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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 phytocompounds (Cheesman *et al.,* 2017). Plants possess an array of phytocompounds 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

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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 phytocompounds, 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 4ÚC 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 evaluated 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 37ºC for 24 hours. After this, cultures were adjusted to the 0.5 McFarland (1.5×10^8 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 37ºC 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 (1.5×10^8 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

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:

$$\% \text{ inhibition} = Abs\,\text{Control} - Abs\frac{\text{Sample}}{Abs}\text{Control} \times 100$$

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 4ºC. 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_2O_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_2O_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 of 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 (13mM), 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. Glycosides 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.

S.No.	Phytochemicals	Plant extracts			
		Methanol	Acetone	Chloroform	Petroleum ether
1	Alkaloids	+	+	+	+
2	Flavanoids	+	+	+	+
3	Glycosides	+	+	+	-
4	Phenols	+	+	+	+
5	Saponins	+	-	-	-
67	SteroidsTerpenoids	—	+-	-+	++

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

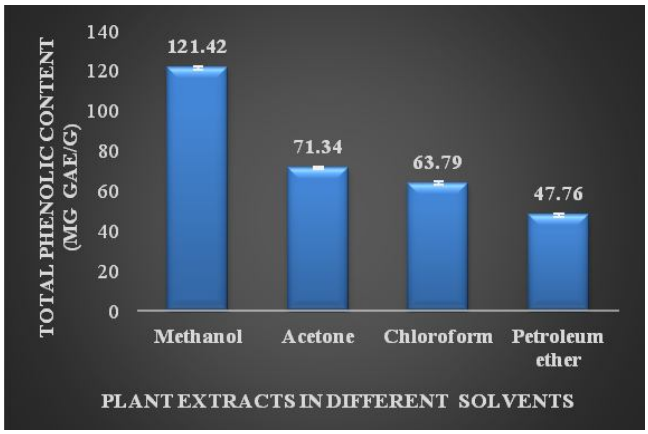


Fig. 1: TPC of *V. cinerea.*

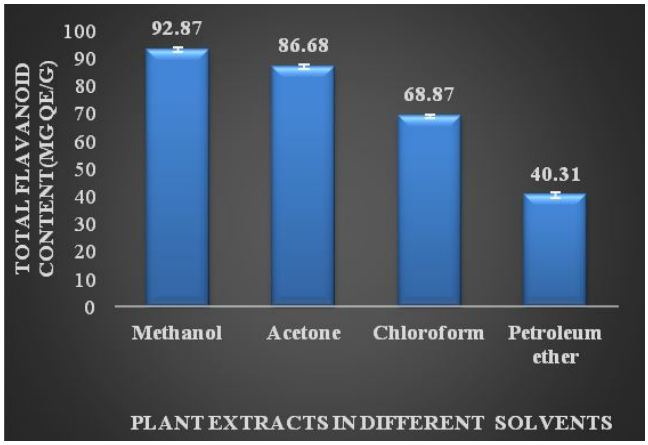


Fig. 2: TFC of *V. cinerea.*

plant extracts are depicted graphically in (Fig. 1 and 2). The maximum phenolic content was exhibited by methanolic extract (121.24± 0.14), followed by acetonic extract (71.34± 0.32). Similar to the TPC, the highest amount of TFC was also obtained in methanolic plant extract (92.87 ± 0.41) followed by acetonic extract (86.68 ± 0.21). Least phenolic (47.76± 0.23) and flavonoid content (40.31± 0.11) was determined in the plant extract prepared in the petroleum ether.

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 *P.aeruginosa (*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.

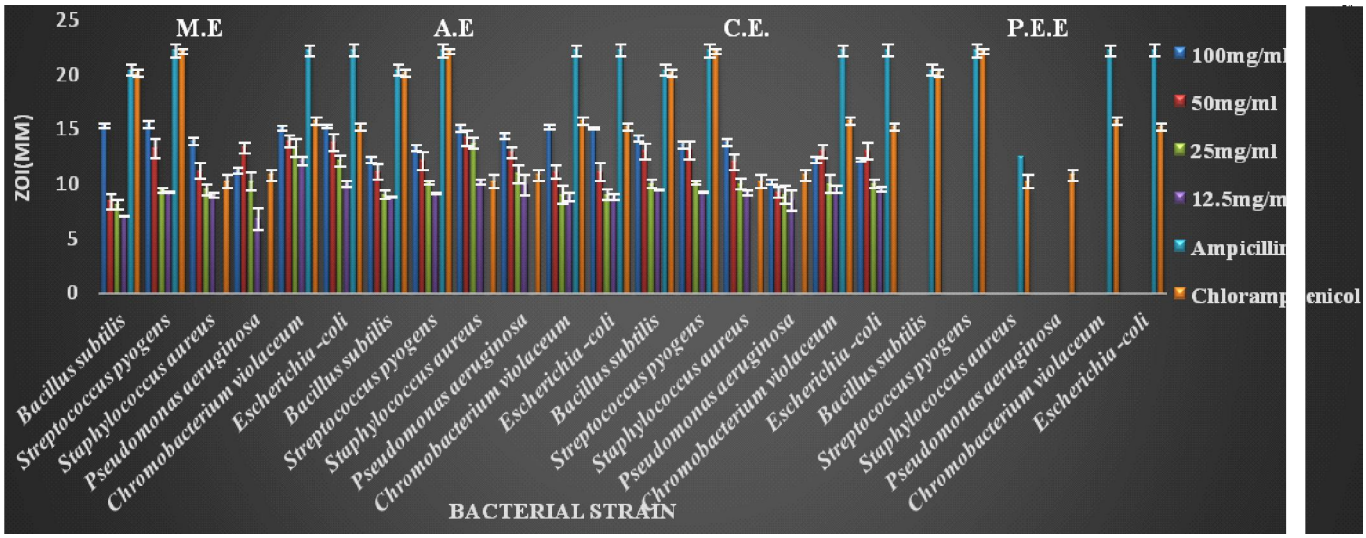
MIC values of plant extracts of *V.cinerea* (mg/ml)

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.

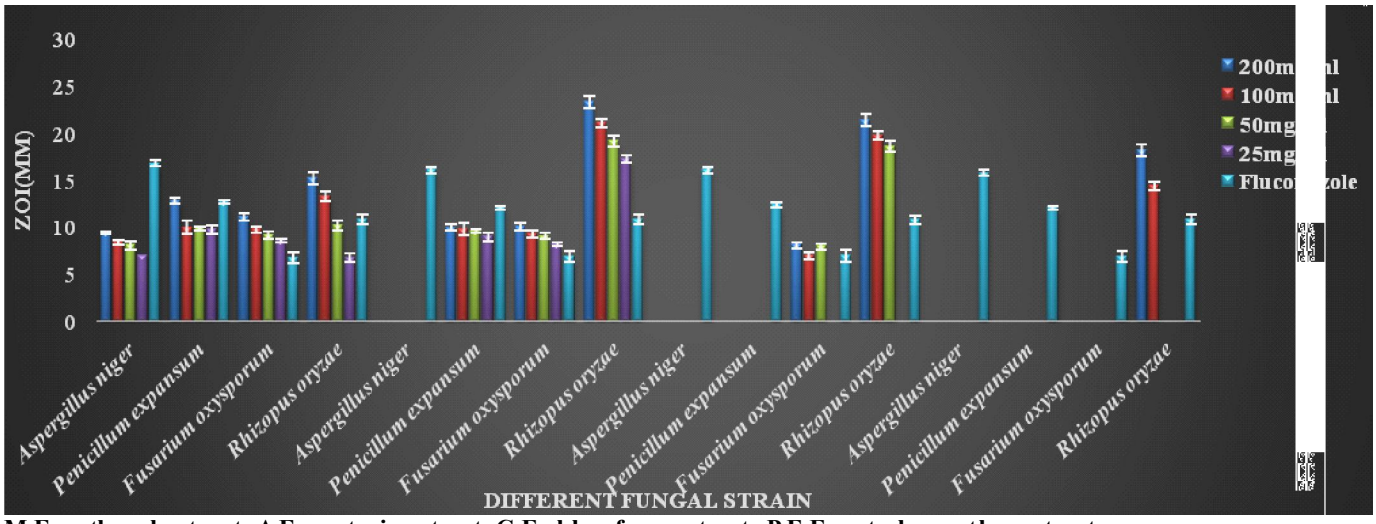
Antifungal activity

Results of the disc diffusion assay are presented graphically in fig.4**.** Methanolic and acetonic 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 extracts of *V. cinerea* and positive control 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 ± 1.25mm (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 the (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 acetonic 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.

Microorganisms	Extracts					
	M.E	A.E	C.E	P.E.E	A	C
B.subtilis	0.78	12.5	3.125	NA	0.05	0.025
S.pyogenes	0.78	3.125	1.56	NA	0.05	0.05
S.aureus	3.125	1.56	1.56	NA	NA	0.025
C.violaceum	6.25	12.5	12.5	NA	0.1	0.1
E.coli	3.125	3.125	6.25	NA	0.1	0.025
P.aeruginosa	3.125	6.25	6.25	NA	NA	0.05

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 from0.1mg/ml up to all 12 wells.

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

Microorganisms	Extracts				
	M.E	A.E	C.E	P.E.E	Fluconazole
P.expansum	3.125	12.5	12.5	NA	0.00025
F. oxysporum	12.5	NA	NA	NA	0.000125
R. oryzae	3.125	6.25	6.25	25	0.000125
A. niger	6.25	NA	NA	NA	0000.05

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.

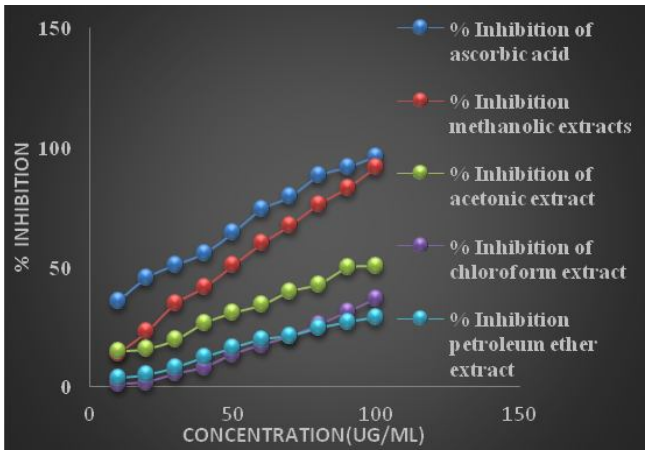


Fig. 5: Percentage inhibition of DPPH radical by different plant extracts of *V. cinerea*.

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).

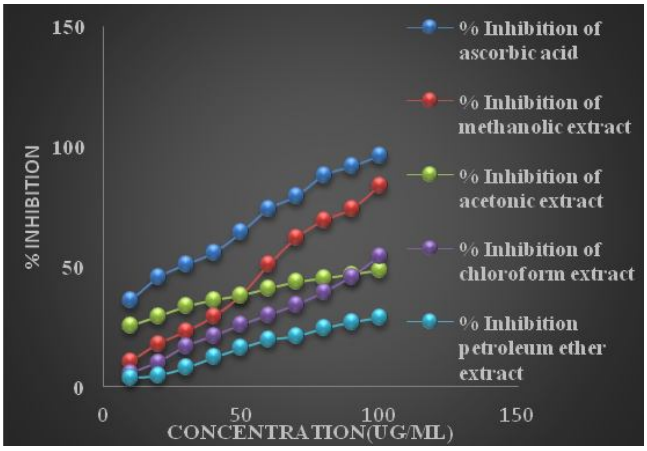


Fig. 6: Percentage inhibition of ABTS radical by plant extracts of *V. cinerea*.

The enzyme activity was found to be 11.07±0.23µ mole of H_2O_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

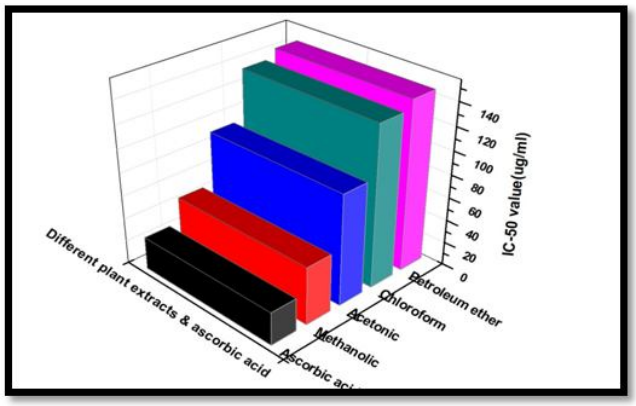


Fig. 7: IC_{50} values of plant extract of *V.cinerea* (DPPH).

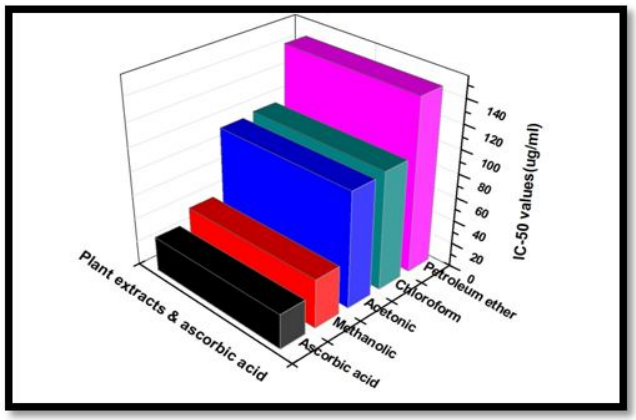


Fig. 8: IC_{50} values of plant extract of *V.cinerea* (ABTS).

Table 4: Correlation between IC_{50} Values, TPC and TFC.

Pearson correlation coefficient (r)				
TPC & TFC	**TPC & IC_{50} (ABTS)**	**TFC & IC_{50} (ABTS)**	**TPC & IC_{50}((DPPH)**	**TFC & IC_{50} (DPPH)**
0.807	-0.968	-0.896	-0.948	-0.967

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

Table 5: Antioxidant enzyme activities of *V.cinerea.*

Antioxidant enzymes Activity	
Catalase	11.07±0.23
Glutathione-S- Transferase	15.40±0.15
Superoxide dismutase	51.94±0.41

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 H_2O_2 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 1.0imol 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, acetonic extract showed seven phytoconstituents, chloroform revealed six while petroleum ether extract showed maximum twelve phytoconstituents predominantly.

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 acetonic extract showed the maximum inhibition against *S.aureus* (15.2± 0.20 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 *P.aeruginosa* 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

Table 6: Phytocompounds detected from different plant extract of aerial vegetative parts of *V. cinerea* by GC-MS.

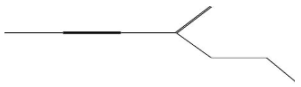
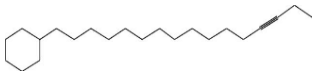
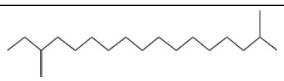
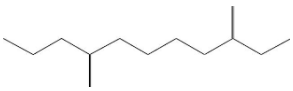
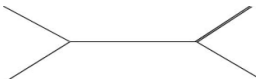
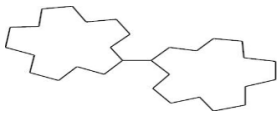
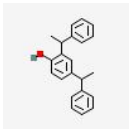
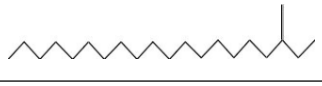
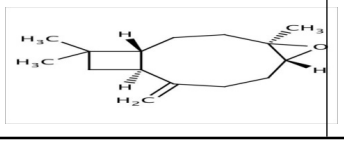
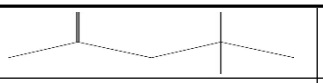
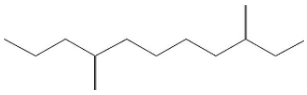
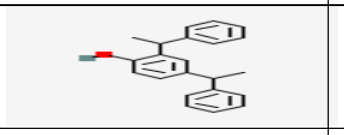
S.No	RT	Compound	MF	SI	RSI	Structure	Area%
Methanolic Extract							
1	19.90	Ethyl 2-butynoate	$C_6H_8O_2$	563	694		0.64
2	20.49	2H-Pyran, 2,2'-[1,10-decanediylbis (oxy)] bis [tetrahydro-	$C_{20}H_{38}O_4$	625	629		0.84
3	22.14	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	648	679		1.02
4	22.57	Hexadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	883	903		2.83
5	23.14	Acetic acid, dichloro-	$C_2H_2C_{l2}O_2$	660	811		0.74
6	24.25	(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	$C_{20}H_{38}O_{10}$	661	694		1.71
7	24.64	Phenol, 2,4-bis(1-phenylethyl)- (Phenolic compound)	$C_{22}H_{22}O$	724	744		1.72
8	25.52	Methyl strearate	$C_{19}H_{38}O_2$	857	874		2.99
9	25.94	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	926	927		48.46
10	26.88	Caryophyllene-oxide	$C_{15}H_{24}O$	909	912		5.29
Acetonic Extract							
1	9.06	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	815	815		11.19
2	22.57	Hexadecanoic acid, methyl ester	$YC_{17}H_{34}O_2$	808	811		2.98
3	24.67	Phenol, 2,4-bis(1-phenylethyl)- (Phenolic compound)	$C_{22}H_{22}O$	553	604		1.33
4	25.51	Methyl stearate	$C_{19}H_{38}O_2$	850	862		3.34
5	25.93	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	926	926		46.12

Table 6 contd....

Table 6 contd....

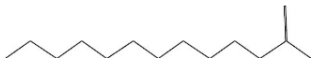
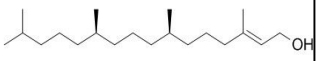
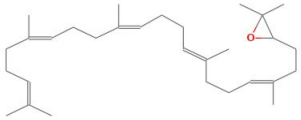
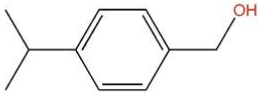
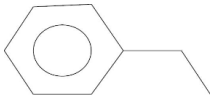
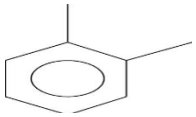
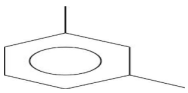
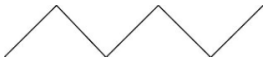
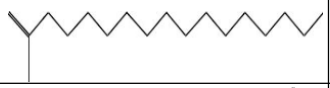
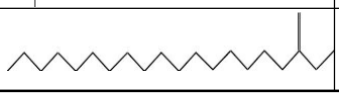
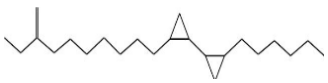
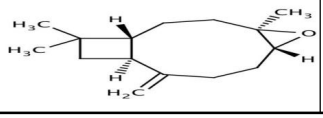
S.No	RT	Compound	MF	SI	RSI	Structure	Area%
6	26.68	Dodecanoic acid	$C_{12}H_{24}O_2$	674	785		2.29
Chloroform Extract							
1	10.22	Sabinene	$C_{10}H_{16}$	913	918		0.40
2	17.13	n-Nonadecanol-1	$C_{19}H_{40}O$	927	928		0.77
3	22.89	Phytol (Diterpene)	$C_{20}H_{40}O$	918	923		0.55
4	23.86	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	924	925		0.92
5	25.93	Oxirane,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosa-pentaenyl)-(all-E)-(Triterpenoids)	$C_{30}H_{50}O$	927	929		2.10
Petroleum Ether Extract							
1	3.27	p-Cymen-7-ol	$C_{10}H_{14}O$	939	941		7.88
2	4.83	Ethylbenzene	C_8H_{10}	942	949		3.55
3	4.96	O-Xylene	C_8H_{10}	943	943		10.86
4	5.82	Benzene, 1,3-dimethyl-	$C_{11}H_{16}O$	912	916		1.88
5	6.29	Ethanol, 2-ethoxy-	$C_4H_{10}O_2$	804	924		1.48
6	9.31	Pentadecane	$C_{15}H_{32}$	850	859		1.84
7	22.57	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2 pentylcyclopropyl) methyl]cyclopropyl] methyl]	$C_{18}H_{38}$	784	794		1.66
8	24.24	cyclopropyl]methyl]-, methyl Ester n-Hexadecanoic acid	$C_{16}H_{32}O_2$	812	837		5.34
9	25.51	Methyl stearate	$C_{19}H_{38}O_2$	796	826		1.90

Table 6 contd....

Table 6 contd....

S.No	RT	Compound	MF	SI	RSI	Structure	Area%
10	25.93	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	904	908		24.12
11	26.88	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	778	817		2.23
12	27.54	Caryophyllene-oxide	$C_{15}H_{24}O$	700	720		1.93

RT- Retention time; MF- Molecular formula; SI-Similarity index; RSI- Reverse similarity index, CAS#-Chemical abstracts services; Area%-peak area.

present in methanolic extract.

Similar to the results obtained with antibacterial properties, both methanolic and chloroform extracts also exhibited good antifungal efficacy showing a prominent zone of inhibition (15.3±0.21 mm, 23.3±0.39mm) against *P.expansum* and *R.oryzae* respectively strains used in the study. *A. niger* and *F. oxysporum* were found to be resistant to all the extracts except methanolic extract. Other workers have a similar observation that fungi are more resistant to plant extracts than bacteria (Obeidat, 2018 and Papaneophytou *et al.,* 2020) *R. oryzae* was the most sensitive fungal strain and it was inhibited by all the extracts including petroleum ether extract**.** As the plant extracts exhibited good antibacterial and antifungal activities, it shows that *V. cinerea* possesses a broad spectrum of antimicrobial compounds.

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 possesses 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 phytocompounds. 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

P.E-Plant extracts

B.O.D-Biological oxygen demand

DMSO-Dimethyl sulfoxide

EC-Extinction coefficient

H_2O_2-Hydrogen peroxide

V.cinerea- Vernonia cinerea

References

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol*., **4:** 177. doi: 10.3389/fphar.2013.00177

Cheesman, M. J., A. Ilanko, B. Blonk and I.E. Cock (2017). Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution? *Pharmacognosy reviews*, **11(22):** 57–72. doi:10.4103/phrev.phrev_21_17

Pan, S.Y., S.F. Zhou, S.H. Gao, Z.L. Yu, S.F. Zhang, M.K. Tang and K.M. Ko (2013). New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine*, 2013.

Atanasov, A.G., B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin and J.M. Rollinger (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology advances*, **33(8):** 1582-1614.

Prasopthum, A., P. Pouyfung, S. Sarapusit, E. Srisook and P. Rongnoparut (2015). Inhibition effects of Vernonia cinerea active compounds against cytochrome P450 2A6 and human monoamine oxidases, possible targets for reduction of tobacco dependence. *Drug metabolism and pharmacokinetics*, **30(2):** 174-181.

Youn, U.J., G. Miklossy, X. Chai, S. Wongwiwatthananukit, O. Toyama, T. Songsak and L.C. Chang (2014). Bioactive sesquiterpene lactones and other compounds isolated from Vernonia cinerea. *Fitoterapia*, **93:** 194-200.

Inpuron, S., L. Musikachat, W. Boonmark, K. Jarmkom, S. Suchaitanavanit and P. Eakwaropas (2013). Antioxidant activities of Vernonia cinerea and Murraya siamensis. In *Ist Academic Science and Technology Conference ASTC2013*, 13-15.

Alara, O.R., N.H. Abdurahman and O.A. Olalere (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. *Food and Bioproducts Processing*, **107:** 36-48.

Gul, R., S.U. Jan, S. Faridullah, S. Sherani and N. Jahan (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. *The Scientific World Journal*, 2017.

Thangaraj, P. (2016). *Pharmacological assays of plant-based natural products*. Geneva, Switzerland: Springer.

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*.

Performance, C.L.S.I. (2017). Standards for antimicrobial susceptibility testing.

Fabry, W., P.O. Okemo and R. Ansorg (1998). Antibacterial activity of East African medicinal plants. *Journal of ethnopharmacology*, **60(1):** 79-84.

Adebiyi, O.E., F.O. Olayemi, T. Ning-Hua and Z. Guang-Zhi (2017). *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of Grewia carpinifolia. *Beni-Suef University Journal of Basic*

and Applied Sciences, **6(1):** 10-14.

Aebi, H. (1983). Catalase. In: Bergmeyer H.U. (ed). Methods of Enzymatic Analysis. Verlag Chemie, Weinhan : 673-84.

Misra, H.P. and I. Fridovich (1972). The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *J. Biol. Chem.*, **247:** 3170-3175.

Habig, W.H., M.J. Pabst, G. Fleischner, Z. Gatmaitan, I.M. Arias and W.B. Jakoby (1974). Glutathione S-Transferases: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem*., **249:** 7130-7139.

Wu, Z.N., Y.B. Zhang, N.H. Chen, M.J. Li, M.M. Li, W. Tang and G.C. Wang (2017). Sesquiterpene lactones from Elephantopus mollis and their anti-inflammatory activities. *Phytochemistry*, **137:** 81-86.

Ruwali, P. and D. Negi (2019). Phytochemical analysis and evaluation of antioxidant activity of Premna latifolia Roxb. A medicinal plant (Family: Lamiaceae). *The Pharma Innovation Journal*, **8(5):** 13-20.

Oribayo, O.O., M.A. Owolabi, G.E. Ukpo and F.O. Shode (2018). Antioxidant activity of some Nigerian medicinal plants used in malaria treatment. *Trop. J. Nat. Prod. Res.*, **2(1):** 18-22.

Lefahal, M., N. Zaabat, R. Ayad, E.H. Makhloufi, L. Djarri, M. Benahmed and S. Akkal (2018). *In vitro* Assessment of Total Phenolic and Flavonoid Contents, Antioxidant and Photoprotective Activities of Crude Methanolic Extract of Aerial Parts of *Capnophyllum Peregrinum* (L.) Lange (Apiaceae) Growing in Algeria. *Medicines*, **5(2):** 26.

Tongur, T., N. Erkan and E. Ayranci (2018). Investigation of the composition and antioxidant activity of acetone and methanol extracts of *Daphne Sericea* L. and *Daphne Gnidioides* L. *Journal of food science and technology*, **55(4):** 1396-1406.

Oyedemi, B.O., S.O. Oyedemi, S.S. Swain, J.M. Prieto and P. Stapleton (2020). Bactericidal and antibiotic-modulation activities of methanol crude extracts of Ligustrum lucidum and Lobelia inflata against MRSA phenotypes: Molecular docking studies of some isolated compounds from both plants against DNA gyrase A. *South African Journal of Botany*, **130:** 54-63.

Markandan, S., A. Abdullah, K.H. Musa, V. Subramaniam and K. Stockham (2016, November). Determination of antioxidant activities, total phenolic and flavanoid contents in Bougainvillea glabra bracts at various methanol concentrations. In *AIP Conference Proceedings,* **1784(1):** 030-038. AIP Publishing LLC.

Rizvi, S.M.D., D. Biswas, J.M. Arif and M. Zeeshan (2011). In-vitro antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. *Int. J. Pharm. Sci. Rev. Res*., **9:** 164-169.

Obeidat, M. (2018). Antimicrobial activities from extracts of seven medicinal plant species against multidrug-resistant bacteria and fungi.

Papaneophytou, C., I. Giannenas and C. Dragomir (2020). Resistance of bacteria, fungi, and parasites to antibiotics or natural substances of botanical origin. In *Feed Additives*, 339-354. Academic Press.

Mahboubi, A., J. Asgarpanah, P.N. Sadaghiyani and M. Faizi (2015). Total phenolic and flavonoid content and antibacterial activity of *Punica Granatum* L. Var. Pleniflora flowers (Golnar) against bacterial strains causing foodborne diseases. *BMC compl. and Alt. Med*., **15:** 366.

Daffodil, E.D. and V.R. Mohan (2013). Study of whole plant of *Vernonia cinerea less*. For *In vitro* antioxidant activity. *World J. Pharm. Pharmaceu. Sci*., **2:** 3710-3722.

Rajamurugan, R., N. Selvaganabathy, S. Kumaravel, C.H. Ramamurthy, V. Sujatha and K.M. Suresh (2011). Identification, quantification of bioactive constituent's evaluation of antioxidant and *in vivo* acute toxicity property from the methanol extract of Vernonia cinerea leaf. *Pharm. Biol*., **49:** 1311-20.

Maity, S., R. Banerjee, P. Goswami, M. Chakrabarti and A. Mukherjee (2018). Oxidative stress responses of two different ecophysiological species of earthworms (Eutyphoeus waltoni and Eisenia fetida) exposed to Cd-contaminated soil. *Chemosphere*, **203:** 307-317.

Abirami, P. and A. Rajendran (2011). GC-MS determination of bioactive compounds of Indigoferaaspalathoides. *J. Nat. Prod. Plant Resour.*, **1:** 126–130.

Kala, S.M.J., T. Balasubramaniam, P.T. Soris and V.R. Mohan (2011). GC-MS determination of bioactive components of Eugenia singampattianaBedd. *Int. J. Chem.Tech. Res*., **3:** 1534–1537.

Parthiban, B., M.G.T. Suky and V.R. Mohan (2015). GC-MS analysis of phytocomponents in *Pleiospermium Alatum (*Wall.Ex Wight &Arn.) Swingl (Rutaceae). *J. of Pharmacogn. Phytochem.,* **4:** 216-222.

Kang, S.N., Y.M. Goo, M.R. Yang, R.I.H. Ibrahim, J.H. Cho and I.S. Kim *et al*. (2013). Antioxidant and Antimicrobial Activities of Ethanol Extract from the Stem and Leaf of *Impatiens Balsamina* L. (Balsaminaceae) at Different Harvest Times. *Molecules,* **18:** 6356-6365.

Shukla, A. and A. Kaur (2018). A systematic review of traditional uses bioactive phytoconstituents of genus Ehretia. *Asian Journal of Pcal and Clinical Research*, **11(6):** 88-100.

Asif, S., M. Ahmad, M. Zafar and N. Ali (2017). Prospects and potential of fatty acid methyl esters of some non-edible seed oils for use as biodiesel in Pakistan. *Renewable and Sustainable Energy Reviews*, **74:** 687-702.