**ABSTRACT**

To perform preliminary phytochemical screening, GC-MS analysis and to study the antimicrobial activity of Petroleum ether, ethanolic and aqueous extracts of *Vernonia cinerea* leaves. Leaves of *Vernonia cinerea* was shade dried, powdered and extracted with respective solvents. The extracts were then screened for phytocompounds, the petroleum ether and ethanol extracts were analysed by GC-MS to detect the compounds present in the polar and non polar solvent extracts of the leaves. Antimicrobial potential of the extracts was detected against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Ethanol and aqueous extracts shows more activity at different concentration. Antifungal activity against *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Candida albicans* and *Monosuc purpures* was evaluated. The extracts showed significant activity against the fungal species. The leaves of *Vernonia cinerea* possess certain bioactive compounds that contribute to its antimicrobial property.

**INTRODUCTION**

Traditional medicinal system is still on road due to their wide application in health and wellness system. In India Ayurvedic medicinal system is practiced for almost 5000 years and till now it is a major health care system. Many folklore herbal preparations are followed because of its efficacy in treatment of certain diseases. Drugs derived from plants are safe, cost effective and readily available. (Yadav et al., 2011). Nature has been gifted with valuable sources of medicinal agents. Medicinal plants are found to possess chemical substances with potential therapeutic effects contributing substantially to health, cultural integrity and local economies. Natural medicines are more acceptable to human body with fewer side effects when compared with modern synthetic drug system (Egharevba and Kunle, 2010). The most eminent source of drugs for world’s population is plants and 25% of prescribed medicines in today’s world are derivatives of plant compounds (Sonam Rajwar et al., 2011). Infections caused by microorganisms remain one of the major threats to human health. In developing countries, infectious diseases have become an important cause of morbidity. There are various infectious diseases which has even taken lives of certain population. Plant extracts can be directly analyzed for the presence of their compounds by GCMS technique, which is a major tool for the separation of volatile techniques.

This chromatography technique mainly focuses on compound purification and in qualitative and quantitative analysis of mixtures. Gas chromatography mass spectroscopy was first described by James and Martin in 1952. Integration of mass spectrometry to gas chromatography helps in direct identification of unknown compounds even at very low concentrations. In gas chromatography analysis, sample is vaporised and injected onto the head of the chromatographic columns. Sample is transported through the column by flow of inert gaseous mobile phase. The column contains a liquid stationary phase which is adsorbed onto the surface of inert solid (Sermakkani et al., 2012). GCMS technique is vastly applied for analysing essential oils, fatty acids, lipids and non polar compounds (Sivakumar et al., 2015). A number of antimicrobial agents have been developed and used to defend pathogenic microorganisms. Antimicrobial resistance is a major problem. Use of antimicrobials extensively has lead to the development of multidrug resistant strains, which raised the risk of certain infectious diseases. Problems with infectious microorganism have led to the emergence of resistant strains due to use of certain antibiotics. Thus plant derived drugs has to be explored to treat infectious diseases effectively. Medicinal plants have been used for centuries in treatment of infectious diseases. Medicinal plants provide new sources of active constituents that can defend against microorganisms. *Vernonia cinerea* is a common plant well distributed in India and is used in some folk medicinal preparations. They belong to the asteraceae family and are considered among one of the most advanced family from the dicotyledonos.
Vernonia cinerea is an annual herb with flat topped arrays of numerous flower heads, with pinkish ray florets. The plant possesses medicinal value in diverse traditional usage. The whole plant is used to treat fever and eye infections. It has been used as remedy for spasms of the urinary bladder and strangury. Seeds are used as a source for alexipharmic and anthelmintic drugs. Leaves of Vernonia cinerea have analgesic, antipyretic and anti-inflammatory effects. The whole plant is used for kidney disorders, stomach pain, diarrhoea, eczema, menstrual pains and decoction for diuretic. Juice of this plant is given to children to treat bed-wetting (Suresh et al., 2015). The present study was done to screen the phytoconstituents and to check the antimicrobial activity of Vernonia cinerea leaf extracts.

**MATERIALS AND METHODS**

**Preparation of plant extract**

Fresh leaves of Vernonia cinerea was washed thoroughly, shade dried and powdered. The plant powder was then kept in contact with petroleum ether, ethanol and distilled water separately in a stoppered container for a defined period with continuous agitation. The extract is then filtered, condensed and stored for further studies.

**Phytochemical Screening**

**Test for Phlobtannins**

To each plant extract 1% hydrochloric acid solution was added and boiled in a water bath. Formation of red colour precipitate indicates the presence of phlobtannins (Abdul Wadood et al., 2013).

**Test for alkaloids**

Dragendroff’s test

Each extract was treated with Dragendroff’s reagent. Formation of red precipitate indicates positive result.

Mayer’s test

Each extract was treated with 2ml of Mayer’s reagent. Formation of yellow coloured precipitate indicates the presence of alkaloids.

**Test for proteins (ninhydrin test)**

Each extract was treated with 2ml of 0.2% ninhydrin solution. Presence of violet colouration indicates amino acids and proteins.

**Test for carbohydrates**

Fehling’s test

Equal volume of Fehling A and Fehling B was mixed, 2ml of this solution was added to each extract and boiled. Formation of red brick precipitate at the bottom of the test tube indicates the presence of carbohydrates.

Benedict’s test

2ml of Benedict’s solution was added to each extract and boiled. Formation of reddish brown precipitate indicates the presence of carbohydrates.

**Iodine test**

2ml of iodine solution was treated with each extract. Dark blue or purple coloration indicates the presence of carbohydrates.

**Test for phenols**

Fehling’s test

2ml of 2% ferric chloride solution was added to each extract. Blue green or purple coloration indicates the presence of phenols.

**Test for flavonoids**

**Alkaline reagent test**

Each extract was mixed with 2ml of 2% NaOH solution. Formation of intense yellow colouration turned colourless on addition of few drops on dilute acid indicating the presence of flavonoids.

**Test for saponins**

**Foam test**

Each extract was mixed with 2ml of chloroform and 2ml of concentrated sulphuric acid. A red colour formed at the chloroform layer indicates the presence of steroids.

**Salkowski’s test**

2ml of chloroform was mixed with each extract. 2ml of concentrated sulphuric acid was added and shaken gently. Reddish brown colour indicates the presence of glycosides.

**Liebermann’s test**

2ml of chloroform was mixed with each extract. 2ml of concentrated sulphuric acid was added and shaken well. Formation of reddish brown colour indicates the presence of terpenoids.
Gas Chromatography Mass spectroscopy Analysis

GC-MS analysis was carried out on Thermo GC Trace Ultra Ver-5.0 system and gas chromatograph interfaced to a mass spectrometer (GC-MS) employing the following conditions: DB5- MS Capillary standard column (30 x 0.25 mm ID x 1μm df), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow rate of 1 mL/minute and an injection volume of 1 μL was employed. The oven temperature was programmed from 70 °C (isothermal for 2 min), then raised to 260°C at 6 °C/min. The total GC running time was 37.53 minutes.

Preparation and standardization of inoculums

All the bacterial and fungal cultures were transferred into 100 ml of nutrient broth (NB). The inoculated broths were incubated at 37°C for 24 hours and at 27°C for 72 hours in the case of bacteria and fungi, respectively. Antibacterial activity (Bauer et al., 1966)

Nutrient agar medium was prepared and transferred into sterile petriplates. 25μl of the standardized bacterial inoculum was spread on agar medium using sterile cotton swab. The discs impregnated with extracts were placed on the inoculated agar medium. Amoxicillin (10μg/disc) was used as standard to determine the sensitivity of each microbial species. All the petriplates were incubated at 37°C for 24 hours. After the incubation period, diameter of zone of inhibition was measured.

Antifungal activity

Potato dextrose medium was prepared and transferred into sterile petriplates. 200μl of the standardized fungal inoculum was spread on agar medium using sterile cotton swab. The discs impregnated with extracts were placed on the inoculated agar medium. Tetracycline (10μg/disc) was used as standard to determine the sensitivity of each microbial species. All the petriplates were incubated at 37°C for 24 hours. After the incubation period, diameter of zone of inhibition was measured.

Statistical analysis

The data were reported as mean ± standard deviation (n=3).

RESULTS

Phytochemical Screening of Vernonia cinerea

The phytochemical screening of petroleum ether, ethanol and aqueous extracts of Vernonia cinerea revealed the presence of certain phytocompounds which is summarized in Table 1. The results shows the presence of certain bioactive compounds in leaf extracts of Vernonia cinerea. Petroleum ether extract shown the presence of alkaloids, tannins, saponins and glycosides.

<table>
<thead>
<tr>
<th>Table 1. Phytochemical Screening of Vernonia cinerea</th>
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<tr>
<td>Alkaloids</td>
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<td>Phlobtannins</td>
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<td>Terpenoids</td>
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<td>+ = presence of compound, - = absence of compound</td>
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Alkaloids, phenols, tannins, steroids, glycosides, flavonoids, carbohydrates and terpenoids were present in ethanolic extracts of V. cinerea. Aqueous extracts were found to have alkaloids, phenols, saponins and phlobtannins.

GC-MS analysis of Vernonia cinerea

The GC-MS analysis of petroleum ether and ethanolic extracts of Vernonia cinerea revealed the presence of certain phytoconstituents, these are tabulated with their retention time, molecular formula, molecular weight and peak area (Table 3 & 4). GC-MS analysis of petroleum ether showed the presence of biocompounds like Junipene, α-Humulene, Zingiberene, α Sesquiphilandrene, Isoxazole, carophyllene oxide, Cis-Asarone, α – Tumerone, Ethyl ρ-methoxy cinnamate, Neophytadiene, hexadecanoic acid, Vetricellol, Thunbergol, 9,12- Octadecadienoic acid, Phyto, Ethyl iso-allocholate, Quercetin, Squalene, Stigmastera, Luconin and silane(Table 3).

Ethanolic extract found to have 1-Tridecanol, n- Nonadecanol-1, Neophytadine, Ninacosane, 3, 7, 11, 15- Tetramethyl-2-hexadecen-1-ol, 3-Eicosyne, Hexadecanoic acid, phytol, 9, 12-Octadecanoic acid ethyl ester, Pentacosane, Farnesyl Acetone, 3-phenyl-2-cholesten-5α-ol, Stigmast-5 en-3-ol, Ethyl iso-allocholate, and Myrisiti (Table 4).

Antimicrobial Activity of Vernonia cinerea

Table 4 shows the zone of inhibition of petroleum ether, ethanol and aqueous extracts of Vernonia cinerea leaf extracts against Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa. Petroleum ether extract exhibited minimum antibacterial activity. Petroleum ether extracts shows inhibition against E.coli and S.aureus. Ethanol and aqueous extracts has antibacterial property against all the five test species. The zone of inhibition was measured in mm for different concentrations of extracts (40, 60, 80 and 100mg/ml). Amoxicillin (10μg) disc was used as the positive control. Among the three extracts of Vernonia cinerea, aqueous extract possess high antibacterial potential. Petroleum ether, ethanol and aqueous extracts of Vernonia cinerea leaves has revealed its antifungal potential against Aspergillus niger, Aspergillus fumigatus, Aspergillus parasiticus, Candida albicans and Monosoc purpures. The antifungal activity was expressed by means of zone of inhibition in mm. The results (Table 5) show that the inhibition zone increased with increasing concentration of the extracts. Tetracycline discs (10μg) were used as the positive control. Petroleum ether extract has no inhibition against A.fumigatus, whereas shown high activity against A.parasiticus, C.albicans and M. purpures when compared to ethanol and aqueous extracts.
Ethanol extracts of *V. cinerea* possess high activity against *A. niger* and aqueous extract shows increased activity against *A. fumigatus* species.

**DISCUSSION**

**Phytochemical Screening:** Medicinal plants are reservoirs of potentially useful compounds that serve as clue for drug development.
designing. Most important bioconstituents are alkaloids, tannins, terpenoids, steroids, flavonoids and phenolic compounds. Correlation between plant bioconstituents and bioactivity is desirable for the synthesis of compounds with specific activity that could treat various diseases. Preliminary phytochemical screening is needed to discover new therapeutic drugs (Manjulika Yadav et al., 2014). Preliminary phytochemical screening of the extracts has shown the presence of Alkaloids, Phenols, Tannins, Saponins, Steroids, Glycosides, Carbohydrates and terpenoids (Table 1). In-vitro screening methods provide required preliminary observations to select a plant with potentially useful properties for further

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Extract</th>
<th>Concentration (mg/L)</th>
<th>PETROLEUM ETHER</th>
<th>ETHANOL</th>
<th>AQUEOUS</th>
<th>CONTROL</th>
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</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>Zone Diameter (mm)</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
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<tr>
<td>B. subtilis</td>
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<tr>
<td>S.aureus</td>
<td>9.083±0.076</td>
<td>10.067±0.115</td>
<td>12.033±0.058</td>
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<td>S.flexues</td>
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<td>P.aeruginosa</td>
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<tr>
<td>Table 4. Anti bacterial activity of Petroleum ether, Ethanol and Aqueous extracts of Vernonia cinerea</td>
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<th>Fungi</th>
<th>Extract</th>
<th>Concentration (mg/L)</th>
<th>PETROLEUM ETHER</th>
<th>ETHANOL</th>
<th>AQUEOUS</th>
<th>CONTROL</th>
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<tbody>
<tr>
<td>A.niger</td>
<td>Zone Diameter (mm)</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
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<tr>
<td>A.fumigates</td>
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<tr>
<td>A.parasiticus</td>
<td>10.100±0.173</td>
<td>11.100±0.100</td>
<td>13.133±0.153</td>
<td>14.067±0.058</td>
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<tr>
<td>C.albicans</td>
<td>12.100±0.100</td>
<td>13.333±0.153</td>
<td>16.100±0.100</td>
<td>17.150±0.132</td>
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<tr>
<td>M. purpures</td>
<td>13.083±0.076</td>
<td>14.033±0.058</td>
<td>14.967±0.058</td>
<td>15.567±0.058</td>
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<tr>
<td>Table 5. Antifungal activity of Petroleum ether, Ethanol and Aqueous extracts of Vernonia cinerea</td>
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Values are mean inhibition zone (mm)± SD of three replicates; - = no inhibition.
pharmacological studies (Nandha Kumar and Nivetha., 2015). In the present study more phytoconstituents were found in the ethanolic extract of *V. cinerea* leaves.

**GC-MS analysis**

Knowledge on chemical constituents of plant is needed for the development of therapeutic agents and to isolate compounds that can treat the root cause of a disease. GC-MS is used for direct analysis of chemical constituents present in medicinal plants. GC-MS studies have been widely applied in plant analysis as this technique has proved to be a valuable method because of its simplicity, sensitivity and effectiveness in separating components of mixture (Sermakkani and Thangapandian., 2012). In this work GC-MS analysis revealed the phytoconstituents present in the petroleum ether and ethanolic extracts of *V. cinerea* leaves (Table 2 and 3).

The analysis shows the presence of certain terpenes, sterols, essential oils and flavonoids. Compounds like Neophytadiene, hexadecanoic acid, Verticellol, 9, 12- Octadecanoic acid, Phytol, Ethyl iso-allocholate, Squalene, 3, 7, 11, 15-Tetra methyl-2-hexadecen-1-ol, Nonacosane, and Pentacosane are some biocompounds that are found to have anti-microbial property from previous reports. (Parthiban et al., 2015). Neophytadiene, 9, 12- Octadecanoic acid, Phytol, Ethyl iso-allocholate, Hexadecanoic acid and Stigmasta are compounds present in both the petroleum ether and ethanol extracts.

**Antimicrobial Activity**

Many medicinal plants are used as traditional medicines to treat infectious diseases. Microorganisms have developed resistance to many commercial antibiotics due to indiscriminate use of antibiotics.
Investigation of medicinal plants against microbial species has become desirable to evaluate its antimicrobial potential (S. Vijayanand and E. G. Wesely., 2014). The present study shows the antimicrobial activity of the leaf extracts. Ethanolic and aqueous extracts was found to have more antibacterial activity than petroleum ether. Petroleum ether extract shows activity only against *E.coli* and *S.aureus* (Table 4). Antifungal activity was exhibited in all the three extracts where petroleum ether extract doesn’t shown activity against *A.fumigatus* (Table 5). The antimicrobial activity of *Vernonia cinerea* is contributed by the presence of phytoconstituents present in the plant.

**Conclusion**

The present study revealed the presence of certain terpenes, sterols, flavonoids and phenols in the leaves of *Vernonia cinerea*. GC-MS analysis of the extracts was found to possess bioactive phytoconstituents, of which some compounds are reported earlier for their activity. Petroleum ether, ethanol and aqueous extracts of *V. cinerea* were found to have antimicrobial potential against some species of bacteria and fungi. Further studies can be done to study the activity of each compound, isolate and purify those compounds. This could provide limelight to the development of new drug.

**REFERENCES**


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