-1-phenyl-2-picryl hydrazine
1(4-Nitrophenyl)-1-phenyl-2-picryl hydrazine

l_{max} 517 nm

Procedure

The stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical's ability to scavenge radicals was used to evaluate the plant extract's antioxidant activity. The DPPH content in MeOH was maintained at 300 µM. MeOH was used to dissolve the extracts [50]. In a 96-well microliter plate, 10 µl of each extract solution was left to react with 200 µl DPPH for 30 minutes at 37 °C. A microplate reader was used to measure each solution's decrease in absorbance at 490 nm following incubation. The standard was ascorbic acid.

ABTS assay (Rajaei et al, 2010):

Principle

[2, 2-azino bis (3-ethyl benzo thiazoline-6-sulphonic acid)] is the chemical name for ABTS. At 734 nm, the test compound's elimination of free radicals using ABTS is detected.

Procedure

Stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared for the ABTS (2,20-Azinobis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt) assay. The working solution was then made by combining the two stock solutions in equal amounts and letting them react for 16 hours at room temperature in a dark environment [51]. After that, methanol was added to the diluted ABTS_+ solution to get an absorbance of 1.00 ± 0.02 units at 734 nm using a spectrophotometer. For every assay, a new ABTS_+ solution was made. Ascorbic acid standard solutions at varying concentrations were made [52]. For two hours in a dark environment, the 200 ml of crude and refined extracts were left to react with 4 ml of ABTS_+. At 734 nm, the absorbance was then measured.

Nitric oxide scavenging activity (Nagda et al., 2009):

The Griess Illosvoy reaction can be used to assess whether sodium nitroprusside in aqueous solution at physiological pH spontaneously produces nitric oxide, which then reacts with oxygen to produce nitrite ions [53]. 0.5 ml of extract at different concentrations was combined with 2 ml of 10 mM sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH 7.4), and the combination was incubated at 25°C for 150 minutes. Five minutes were spent at room temperature after 0.5 ml of the incubated mixture was removed and mixed with 1.0 ml of sulfanilic acid reagent (33% in 20% glacial acetic acid) [54]. Lastly, 1.0 ml of 0.1% w/v

naphthylethylenediamine dihydrochloride was combined and allowed to sit at room temperature for half an hour. A spectrophotometer was used to measure the absorbance at 540 nm. The following formula was used to determine the nitric oxide radical scavenging activity:

% Inhibition = $[(A_0 – A_1) / A_0 \times 100]$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract/Standard.

Total antioxidant capacity (Prieto et al, 1999):

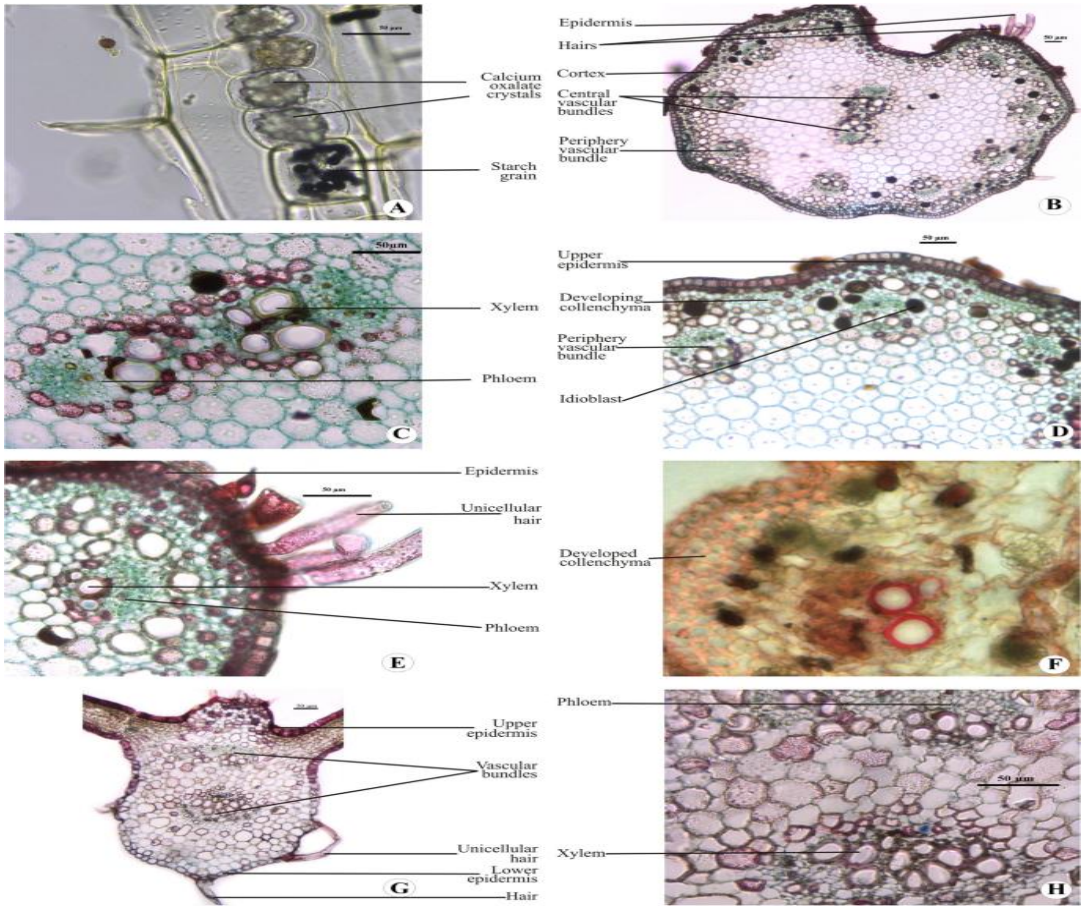
With minor adjustments, the method described by Prieto et al. was used to determine total antioxidant capacity [55]. To put it briefly, 100 µg of extract and 100 µg of ascorbic acid (as standard) were taken in 0.1 ml of alcohol and individually mixed with 1.9 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in an Eppendorf tube. After being sealed, the tubes were incubated for 90 minutes at 95°C in a thermal block. Following the samples' cooling to room temperature, each sample's aqueous solution's absorbance at 695 nm was measured in comparison to a blank. 1.9 ml of reagent solution and the proper volume of the same solvent used for the sample were included in a standard blank solution, which was incubated in the same manner as the other samples [56]. Water-soluble antioxidant capabilities are reported as ascorbic acid equivalents for materials with unknown compositions. The standard ascorbic acid graph was used to compute ascorbic acid equivalents. Values are reported as ascorbic acid equivalents in µg per milliliter of extract, and the experiment was carried out in triplicate.

3. Results

Macroscopy

Grown as a weed, Vernonia cinerea L. (Asteraceae) is an annual herbaceous plant that is found all throughout India. It is a 1.3-meter-tall annual herb that stands upright, thin, and rarely branches. The stems are glandular and coarsely pilose. The leaves alternate, with the upper ones being reduced and sessile and the lower ones petiolating. They are roughly thickly and delicately hairy and range in length from 2 to 6 cm. The heads are tiny, measuring only 2.5 mm in diameter and 7 mm in length. Each head contains roughly 20 tubular blooms that are twice as long as the involucral bracts and are quite brilliant purple, pink, or white. The bristles of the pappus are 3–5 mm long. The spherical, almost ribless achenes are around 1.5 mm long.

Microscopy



Physicochemical constants of leaves of Vernonia cinerea

- Ash values

Ash values

Parameters	Value
Total ash	3.7 %w/w
Acid insoluble ash	0.65 %w/w
Water soluble ash	2.37 %w/w
Sulphated ash	2.80 %w/w

- Extractive values

Table Extractive values

Parameters	Value
Water soluble extractive	16.7 %w/w
Ethanol soluble extractive	5.6 %w/w
Ether soluble extractive	3.2 %w/w

- Moisture content

Loss on drying at 105 ºC: 15.45 %w/w

- Foaming index

Foaming index of stem powder: NIL

- Tannin content

Tannin content of stem powder: 9.0 % w/w

- Swelling index

Swelling index of stem powder: 3

Phytochemical study of stem of Vernonia cinerea

- Preliminary phytochemical screening

Successive solvent extraction

S. No.	Solvent	Color	Weight of the extract(g)
1.	Petroleum ether (60-80 °C)	Dull yellowish green	1.17
2.	Benzene	Dark greenish brown	1.24
3.	Chloroform	Dark green	1.73
4.	Acetone	Dull green	1.64
5.	Ethanol (95%)	Blackish green	4.23
6.	Chloroform:Water (1:99)	Dark Brown	5.60

Phytochemical analysis of different extracts (Qualitative chemical tests)

Qualitative chemical tests of successive extracts

Test	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Water
Alkaloids	-	-	+	-	+	+

Carbohydrates	+	-	-	-	+	+
Phytosterols	+	-	-	+	+	+
Fixed oils and fats	+	+	-	-	-	+
Saponins	-	-	-	-	-	-
Phenolic compounds and tannins	-	-	-	-	+	+
Proteins	-	+	-	+	-	+
Gums and mucilages	-	-	-	-	-	-
Flavonoids	-	-	-	+	+	+

(+ Present, - Absent)

In vitro antioxidant study:

Scavenging of DPPH free radical by various extracts

Concentration (µg/ml)	Methanolic extract % scavenging	Aqueous extract % scavenging	Standard (Ascorbic acid) % scavenging
5	12.36	16.95	37.69
10	21.82	26.17	56.21
25	30.72	35.32	80.57
50	38.63	42.76	88.55
100	46.79	49.80	92.09
200	57.38	61.34	95.22

Interaction of different extracts with ABTS radical

Concentration (µg/ml)	Methanolic extract % scavenging	Aqueous extract % scavenging	Standard (Ascorbic acid) % scavenging
5	9.60	13.53	28.67
10	17.76	22.32	45.51
25	25.85	31.41	65.32
50	33.41	40.76	77.67
100	44.65	47.91	85.45
200	53.45	56.47	89.76

Nitric oxide scavenging activity of various extracts

Concentration (µg/ml)	Methanolic extract % scavenging	Aqueous extract % scavenging	Standard (Ascorbic acid) % scavenging
5	1.19	1.10	4.83
10	2.25	2.38	18.06
25	8.37	6.19	48.22
50	21.26	18.80	68.13

100	40.20	36.18	82.78
200	52.09	50.96	88.33

Total antioxidant activity

Concentration (µg/ml)	Standard Absorbance
25	0.167
50	0.324
100	0.604
200	1.084
400	2.005
800	3.489

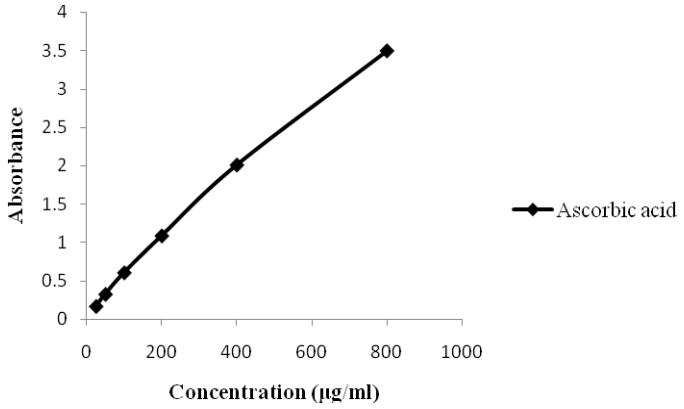


Figure-19: Standard plot of ascorbic acid

4. Conclusion

With the rising interest in natural antioxidants for health promotion and disease prevention, understanding the phytochemical and antioxidant properties of medicinal plants like Vernonia cinerea is of paramount importance. This study seeks to bridge the gap between traditional knowledge and scientific validation by systematically analyzing the bioactive components and their role in antioxidant mechanisms. The results will contribute to the growing body of evidence supporting plant-based therapeutics, paving the way for potential applications in pharmaceutical and nutraceutical industries.

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