IN-VITRO BIOLOGICAL ACTIVITIES OF VERNONIA CINEREA (L.)

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Abstract

The use of natural products with therapeutic properties is as ancient as human civilization with plant products being the main source of drugs. Plants are thus rich storehouse of bioactive compounds which contributes significantly to human health and wellbeing. Therefore, there is always an urgent need to investigate more and more plants for their beneficial properties. The present investigation was planned to assess the phytoconstituents, antimicrobial and antioxidant potential of plant extracts of V. cinerea prepared in methanol, acetone, and chloroform and petroleum ether. The antimicrobial potential of the extracts was evaluated against six bacterial and four fungal strains by disc diffusion assay and minimum inhibitory concentration (MIC) was also determined. All the plant extracts except petroleum ether extract inhibited the growth of bacterial and fungal strain; the MICs of methanolic extract were found to be the least. Antioxidant potential of the plant extracts was evaluated by both enzymatic assays (Catalase, Glutathione S-transferase, and Superoxide dismutase) and non-enzymatic assays (DPPH, ABTS). The lowest IC_{50} value was exhibited by methanolic extract followed by acetonic extract. The activity levels of antioxidant enzymes of the plant extracts were found to be satisfactory. The antimicrobial potential of the plant extracts was found to be positively correlated with the total phenolic and flavonoid content. Ten phytoconstituents in methanolic extract, six each in acetonic and chloroform extracts, while maximum of twelve compounds were predominantly identified in the petroleum ether extract by GCMS. Some of these compounds are known to possess antimicrobial properties.

Key words: Phytoconstituents, plant extracts, antimicrobial, antioxidant, and MIC.

Introduction

Plants have been utilized for the wellbeing of mankind since time immemorial due to their therapeutic potential. As per WHO, nearly 80% of the world population relies upon plant-based products for their primary health care (Ekor, 2014). There is an increased emphasis on plant-based drugs due to inadequate supply, high cost, and side effects of synthetic drugs. Moreover, the development of multidrug-resistant strains and the emergence of infectious diseases has also generated a lot of interest in phytochemicals (Cheesman et al., 2017). Plants possess an array of phytochemicals that provides them the biological properties for the amelioration of human beings. In fact, plants are one of the richest bio-resources that have considerable importance not only in modern medicine but also as nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical individual for synthetic drugs (Pan et al., 2013).

Although extensive research on medicinal plants is being carried out globally, only a few plants have been comprehensively evaluated for pharmacological activity (Atanaov et al., 2015) Hence, there is urgent need to explore more and more plants for identifying novel compounds for drug development. Moreover, the emergence of multidrug-resistant strains has become a challenge for mankind. As plant-derived products, are chemically complex mixtures containing multiple major and minor constituents with multiple potential targets and mechanisms, hence they offer tremendous opportunities for the development of new types of phytomedicine.

Vernonia cinerea (L). (Family: Asteraceae) is an erect, annual herb. It is widely prevalent in India including Haryana (Prasopthum et al., 2015). The Ayurvedic Pharmacopoeia recommends this plant for the treatment of inflammation, arthritis, asthma, and many more human ailments (Prasopthum et al., 2015 and Youn et al., 2014). The plant is also a potential source of compounds like...
sterols, flavonoids, sesquiterpene lactones, terpenoids, and lupeol acetate which largely contribute to various biological properties in traditional as well as in some modern therapeutic principles (Inpuron et al., 2013 and Alara et al., 2018). Looking at the ethnomedicinal values and scanty information available in the literature, the present study was planned to investigate the plant extracts of V. cinerea for their phytochemicals, antimicrobial, and antioxidant appraisal. Efforts were also been made to identify the phytoconstituents with the help of GC-MS.

**Materials and Methods**

**Preparation of plant extracts**

The vegetative aerial parts (stem and leaves) of *V. cinerea* were collected from district Bhiwani, Haryana (India). They were washed and dried and dried plant material (50gm) was extracted (1:5W/V) in a Soxhlet apparatus by using four different solvents (250ml) petroleum ether (polarity index- 0.1), chloroform (4.1), acetone (5.1) and methanol (5.1) The plant extracts were filtered through a Whatman filter paper and the solvent was evaporated by using a rotary evaporator. The extracts were preserved in airtight containers and stored at 4ºC until further use.

**Phytochemical screening**

The plant extracts prepared in different solvents were screened qualitatively for the presence of various phytochemicals as per standard methods (Gul et al., 2017, Thangaraj, 2016 and Barbouchi et al., 2018).

**Total phenolic (TFC) and flavonoid CONTENT (TFC)**

TPC and TFC were determined by the Folin-ciocalteu method and Aluminium Chloride Colorimetric method, respectively (Barbouchi et al., 2018). In the case of TPC, Gallic acid was used as standard, and absorbance was measured at 620 nm and results were expressed in terms of mg of gallic acid equivalent (GAE)/ gram dry weight of the sample. For TFC the absorbance was measured at 420 nm with quercetin as standard. Results were expressed as mg of quercetin equivalent (QE)/ gram dry weight of the sample. All tests were performed in triplicates.

**Antimicrobial studies**

Antimicrobial potential of all the plant extracts was measured by the disc diffusion method and micro broth dilution assay against six bacterial strains and four fungal strains. Plant extracts were reconstituted in DMSO to obtain five concentrations of 200 mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml for antimicrobial studies.

**Inoculum preparation**

The antimicrobial activity of plant extracts against six different bacterial strains “(*Staphylococcus aureus* (MTCC-96), *Bacillus subtilis* (MTCC-2057), *Escherichia coli* (MTCC-41), *Streptococcus pyogenes* (MTCC-890), *Pseudomonas aeruginosa* (MTCC-2453), and *Chromobacterium violaceum* (MTCC-2656)" was assessed. Four different fungal strains used were “*Aspergillus niger* (MTCC- 3002) Rhizopus oryzae (MTCC-262) Fusarium oxysporum (MTCC-7392) and *Penicillium expansum* (MTCC- 2818)”. Active cultures for experiments were prepared by dissolving the NB (6.5 gm, Himedia) in distilled water (500ml) and sterilized by autoclaving at 121º C for 15 minutes. A loopful sterilized powder was added to 10 ml of sterile nutrient broth and was incubated at 37°C for 24 hours. After this, cultures were adjusted to the 0.5 McFarland (1.5 × 10⁶ CFU/ml) and used for further experiments.

**Disc diffusion method**

Antimicrobial investigations were carried out by disc diffusion assay as per CLSI, 2017 (Performance, 2017). 6 mm sterilized discs were kept over the nutrient agar media (Himedia) petri plates already spread with 100µl bacterial inoculum. CZ and PDA petriplates, already spread with 20µl fungal strains were used for antifungal studies. 10µl plant extract of different concentrations was loaded on the discs. Petri plates were then incubated at 37°C in the BOD incubator and the diameter of the ZOI was measured. Ampicillin and chloramphenicol (0.1mg/ml) were taken as positive controls for antibacterial assay while fluconazole (0.1mg/ml) as a positive control for the antifungal assay. DMSO acted as a negative control in both cases. The assay was repeated thrice.

**Minimum inhibitory concentration (MIC)**

MIC’s of *V.cinerea* were determined by micro broth dilution assay using two-fold serial dilutions of plant extracts (Fabry et al., 1998). 100µl of NB and 100 µl PE was added to each well of a 96 welled microtiter plate (12x8). 10µl of standardized inoculum (1.5 × 10⁶ CFU/ml) and 10µl of resazurin dye (0.2% W/V) were added in each well and petriplates were incubated at 37°C for 24 hours to find out the bacterial MIC. Similarly, MIC assay was also performed against fungal strains and petriplates were incubated for 72 hours. The lowest concentration of plant extract that inhibited the test organism was recorded as MIC.

**Antioxidant studies**

**DPPH and ABTS assay**

Antioxidant potential of plant extracts was measured

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In-Vitro biological activities of Vernonia cinerea (L.)

using DPPH (1, 1-diphenyl-2-picryl-hydrazyl) assay [14] and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay (Adebiyi et al., 2017). Ascorbic acid was used as standard in both the assays. The absorbance was measured at 517 nm in the case of DPPH assay and 745nm in case of ABTS assay by UV-Vis spectrophotometer (SHIMADZU 1800, Japan). Experiments were performed in triplicates and mean value was recorded. Antioxidant potential was calculated using the formula given below:

\[
\text{% inhibition} = \frac{Abs_{\text{Control}} - Abs_{\text{Sample}}}{Abs_{\text{Control}}} \times 100
\]

Preparation of plant extract for enzymatic assay

Plant extract was prepared by homogenization of freshly collected plant material (0.5g) in 5ml of cold extraction buffer containing 100mM potassium phosphate buffer (pH 7.0) and 0.1mM sodium ethylene diamine tetraacetic acid (EDTA). The homogenate was filtered through three layers of muslin cloth, and the filtrate was centrifuged at 14000 rpm for 20 minutes at 4°C. The supernatant obtained was used for all the enzymatic assays.

Catalase assay

Catalase activity was determined by the method of Aebi et al., 1983. 3ml reaction mixture containing 100µL plant extract, 10 mM H$_2$O$_2$ and 50 mM (pH-7.0) potassium phosphate buffer was prepared. The absorbance of the reaction mixture was recorded at 240 nm every 30 seconds continuously for 5 minutes. Enzyme activity was expressed as µmoles of H$_2$O$_2$ decomposed mg-1 fresh weight minutes-1.

GST assay

Glutathione S-transferase activity was estimated by the method of Habig et al., 1974. 3 ml reaction mixture containing 97mM potassium phosphate buffer (pH-6.5), 1mM EDTA (in DW), 30mM CDNB (in 95% ethanol) and GSH 75 mM (in Buffer) was prepared. The absorbance of the reaction mixture was recorded at 340 nm every 30 seconds till 5 minutes.

SOD assay

Superoxide dismutase (SOD) activity was assessed by the method of Misra and Fridovich, 1972. 3ml reaction mixture containing potassium phosphate buffer (50mM), methionine (15mM), NBT (75 mM), riboflavin (2µM), EDTA (0.1mM) and 100µl plant extract was prepared. The reaction mixture was transferred into a test tube and placed in a light intensity of 5000 lux for 25 minutes. The absorbance was measured at 560 nm. The reaction mixture placed in the dark did not produce any color and served as the control.

GC-MS analysis

The plant extracts were also analyzed by GC-MS equipment (Thermo Scientific Co. Trace 1300) to identify various phytoconstituents. The temperature was set as, 70°C and hold for 2 minutes and then the temperature was increased at 7°C per minutes up to 200°C and then accelerated at 5°C per minutes up to 220°C with 5 minutes hold. The temperature when samples were injected was set at 220°C. The mass scanning range was from 35 to 400 (m/z). The control of the GC-MS system and data peak processing was controlled using Xcalibur (software). Phytoconstituents identification was verified based on the relative retention time and their peak area with the NIST library in Xcalibur software and also compared with the available literature. The compound name, molecular weight, molecular formula, peak area percentage of the all the extracts were ascertained.

Statistical analysis

Graphs were plotted with the help of Microsoft Excel and Origin pro-8. Antioxidant activity (IC$_{50}$ values) and TPC/TFC were correlated using Pearson’s correlation coefficient. Statistical analysis was performed using SPSS version 16.0. Data are presented as means ± SD and P values less than 0.05 were taken as statistically significant.

Results

V. cinerea based on its ethnopharmacological significance was selected for the present study for evaluating its various biological properties. Extracts were prepared from aerial vegetative parts (stem and leaves). Plant extracts were further screened out for the presence of various phytochemicals, antimicrobial and antioxidant potential. Efforts have also been made to identify the phytoconstituents present in the plant extracts by GC-MS. The results obtained are described below:

Phytochemical analysis

The plant extracts of V. cinerea prepared in four different solvents were evaluated for the presence of different phytochemicals and the results obtained are given in table -1.

All the four plant extracts showed the presence of alkaloids, phenols, and flavonoids. Methanolic extract showed the maximum number of phytoconstituents followed by acetone and chloroform extract. Glicosides were detected in all the extracts except petroleum ether extract.

TPC and TFC

Total phenolic and flavonoid content calculated in the
Table 1: Phytochemical analysis of plant extracts of *V. cinerea* prepared in different solvents.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>67</td>
<td>Steroids/Terpenoids</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) indicate the presence of phytoconstituents, (-) indicate the absence of phytoconstituents.

Antibacterial activity (disc diffusion assay)

Disc diffusion assay was performed for all the four plant extract against six different bacterial strains and ZOI (in mm) obtained are graphically represented in (Fig. 3). The methanolic extract exhibited the highest ZOI (15.4 ± 1.0 mm) against *S. pyogenes* followed by *B. subtilis* (15.3 ± 0.57 mm). The least diameter of ZOI was observed against methanolic extract against the *Paeruginosa* (11.3 ± 0.23 mm). Acetonic plant extract exhibited maximum inhibition against *S. aureus* (15.2 ± 0.20 mm) whereas the chloroform extract showed the highest ZOI against *C. violaceum* (14.2 ± 0.1 mm). Petroleum ether extract did not show any ZOI against the bacterial strains used in study. Maximum ZOI recorded with the positive controls *i.e.* ampicillin and chloramphenicol was 22.2±0mm and 22.1±0mm against *C. violaceum* and *S. pyogenes*, respectively. *S. aureus* and *P. aeruginosa* were found to be resistant to one of the positive control *i.e.* Ampicillin. Negative control (DMSO) didn’t show any activity against any of the bacterial strains used in the study.

MIC values of plant extracts of *V. cinerea* (mg/ml)

The MIC was recorded for all the plant extracts by micro broth dilution assay and the results obtained are presented in (Table 2).

MIC values obtained for methanolic plant extract were in the range of 0.78-6.25 mg/ml; minimum (0.78 mg/ml) being with both *B. subtilis* and *S. pyogenes* whereas plant extract prepared in acetone showed values in the range of 1.56-12.5mg/ml, least MIC (1.56 mg/ml) was obtained in case of *S. aureus*. MIC values obtained with chloroform extract were from 1.56-12.5 mg/ml maximum inhibition was in the case of *S. pyogenes*. Petroleum ether extract didn’t show any activity against any of the bacterial strains. The positive controls *i.e.* ampicillin and chloramphenicol showed very less values as compared to the plant extract. Ampicillin showed MIC range from 0.05-0.1mg/ml whereas the MIC values obtained with chloramphenicol were less in the range of 0.025-0.1mg/ml.

Antifungal activity

Results of the disc diffusion assay are presented graphically in fig. 4. Methanolic and aceton extract showed significant ZOI (mm) against all the strains whereas petroleum ether extract exhibited poor activity.
M.E methanol extract; A.E- acetonic extract; C.E- chloroform extract; P.E.E- petroleum ether extract

Fig. 3: ZOI obtained with different extract of V. cinerea and positive controls against different bacterial strains.

M.E methanol extract; A.E- acetonic extract; C.E- chloroform extract; P.E.E- petroleum ether extract

Fig. 4: ZOI obtained with different extract of V. cinerea and positive controls against different fungal strains.

except a single strain i.e. R. oryzae. The most susceptible strain towards all the extract was R. oryzae giving a zone of inhibition 23.3 ± 1.0 mm (chloroform extract) while the most resistant strain was A. niger with inhibition zone of 9.4 ± 1.25 mm (susceptible towards methanolic extract only).

**MIC values of plant extracts of V.cinerea (mg/ml)**

Methanolic extract table 3 exhibited the maximum antifungal activity against all the fungal strains hence displaying least MIC values as compared to the other extracts. MIC values of methanolic extract ranged from 3.125-12.5 mg/ml minimum value was obtained with both R. oryzae and P. expansum. In the case of acetonic and chloroform extract maximum inhibition was obtained with R. oryzae while the range in both the extract was 6.25-12.5 mg/ml. Petroleum ether extract showed activity (25mg/ml) only against R. oryzae out of four fungal strains.

**Non enzymatic assays**

**DPPH and ABTS assays**

The results obtained from the DPPH and ABTS assays of all the four extracts are graphically represented in (Fig. 5 and 6). The highest antioxidant activity was obtained with the methanolic extract in both the assays and showed percentage scavenging activity from 13.84% to 92.03% for DPPH and 38.29 % to 69.10 % for ABTS, respectively, for different concentrations. Chloroform and acetonie assays also showed good activity. Least antioxidant potential was obtained with the petroleum ether extract.
Table 2: MIC values of all the plant extracts and positive controls i.e. ampicillin and chloramphenicol against different bacterial strains.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>ME</th>
<th>AE</th>
<th>CE</th>
<th>PEE</th>
<th>A</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.subtilis</td>
<td>0.78</td>
<td>12.5</td>
<td>3.125</td>
<td>NA</td>
<td>0.05</td>
<td>0.025</td>
</tr>
<tr>
<td>S.pyogenes</td>
<td>0.78</td>
<td>3.125</td>
<td>1.56</td>
<td>NA</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>S.aureus</td>
<td>3.125</td>
<td>1.56</td>
<td>1.56</td>
<td>NA</td>
<td>NA</td>
<td>0.025</td>
</tr>
<tr>
<td>C.violaceum</td>
<td>6.25</td>
<td>12.5</td>
<td>12.5</td>
<td>NA</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>E.coli</td>
<td>3.125</td>
<td>3.125</td>
<td>6.25</td>
<td>NA</td>
<td>0.1</td>
<td>0.025</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>3.125</td>
<td>6.25</td>
<td>6.25</td>
<td>NA</td>
<td>NA</td>
<td>0.05</td>
</tr>
</tbody>
</table>

MIC (Minimum Inhibitory Concentration) values are expressed as mean (n=3), Unit is mg/ml; M.E methanol extract; A.E- acetonic extract; C.E-chloroform extract; P.E.E- petroleum ether extract; A-Ampicillin; C-Chloramphenicol; NA- no activity; Ampicillin and Chloramphenicol were used as dilutions from 0.1mg/ml up to all 12 wells.

Table 3: MIC values of plant extracts and positive control (fluconazole) against different fungal strains.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>ME</th>
<th>AE</th>
<th>CE</th>
<th>PEE</th>
<th>Fluconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.expansum</td>
<td>3.125</td>
<td>12.5</td>
<td>12.5</td>
<td>NA</td>
<td>0.00025</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>12.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.000125</td>
</tr>
<tr>
<td>R. oryze</td>
<td>3.125</td>
<td>6.25</td>
<td>6.25</td>
<td>125</td>
<td>0.000125</td>
</tr>
<tr>
<td>A. niger</td>
<td>6.25</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.000005</td>
</tr>
</tbody>
</table>

MIC (Minimum Inhibitory Concentration) value are expressed as mean (n=3), Unit is mg/ml; M.E methanol extract; A.E- acetonic extract; C.E-chloroform extract; P.E.E- petroleum ether extract; NA- no activity; Fluconazole was used as dilutions from 0.1mg/ml.

IC$_{50}$ values

The IC$_{50}$ values were calculated for all the four extracts by using Microsoft Excel and are represented in the fig.7 and 8. A low IC$_{50}$ value shows high antioxidant activity and vice versa. Methanolic extract showed the least value of IC$_{50}$ (49.93µg/ml DPPH) (40.89 µg/ml, ABTS) in both the assays, hence showed highest antioxidant activity and poorest antioxidant activity was shown by petroleum ether extract having highest IC$_{50}$ value 165.32µg/ml DPPH, IC$_{50}$ 145.30 µg/ml value, respectively.

Correlation between IC$_{50}$ values and TPC and TFC

Correlation values (Pearson correlation coefficient = r) between the antioxidant activities (IC$_{50}$ values) and total phenolic and total flavonoid content of the plant extracts are shown in the table 4 given below.

From table 4, it is clear that two variables i.e. TPC/TFC and IC$_{50}$ values are negatively correlated with each other which means plant extracts having high TPC/TFC values showed lower IC$_{50}$ values, lower IC$_{50}$ values means higher antioxidant potential. From the table it is also clear that TFC may have contributed more to the antioxidant potential than the TPC as it showed a very good correlation (R > 0.9) than the TPC (R<0.9).

Enzymatic assays

The antioxidant activity results obtained in the case of antioxidant enzymes namely, Catalase, Glutathione S-Transferase, and Superoxide Dismutase are shown in (Table 5).

Enzymatic assays

The enzyme activity was found to be 11.07±0.23µ mole of H$_2$O$_2$ per minute fresh weight for Catalase, 15.40±0.15µ mol of GS-DNB conjugate/min FW for Glutathione-S- Transferase and 51.94±0.41 SOD (Unit) FW for Superoxide dismutase respectively.

GC-MS characterization

GC-MS analysis of different plant extracts, with their retention time (RT), molecular formula, similarity index
Discussion

In the present investigation, *V. cinerea* was selected for the study as it is a widely distributed plant throughout India. The plant also has been utilized as folklore medicine in various traditional systems of medicine. The extracts of aerial vegetative parts prepared by soxhlet extraction in four different solvents were evaluated for varied biological activities to validate its traditional claims. Phytochemical analysis of the plant extracts showed the presence of phenols, flavonoids, and alkaloids in all the plant extracts. Similar observations have also been reported by other workers (Gul et al., 2017, Wu et al., 2017 and Ruwali & Negi, 2019). The maximum amount of TFC (192.87 mg QE/g) and also the TPC (121.34 mg GAE/g) was shown by the methanolic extract. Plant extracts prepared in the methanolic extract has also been reported to be rich sources of phenolic and flavonoid content by several workers (Oribayo et al., 2018 and Lefahal et al., 2018). Methanol is a good solvent (Tongur et al., 2018) this is probably because more phytoconstituents are extracted in methanol which is a polar organic solvent. Many other workers have also reported methanol to be a good solvent for other plants also (Tongur et al., 2018 and Markandan, 2016).

When the plant extracts were assessed for their antibacterial activity, extracts prepared in methanol, acetone, and chloroform showed inhibition against all the six bacterial strains. The maximum zone of inhibition was obtained against *S. Pyogenes* (15.4 ± 0.22 mm) in methanolic extract followed by *B. subtilis* (15.3 ± 0.22 mm), least ZOI (11.3± 0.23 mm) was observed in case of *P. aeruginosa*. Plant extracts prepared in acetone extract showed the maximum inhibition against *S.aureus* (15.2± 0.17 mm) followed by both *C.violaceum* and *E.coli* (15.1± 0.17 mm). Petroleum ether extract didn’t show any activity against any of the bacterial strains. Several other workers have also reported good antibacterial potential of *V. cinerea* (Youn et al., 2014 and Rizvi et al., 2011). It is interesting to note that *Paeruginosa* presented maximum sensitivity towards methanolic extract even though it was found to be resistant to one of the standard used *i.e.* ampicillin. This clearly shows the medicinal potential of the extract especially in the cases where available antibiotics are not effective against this bacteria. As observed in the present study, methanolic extract is found to exhibit high antimicrobial activity, which may be possible due to the highest phenolic and flavonoid content.

![Fig. 7: IC₅₀ values of plant extract of *V.cinerea* (DPPH).](image)

![Fig. 8: IC₅₀ values of plant extract of *V.cinerea* (ABTS).](image)

<table>
<thead>
<tr>
<th>Table 4: Correlation between IC₅₀ Values, TPC and TFC.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pearson correlation coefficient (r)</strong></td>
</tr>
<tr>
<td>TPC &amp; TFC</td>
</tr>
<tr>
<td>0.807</td>
</tr>
</tbody>
</table>

Correlation is significant at the 0.05 level (2 tailed).

<table>
<thead>
<tr>
<th>Table 5: Antioxidant enzyme activities of <em>V.cinerea</em>.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antioxidant enzymes Activity</strong></td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>Glutathione-S- Transferase</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
</tr>
</tbody>
</table>

Values are expressed as mean (n=3) ±SD.

Units of enzyme activities were expressed as:

CAT- One unit of catalase will decompose 1.0 µmole of H₂O₂ per minute at pH 7.0 at 25 °C under the assay condition.

SOD- One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT per unit time as monitored at 560 nm.

GST-One unit of GST activity is the amount of enzyme which produces 1.0µmol of GS-DNB conjugate/min under the conditions of the assay.

(SI), reverse similarity index (RSI), CAS no, peak area % are presented in (Table 7). The methanolic extract exhibited ten phytoconstituents, aceton extract showed seven phytoconstituents, chloroform revealed six while petroleum ether extract showed maximum twelve phytoconstituents predominantly.
Table 6: Phytocompounds detected from different plant extract of aerial vegetative parts of *V. cinerea* by GC-MS.

<table>
<thead>
<tr>
<th>S.No</th>
<th>RT</th>
<th>Compound</th>
<th>MF</th>
<th>SI</th>
<th>RSI</th>
<th>Structure</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.90</td>
<td>Ethyl 2-butyrate</td>
<td>C\textsubscript{6}H\textsubscript{8}O\textsubscript{2}</td>
<td>563</td>
<td>694</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20.49</td>
<td>2H-Pyan, 2,2'-[1,10-decanediylbis (oxy)] bis [tetrahydro-</td>
<td>C\textsubscript{20}H\textsubscript{38}O\textsubscript{4}</td>
<td>625</td>
<td>629</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22.14</td>
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<td>C\textsubscript{20}H\textsubscript{38}O\textsubscript{10}</td>
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<td>927</td>
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**Acetonic Extract**

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<th>SI</th>
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<th>Area%</th>
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*Table 6 contd...*
Table 6 contd....

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<td>Cyclopropanebutanoic acid, 2-[[2-[[2-[[2-pentylcyclopropyl]methyl]cyclopropyl]methyl]methyl]cyclopropyl[methyl]-, methyl Ester n-Hexadecanoic acid</td>
<td>C_{16}H_{38}O_{2}</td>
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*In-Vitro* biological activities of *Vernonia cinerea* (L.)
Antioxidant potential of V. cinerea extracts prepared in different solvents was assessed both by non-enzymatic (DPPH and ABTS assay) and enzymatic methods. Out of the four plant extracts, the maximum antioxidant potential was exhibited by methanolic extract followed by the acetonic extract by non-enzymatic assay. On a similar pattern of antimicrobial results, antioxidant potential was also least in the case of petroleum ether extracts.

In the present study, a direct relationship between the TPC, TFC, and antioxidant activity was observed. Many other studies have also reported a positive correlation between the total phenolics and antioxidant activity (Mahboubi et al., 2015 and Daffodil & Mohan 2013). Similar to our observations, Inpuron et al., (2013) also reported good antioxidant potential of V. cinerea and observed that the scavenging effect of the extract on the DPPH radical increased with increasing concentration of the plant extract. Rajamurugan et al., (2011) also investigated the antioxidant activities of V. cinerea plant extracts by DPPH and ABTS assay and reported methanolic leaf extracts to possess good free radical scavenging activity as determined.

Enzymatic antioxidants serve as an intrinsic defense tool to resist oxidative damage in plants. Catalase, GST, and Superoxide dismutase are the key enzymes that are involved in cellular defense against reactive oxygen species in living organisms; hence these are an important indicator of antioxidant capacity (Daffodil and Mohan, 2013). The enzymatic assays carried out with the plant extracts showed them to possess good antioxidant enzymatic activity. The results are in conformity with the non-enzymatic assay thus suggesting the antioxidant potential of the plant extracts. Similar to our observation workers from other parts of the country have also reported V. cinerea to exhibit good antioxidant properties carried out by non-enzymatic assays (Inpuron et al., 2013 and Maity et al., 2018).

GCMS analysis of different crude extracts i.e. methanol, acetone, and chloroform and petroleum ether extract of V. cinerea revealed the presence of various volatile phytoconstituents. Methanolic extract of V. cinerea showed ten main phytoconstituents to be present and acetonic extract exhibited seven phytoconstituents. Chloroform extract presented six volatile compounds while petroleum ether exhibited the maximum twelve numbers of compounds. Among the various compounds identified, hexadecanoic acid and Octadecadienoic acid are reported to have antimicrobial, antioxidant, anticancer, and hyper-cholesterolemic properties (Abirami and Rajendran (2011), Kala et al., 2011, Parthiban et al., 2015 and Kang et al., 2013). The maximum amount (48.46%) of Octadecadienoic acid was present in the methanolic extract and lowest (24.12%) in the case of petroleum ether. It could be one of the reasons for the best antimicrobial properties of methanolic extract as compared to other extracts. Octadecadienoic acid is also reported to have the property of anti-inflammatory and antiarthritic activity (Kala et al., 2011 and Shukla & Kaur, 2018). Esters are important organic compounds with an
increasing number of commercial applications (Asif et al., 2017). Similar results were also reported by Daffodil & Mohan (2013) and Rajamurugan et al., 2011 in the methanolic leaf extract and whole plant extract of V. cinerea, respectively.

As the plant parts of V. cinerea possess important bioactive compounds in the different plant extracts, it may be used for various pharmaceutical applications especially the methanolic extract. The plant is thus a potential source of bioactive compounds that can be beneficial for the health maintenance of mankind and this plant is not developed as a drug yet, although it contains lots of novel phytoconstituents with newer biological properties. Further, active elements from the plant should be isolated and assessed for their medicinal properties. Finally, it is anticipated that this investigation may help researchers at the global level to select this plant for detailed pharmacological and phytochemical investigations. Further investigations, when successfully implemented on V. cinerea, would hopefully lead to the isolation of new phytoconstituents(s) with the potential to be developed as effective phytodrug(s).

**Abbreviations**

- TPC-Total phenolic content
- TFC-Total flavonoid content
- ROS- Reactive oxygen species
- MIC-Minimum inhibitory concentration
- ZOI-Zone of inhibition
- NB-Nutrition broth
- NA-Nutrition Agar
- PDA- Potato dextrose agar
- CZ- Czapekdox
- PE-Plant extracts
- B.O.D-Biological oxygen demand
- DMSO- Dimethyl sulfoxide
- EC-Extinction coefficient
- \( H_2O_2 \)-Hydrogen peroxide

**V. cinerea** - Vernonia cinerea

**References**


Barbouchi, M., K. Elamrani and M. El Idrissi (2018). A comparative study on phytochemical screening, quantification of phenolic contents and antioxidant properties of different solvent extracts from various parts of *Pistacia lentiscus L.* *Journal of King Saud University-Science*.


