Bioactive constituents of different solvent extracts of *Vernonia cinerea* (L.) whole plant and their pharmacological properties

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Abstract

The *Vernonia cinerea* plant is found all throughout India and is a species of the Asteraceae family. Folk medicine uses *Vernonia cinerea* (L.) as a treatment for a number of ailments. The plant is advised in cases of intermittent fever, filariasis, blisters, boils, etc. by the Indian Ayurvedic Pharmacopoeia. Therefore, the primary goals of the current work are to screen different phytochemicals for antioxidant and antimicrobial activities in various *Vernonia cinerea* extracts. *Vernonia cinerea's* phytochemical analysis revealed that its ethanol and chloroform extracts included alkaloids, flavonoids, tannins, terpenoids, glycosides, phenols, saponins, steroids, proteins, and carbohydrates. Alkaloid, Flavonoid, Tannin, Terpenoid, Glycoside, Phenol, and Steroid were all found in hexane extracts. The presence of these substances strongly suggests that V. cinerea has a variety of therapeutic characteristics. The 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), hydroxyl radical, superoxide radical, and ferric reducing antioxidant potential (FRAP) assays were used to measure antioxidant activity, with ascorbic acid serving as the reference antioxidant. It was discovered that the extract effectively scavenges free radicals. Particularly, the ethanol extracts demonstrated more DPPH and hydroxyl radical scavenging inhibition than the standard treatment. Using the agar diffusion technique, antimicrobial activity were evaluated against microorganisms. All of the extracts had a high level of antimicrobial activity against the pathogens that were subjected to the test, with zones of inhibition ranging from 7.0 to 20 mm. The findings of this study supported the traditional medical practice of using *V. cinerea* to treat a variety of diseases by demonstrating that the plant's solvent extracts have antimicrobial and antioxidant properties.

Keywords: Antioxidant activity, Asteraceae, DPPH, Microorganisms, *Vernonia cinerea*

1. Introduction

Ayurvedic medicine has been practised in India for more than 3,000 years. Two of the oldest Indian writers, Charaka and Susruta, were sufficiently knowledgeable about the characteristics of Indian medicinal herbs. The principles of nature, which contend that life is a synthesis of the senses, intellect, body, and soul, control the medical form. Ayurvedic remedies are becoming widely accepted due to this comprehensive approach (Neethu Vijayakumar and Gangaprasad, 2019).

Recently, the field of phytochemistry, sometimes known as plant chemistry, has emerged as a separate academic field that lies in the intersection of plant biochemistry and natural product organic chemistry. It addresses the immense range of organic compounds that plants produce and

accumulate, as well as their chemical compositions, biosynthesis, turnover, and metabolism, as well as their natural distribution and biological functions (Neethu Vijayakumar and Gangaprasad, 2019). Secondary metabolites are abundant in the medicinal plants that are utilised as traditional medicine across the world. Nowadays, there is a significant amount of study being done on various plant species and the medicinal principles they possess, which has revealed the traditional medicine used around the world. Phytochemicals found in plants have a range of bioactivities, including antioxidant, anti-inflammatory, and anticancer effects.

Free radicals that are detrimental to human body are scavenged by natural antioxidants found in plants. Any entity capable of independent life that has one or more unpaired electrons reacts with other molecules by either taking or donating electrons, and is involved in a variety of pathological diseases, is referred to as a free radical (Madhavi et al., 1996). Free radicals are chemical entities with an unpaired electron that play a significant role in human health and are helpful in the treatment of a number of ailments, including inflammation, lung damage, and cardiovascular issues. These free radicals are extremely unstable, and when their levels in the body are too high, they can harm cells and tissues and contribute to a number of disorders. Antioxidants of natural origin are therefore necessary since they can shield the body from illnesses brought on by free radicals (Updhaye et al., 2009; Mehta et al., 2019).

The spices and medicinal herbs are believed to be the plants that are most frequently examined for their therapeutic potential. Although plant antimicrobial compounds are widely employed in the design and development of novel medications for human treatment, they also hold promise for future plant disease controlling agents. Although the concept of using plant-based products as antimicrobial agents is not novel (Cowan, 1999), recent research in the field is garnering more attention. In order to treat infectious illnesses in light of the acquired pathogen resistance to antibiotics now available on the market, new options need be developed. The potential of plant extracts for the creation and design of novel antimicrobial agents should thus be investigated, since this might provide a remedy for the medical and phytopharmaceutical industries (Ahmed et al., 2015; Acharya et al., 2019).

Terrestrial annual erect plant *Vernonia cinerea* is a member of the asteraceae family. It may reach a height of 80 cm. Roadsides, open waste areas, dry grassy grounds, and perennial crops during planting are all possible places to find it. It is mostly found in many Asian nations including India, Bangladesh, and Nepal. *V. cinerea* is a significant medicinal plant used in the treatment of cancer, several gastrointestinal problems, and abortion (Yusuf et al., 1994). The goal of the current study was to analyse the phytochemical makeup of *Vernonia cinerea's* whole plant and assess its antimicrobial and antioxidant properties.

2. Materials and methods

2.1. Plant collection

The whole Vernonia cinerea plant was gathered in Tamil Nadu, India, at Tholayavattam, Kanyakumari District.

2.2. Sample preparation and Extraction

The whole Vernonia cinerea plant was cleaned with running tap water and then rinsed with distilled water to get rid of the dirt and dust that was on it. After being thoroughly cleaned, the leaves were shade dried and ground into a powder.

According to the kind of solvent (non-polar to polar = Hexane > Chloroform > Ethanol), about 250–300 ml of solvent is placed in the round bottom flask of the Soxhlet apparatus. In order to prevent the powdered sample from settling in the round bottom flask and the syphon

tube, cotton is used to plug the opening of the syphon or capillary tube. A cotton rag was used to roll up the powdered sample before putting it in the soxhlet thimble. Once flowing tap water has been poured into the condenser. The solvent is evaporated using an isomantle (heat source), with the temperature set at the solvents' desired heating points. Running the soxhlet equipment causes the extraction to be done fifteen times (24 hours for each solvent extracts). Crude solvent extracts of all species were kept at 4-5°C (refrigeration) until required.

2.3. Phytochemical screening

Alkaloids, saponin and glycosides (Ansari, 2006), flavonoids (Gul et al., 2017), tannin (Singh and Kumar, 2017), phenol (Mukherjee, 2002), carbohydrates (Brain and Turner, 1975), steroids (Indian Pharmacopoeia, 1996), terpenoids (Horbone, 1984) and Proteins (Vishnu Balamurugan et al., 2019) were identified in the whole plant extract using a conventional technique.

A standard procedure was used to determine the amounts of alkaloids, saponin and steroids (Devanaboyina et al., 2013), flavonoids, tannin and phenol (Naima Saeed et al., 2012), and terpenoids (Ghorai et al., 2012), carbohydrates (Roe, 1955), and glycosides (Solich et al., 1992), protein (Lowry et al., 1957) in ethanol, chloroform and hexane extracts.

2.4. Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of the fractions was measured in vitro by 2,2′ -diphenyl-1-picrylhydrazyl (DPPH) assay according to the standard method (Williams et al., 1995). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml of ethanol stored at 20°C until required. The working solution was obtained by diluting DPPH solution with ethanol and 3 ml aliquot of this solution was mixed with 1 ml of sample at various concentrations (100, 200 and 300 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared without any sample and scavenging activity was estimated based on the percentage of DPPH radical scavenging as the following equation.

$$Percentage\ of\ inhibition = \left[\frac{(Control\ OD - Sample\ OD)}{(Control\ OD)}\right] \times 100$$

Superoxide anion scavenging activity

The assay for superoxide anion radical scavenging activity was supported by riboflavinlight-NBT system (Beauchamp and Fridovich, 1971). 1 ml of extracts was taken at different concentrations (100, 200 and 300 µg/ml) and mixed with 0.1 ml of Riboflavin solution (20 µg), 0.2 ml of EDTA solution (12 mM), 0.2 ml of methanol and 0. 1 ml of Nitro-blue tetrazoliumn (0.5 mM) were mixed in test tube and reaction mixture was diluted up to 3 ml with phosphate buffer (50 mM). After 20 min of incubation at room temperature, the absorbance was measured at 560 nm. Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

$$Scavenging\ effect = \left[\frac{(Control\ OD - Sample\ OD)}{(Control\ OD)}\right] \times 100$$

Hydroxyl radical scavenging activity

The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L-1, pH 7.4), 0.2 mL of a sample of different concentrations (100, 200 and 300 µg/ml), 0.2 mL of EDTA (1.04 mmol L-1), 0.2 mL of FeCl3 (1 mmol L-1), and 0.2 mL of 2-deoxyribose (60 mmol L1). The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 mL of ascorbic acid (2 mmol L-1) and 0.2 mL of H2O2 (10 mmol L-1). After incubation at 37 °C for 1 h, 2 mL of cold thiobarbituric acid (10 g L-1) was added to the reaction mixture followed by 2 mL of HCl (25%). The mixture was heated at 100 °C for 15 min and then cooled down with water. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation on hydroxyl radicals (Halliwell and Arnoma, 1987). The scavenging percentage was calculated according to the following formula:

$$Scavenging\ effect = \left[\frac{(Control\ OD - Sample\ OD)}{(Control\ OD)}\right] \times 100$$

Ferric Reducing Antioxidant Potential (FRAP) Assay

The ferric reducing power of plant extracts was determined using a modified version of the FRAP assay (Stephanie et al., 2009). This method is based on the reduction, at low pH, of a colorless ferric complex (Fe^{3+}-tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants. The reduction is monitored by measuring the change of absorbance at 593 nm. The working FRAP reagent was prepared daily by mixing 10 volumes of 300 mM acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM hydrochloric acid with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of $FeSO_4 \times 7H_2O$.

All solutions were used on the day of preparation. One hundred microliters of sample solutions and 300 µL of deionized water were added to 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37°C in a water bath. Then, the absorbance of the samples was measured at 593 nm. A sample blank reading using acetate buffer was also taken. The difference between sample absorbance and blank absorbance was calculated and used to calculate the FRAP value.

In this assay, the reducing capacity of the plant extracts tested was calculated with reference to the reaction signal given by a Fe^{2+} solution. FRAP values were expressed as mmol Fe^{2+}/g of sample. All measurements were done in triplicate.

2.5. Antimicrobial activity

Test Organisms

The test microorganisms used for antimicrobial analysis Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger and Aspergillus flavus were purchased from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA) and the fugal strains are maintained on Sabouraud Dextrose Agar (SDA).

Antibacterial Test

The medium was prepared by dissolving 38 g of Mueller-Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure

at 121ºC for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Finally, The Sample or Sample loaded Disc was then placed on the surface of Mueller-Hinton Agar medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Mathabe et al., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm.

Antifungal assay

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method (Bauer et al., 1966). Fungal strains such as *Aspergillus niger, Aspergillus flavus* and *Candida albicans* were swabbed using sterile cotton swabs in SDA agar plate. Up to 40 µl of each concentration of the extract were respectively introduced in the sterile discs using sterile pipettes. The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres.

3. Results

3.1. Phytochemical constituents

The preliminary Phytochemical screening of the plant showed the presence of Alkaloid, Flavonoid, Tannin, Terpenoid, Glycoside, Phenol, Saponin, Steroid, Protein and Carbohydrate (Table 1).

Table 1. Results of preliminary phytochemical screening of various extracts of *Vernonia cinerea*

Phytochemical constituent	Solvent extracts of *Vernoniacinerea*		
	Hexane	**Chloroform**	**Ethanol**
Alkaloid	+	+	+++
Flavonoid	++	+++	+++
Tannin	+++	+++	+++
Terpenoid	+	+	+
Glycoside	++	+++	+++
Phenol	++	++	+++
Saponin	-	+++	+++
Steroid	+++	+++	+++
Protein	-	++	+++
Carbohydrate	-	++	+++

Figure 1 shows the quantitative phytochemical analysis of *Vernonia cinerea* using various extracts. The maximum quantity of tannin (750.24 ± 0.08 mg/g), Glycoside (500.12 ± 0.01 mg/g), Flavonoid (456.16 ± 0.13 mg/g), Steroid (417.26 ± 1.58 mg/g), Phenol (380.13 ± 0.13 mg/g), Saponin (380.33 ± 0.008 mg/g),

Alkaloid (348.42 ± 0.12 mg/g), Carbohydrate (255.44 ± 0.004 mg/g) and Protein (214.31 ± 0.004 mg/g) were found in the ethanol extract of *Vernonia cinerea*. Chloroform has the highest concentration of Saponin, Steroid, Flavonoid, tannin and Glycoside (385.82 ± 0.28, 348.55 ± 2.9, 304.36 ± 0.04, 304.35 ± 0.26 and 301.59 ± 1.72 mg/g). The presence of the highest quantity of Tannin (274.23 ± 0.21 mg/g), Steroid (211.84 ± 1.76 mg/g), Flavonoid (148.58 ± 1.72 mg/g) and Glycoside (104.11 ± 0.008) was found in the Hexane extract.

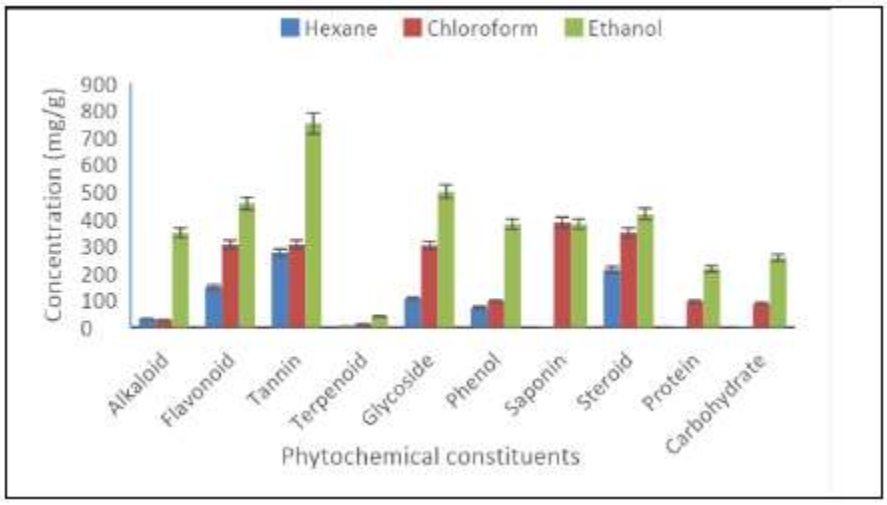

Fig. 1. Results of quantitative phytochemical screening of various extracts of *Vernonia cinerea*

3.2. Antioxidant Activity

Since the DDPH assay provides a simple method to test the antioxidant property, it was employed to measure the antioxidant activity of the plant extract. We looked at the antioxidant values (% of inhibition) of the crude solvent extracts. Whole plant extracts of ethanol, hexane, and chloroform have IC_{50} values of 163.496, 214.932, and 418.51 µg/ ml, respectively (Fig. 2). The ability of each extract to inhibit is greater than that of the reference medicine, ascorbic acid (458.875 µg/ ml).

The capacity of the superoxide radicals to degrade NBT may be used to gauge how much dissolved oxygen they produce. At three different concentrations (100, 200, and 300 µg/ml), all extracts were evaluated for their ability to scavenge superoxide. The results of this evaluation were compared and summarized in Figure 3. The capacity to inhibit is greatest for the ethanol extract (119.774 µg/ ml), followed by the chloroform (670.697 µg/ ml), hexane (916.962 µg/ ml), and the reference treatment, ascorbic acid (146.061 µg/ ml) extracts.

The ability of the extract and regular Ascorbic acid to inhibit hydroxyl radical is demonstrated by their scavenging of hydroxyl radicals. The outcomes are displayed in figure 2. Hexane, chloroform, ethanol, and the standard all had IC_{50} values in this test of 319.023, 326.64, 328.634, and 237.444 µg/ml, respectively (Fig. 4). All extracts showed antioxidant activity as determined by the FRAP technique (Fig. 5). The maximum inhibitory capacity is found in the ethanol extract (215.026 µg/ ml), which is higher than the chloroform (734.571 µg/ ml), hexane (484.817 µg/ ml), but not the ascorbic acid (173.727 µg/ ml) standard medication.

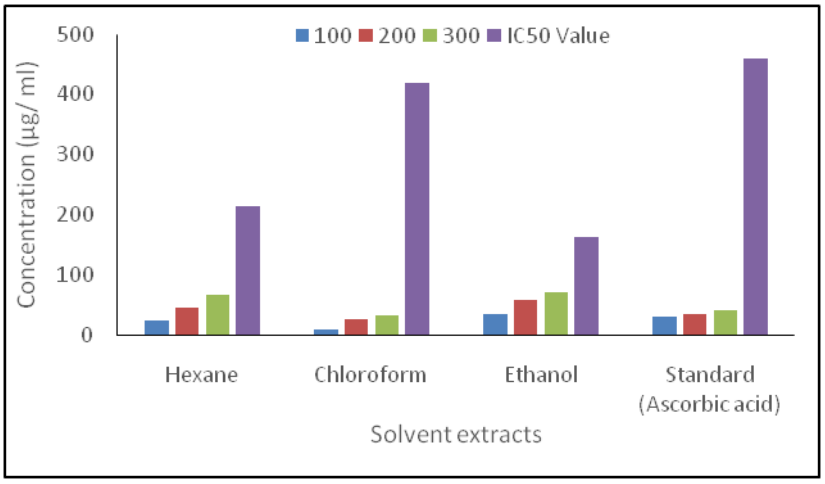

Fig. 2. DPPH radical scavenging activity of various extracts of *Vernonia cinerea*

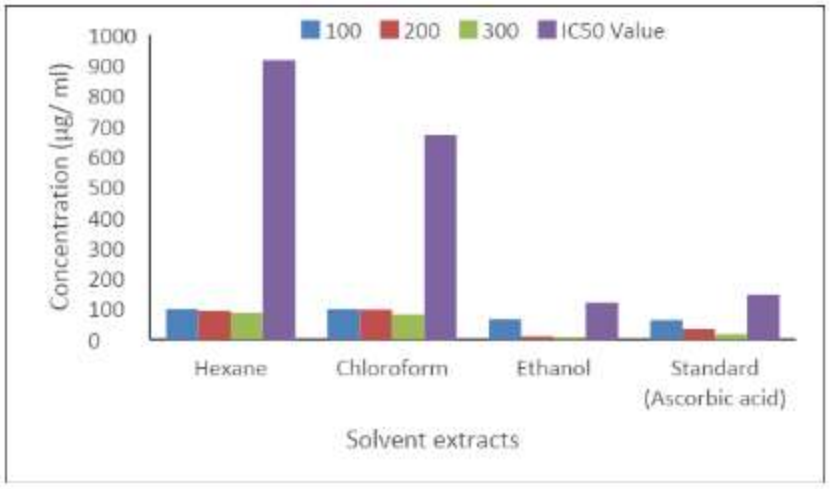

Fig. 3. Superoxide radical scavenging activity of various extracts of *Vernonia cinerea*

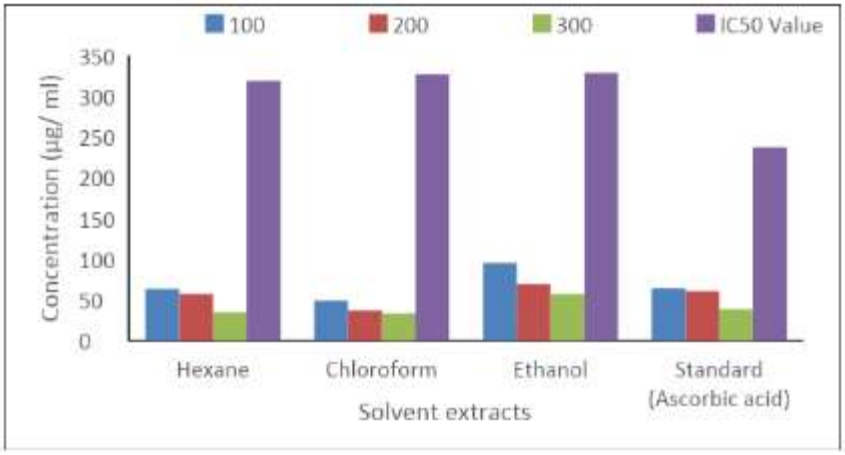

Fig. 4. Hydroxyl radical scavenging activity of various extracts of *Vernonia cinerea*

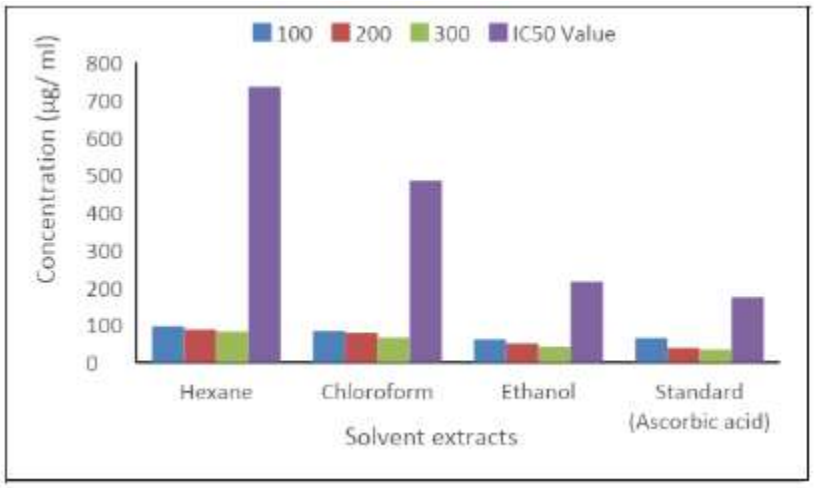

Fig. 5. Ferric Reducing Antioxidant Potential (FRAP) Assay of various extracts of *Vernonia cinerea*

3.3. Antimicrobial activity

The in vitro antimicrobial activities of the extracts of the plant were investigated separately using well diffusion method against six bacterial strains including three gram negative (Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris), three gram positive bacteria (Staphylococcus aureus, Bacillus subtilis and Enterococcus faecalis) and three fungi such as Aspergillus niger, Aspergillus flavus and Candida albicans. The existence or absence of an inhibitory zone and the zone's diameter were used to determine an extract's efficacy. In the current investigation, all of the examined extracts had a higher zone of inhibition against gram negative than gram positive (Fig. 5). All of the extracts exhibited a modest amount of fungi-inhibiting action.

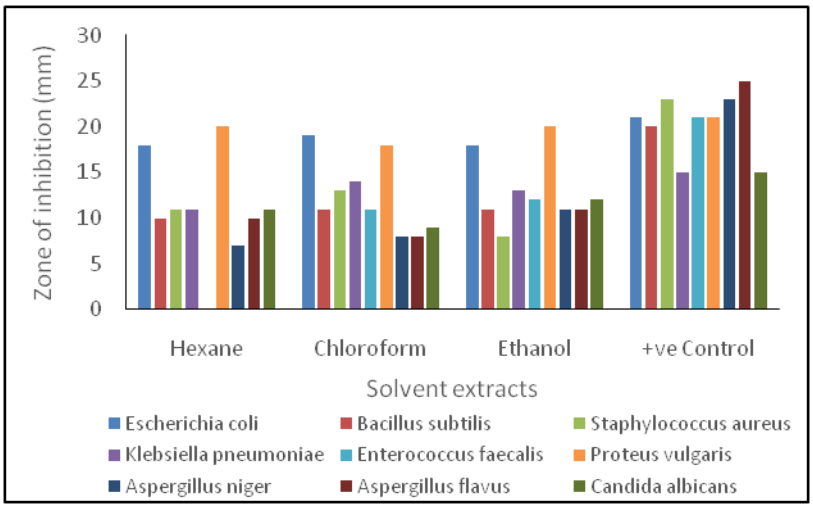

Fig. 5. Antimicrobial activity of three different solvent extracts of *Vernonia cinerea*

4. Discussion

The common people employ medicinal herbs, which are therapeutically effective, as a home cure for a variety of illnesses, including fever, the common cold, dysentery, and others. Especially in underdeveloped nations, herbal medicines constitute a crucial part of healthcare programmes. Some function in a plant's defence system is played by secondary metabolites. The extraction process's use of solvent aids in the subsequent isolation of botanical compounds from plant material (Neethu Vijayakumar and Gangaprasad, 2019).

Phytochemical research confirmed the presence of substances that are known to have physiological and therapeutic effects. The discovery of active chemicals benefits greatly from preliminary analysis. The primary source of natural antioxidants is plant phenolic compounds such flavonoids, phenolic acids, tocopherols, etc (Ali et al., 2008). Tannins bind to proline-rich proteins and prevent the creation of new proteins. Plants are known to produce flavonoids, which are hydroxylated phenolic compounds, in response to microbial infection. Flavonoids have been shown to exhibit antibacterial properties *in vitro* against a variety of pathogens Additionally, it was discovered that the plant extracts included saponins, which are known to have an anti-inflammatory impact (Just et al., 2005). Red blood cells can be precipitated and coagulated by saponins. The ability to create foam in aqueous solutions, hemolytic activity, cholesterol-binding abilities, and bitterness are some of the characteristics of saponins (Okwu, 2004). According to reports, steroids have antimicrobial qualities, and they are crucial substances because of their interactions with other substances like sex hormones (Raquel, 2007). For centuries, alkaloids have been used medicinally, and one of its common biological characteristics is their cytotoxicity (Nobori et al., 1994). Alkaloids have been shown to have analgesic, antispasmodic, and antibacterial effects by several researchers (Harborne, 1973; Okwu and Okwu, 2004). Many studies have shown that glycosides can reduce blood pressure (Nyarko and Addy, 1990). The study's findings so imply that the detected phytochemical compounds may be the bioactive components, and these plants are demonstrating their value as a source of bioactive substances with significant medical value. The plant material had alkaloids, flavonoids, tannins, terpenoids, glycosides, phenols, saponins, steroids, proteins, and carbohydrates, according to the phytochemical screening of the current study. Some ethnomedicinal plants' antimicrobial properties are attributed to flavonoids (Han et al., 2007). The detected phytochemical substances may be the bioactive ingredients, according to the findings of this study.

Antioxidants are very significant compounds that have the power to shield the body from harm brought on by oxidative stress brought on by free radicals. Propyl gallate (PG), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), three synthetic antioxidants now employed to combat oxidative stress, have been linked to harmful health consequences such liver damage and cancers. Additionally, both in human and animal models, their effectiveness is limited (Gandomi et al., 2014). Natural antioxidants from plants are becoming more popular as a replacement for synthetic antioxidants due to their increased safety, accessibility, and affordability (Saxena et al., 2012). In the quest for novel bioactive chemicals from natural sources, the antioxidant potential of *V. cinerea* extracts was examined. In terms of DPPH and hydroxyl radical scavenging activity, it was shown that *V. cinerea* exhibited the greatest antioxidant activity when compared to the reference antioxidant ascorbic acid. According to research, antioxidant activity shouldn't be determined using only one antioxidant experimental model (Mwihia et al., 2017). In reality, a variety of in vitro evaluation methods are taken into account while evaluating antioxidant activity (Beatrice Muthoni Guchu et al., 2020). The DPPH,

hydroxyl radical, superoxide radical scavenging, and ferric reducing antioxidant power (FRAP) assays were utilised in the current investigation.

One of the free radicals that is frequently used to check a compound's or a plant extract's potential to scavenge radicals is DPPH. Information on the reactivity of test chemicals with stable free radicals is provided by the DDPH test (Wagner, 1996). The 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), which has an odd electron, produces a strong absorption band at 517 nm (Duh, 1999). Antioxidants scavenge the DPPH radical by donating a proton to generate the reduced DPPH. Widespread in plants, phenolic compounds and flavanoids have been shown to have a variety of biological effects, including antioxidant, anti-inflammatory, anticarcinogenic, and free radical scavenging properties (Miller, 1996). The ethanol extract had an inhibitory concentration of 50% (IC_{50}) of 163.496 µg/ml, followed by the hexane extract with an inhibitory concentration of 50% (IC_{50}) of 214.932 µg/ml, and the chloroform fraction with an inhibitory concentration of 50% (IC_{50}) of 418.51 µg/ml, which had the lowest activity.

Additionally, this study looked at the plant extracts' capacity to scavenge for the hydroxyl radical. The hydroxyl radicals directly denature body enzymes by oxidising thiol (-SH) groups, according to research (Ogasawara et al., 2007). The Fenton reaction produces the hydroxyl radicals: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\cdot$ (Kibiti and Afolayan, 2015a). A substance is regarded as a powerful antioxidant with potential benefits in vivo if it can scavenge for hydroxyl radicals in vitro. According to findings published on both acetone and aqueous whole plant extracts of *Bulbine abyssinica*, the investigated *V. cinerea* extracts showed a concentration-dependent reduction in hydrogen peroxide scavenging activity in this study (Abimbade et al., 2014). On the other hand, several investigations have noted an increase in hydroxyl radical scavenging activity that is concentration-dependent (Agbor et al., 2014). The saturation of hydroxyl radicals' reactive centres by high extract concentrations, which led to low activities, as opposed to diluted concentrations, which ensured an easier and faster reaction, which led to high activity, may have caused the concentration-dependent decrease in hydroxyl radical scavenging activity. According to Fidrianny et al. (2015), the half maximum (IC_{50}) values for *A. hockii, C. volkensii, and V. lasiopus* were less than 50 mg/ml, making them extremely potent.

Cellular constituents are also severely harmed by superoxide anion (Korya-Dahl and Richardson, 1978). Flavonoids are efficient antioxidants, according to Robak and Glyglewski (1988), primarily because they scavenge superoxide anions. The superoxide radical scavenging abilities of the *V. cinerea* extracts and the reference chemical both rise noticeably with concentration, as shown in figure 3. The findings imply that the plant extract is a more effective superoxide radical scavenger. When an antioxidant reacts with a ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex to form a coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ), the FRAP test determines the reducing potential of the antioxidant. The donation of a hydrogen atom breaks the free radical chain. All extracts and the standard, ascorbic acid, both exhibited the FRAP-measured antioxidant activity. The IC_{50} values in ethanol, chloroform, hexane, and standard extracts of *V. cinerea* were 215.026, 484.817, 734.571, and 173.727 µg/ ml, respectively. In fresh primordium and aqueous extracts of fresh fruiting bodies of *Pleurotus ostreatus*, researchers Palma et al. (2016) discovered strong antioxidant concentrations of 166.5 0.10 and 113.9 0.24 M de $FeSO_4$/g.

The most notable antioxidant phytochemicals, flavonoids and phenols, showed significant levels in both qualitative and quantitative analyses, which may have contributed to the antioxidant effectiveness of the examined plant extracts (Moriasi et al., 2020). These phytochemicals' reductive and oxidative abilities, which enable the absorption of free radicals

and the ability to counteract their effects, are regarded to be the source of their antioxidant capacity (Elzaawely and Tawata, 2012). Many of these secondary metabolites have powerful reductive properties, which are thought to be responsible for the decreased prevalence of oxidative stress-related illnesses that cause mortality and misery (Kibiti and Afolayan, 2015b). Due to their biological impacts, our work strongly implies that phenolics are important parts of these plants.

The agar diffusion techniques utilised in this investigation to evaluate the plant extracts' antimicrobial activity have been extensively described in the literature (Das et al., 2010). When tested against recognised commercially available antibiotics, all test microbes displayed distinct resistance patterns. The extracts demonstrated antimicrobial activity against clinical isolates of six bacterial and three fungal pathogens. Since it prevented the growth of disease-causing Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*, and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae,* and *Proteus vulgaris*), as well as fungi (*Aspergillus niger, Aspergillus flavus* and *Candida albicans*), in the study, *V. cinerea's* entire plant exhibited both antibacterial and antifungal properties. All of the extracts are abundant in secondary metabolites; nevertheless, the activity of the plant extracts depends not only on the presence of secondary metabolites but also on their quantity and potential interactions with other components (Dzotam et al., 2016). Since bacterial cell walls are formed of proteins, the antibacterial activity of tannins is related to their capacity to interact with proteins to generate stable water-insoluble components (Dangoggo et al., 2012). Additionally, it may bind to proline-rich proteins and prevent the production of new proteins (Shimada et al., 2006; Kaczmarek, 2020). Furthermore, saponins' antibacterial characteristics may result in the release of proteins and certain enzymes from cells (detergent like properties). While flavonoids' activity is due to their capacity to bind with intracellular and soluble proteins and to bind with bacterial cell walls, and steroids' antibacterial properties are due to their capacity to complex with membrane lipids and exert their action by causing leakage, alkaloids' antibacterial effect is due to their ability to interchelate with DNA of both Gram positive and negative bacteria and interfere with cell division (Bukar et al., 2015). (Majorie, 1999; Vollaro et al., 2020). All the extracts was shown to have the modest antifungal effects against tested fungi. The findings demonstrate that plants high in tannin and phenolic compounds have antimicrobial properties against a variety of microbes.

5. Conclusion

The medicinal herbs under study have historically been utilised to treat a number of conditions linked to oxidative stress. The observed results led to the conclusion that *V. cinerea* extracts in ethanol, chloroform, and hexane exhibit significant antioxidant capacity and antimicrobial activity. It is advised that more research be done with the goal of extracting and defining the pure phytoactive components. The safety and clinical utility of the *V. cinerea* extracts should be determined by toxicology investigations.

6. References

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