Free Radicals and Antioxidants, 2017; 7(2): 178-183
A multifaceted peer reviewed journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.phcog.net

Original Article

Antioxidant Activities of Root, Stem and Leaves of *Vernonia cinerea* (L) Less.

Arti Goggi, Nutan Malpathak*
Department of Botany, Savitribai Phule Pune University, Pune, Maharashtra, INDIA.

ABSTRACT

Background: *Vernonia cinerea* (L) Less has ethnomedicinal importance and is well known for its anti-inflammatory and anticancerous properties. **Objective:** Present study investigates antioxidant activity of various crude extracts prepared from different organs of plant such as Root, Stem and Leaves using polar and nonpolar solvents. **Materials and methods:** The antioxidant activity was determined using different methods which includes radical scavenging assays (DPPH and ABTS) and phosphomolybdenum assay. LC- MS profiling was performed for the extracts showing maximum antioxidant activity to identify bioactive constituents. **Result:** Difference was observed in the antioxidant activities of extracts by different methods. Significant (p< 0.05) antioxidant activity was exhibited by different organs of a plant and different extracts. LC-MS analysis revealed the presence of phenols, terpenoids, quinones and steroids in the extracts. Conclusion: Different antioxidant assays help in knowing the antioxidant potential of different crude extracts. The bioactive constituents identified can be correlated to antioxidant activity and used in drug formulations.

Key words: ABTS, DPPH, LC-MS, Phosphomolybdenum, *Vernonia cinerea*.

Correspondence :

Dr. Nutan Padmanabh Malpathak,
Professor and Head in Department of Botany, Savitribai Phule Pune University, Ganeshkhind Road, Pune - 411 007, Maharashtra, INDIA.
Phone no: +91 98232 80187

E-mail: mpathak@unipune.ac.in
DOI: 10.5530/fra.2017.2.27

INTRODUCTION

The metabolic process of human body in its natural course creates several reactive oxygen species (ROS) in the form of superoxide anion (O_2-.), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{.}$). The generation of excess ROS leads to development of many diseases which include insulin resistance, Diabetes mellitus, sepsis, Obesity[1] and Alzheimer's.[2] In order to treat several such diseases an antioxidant based therapeutics is suggested. Such prominent remedies are commonly used for treatment of diabetic neuropathy,[3] venous insufficiency[4] and neuroprotection[5]

There is a rising interest among the researchers to search and extract antioxidants from various medicinal plants, vegetables and fruits. These antioxidants are free radical scavengers that stop free radical chain reactions and support such treatments. Ethnomedicinal plants are good sources of antioxidants and are a potential source in the development of drugs and the treatment of different ROS generated diseases. Many compounds such as Vitamin C, Vitamin E, carotenoids and polyphenols from different plant sources have capacity to scavenge free radicals and are well recognized as antioxidants.[6]

Vernonia cinerea (L) Less plant is ethnomedicinally important. It is traditionally used as febrifuge, diaphoretic, diuretic, antispasmodic and anthelmintic.[7] Various parts of the plant are reported to contain sterols such as stigmasterols, sitosterols and spinasterols; triterpenoids like amyrin and lupeol; sesquiterpenes, glycosides and flavonoids.[7-10] Experimental evidences strongly suggest the anti-inflammatory and cytotoxic properties of plant extracts.[8,11] The earlier experiments have investigated the antioxidant activities of *V. cinerea* plant using whole plant and leaf extract from single solvent.[11,12]

In the present study we considered the importance of medicinal properties and bioactive compounds of *V. cinerea* and carried out a comprehensive evaluation of antioxidant activities of root, stem and leaf extracts.

Based on earlier studies, it was recommended to use a multiple methods to determine antioxidant activities[13] so as to understand different mechanisms involved in antioxidation process. We used three different assays and compared the antioxidant activities of root, stem and leaf extracts prepared using both polar and non-polar solvents. The LC- MS profiling identified compounds from the extracts showing significant antioxidant activity. This identification of phytoconstituents will help in isolation of compounds which are natural sources of antioxidant molecules.

MATERIALS AND METHODS

2, 2 - Diphenyl- 1- Picrylhydrazyl (DPPH), 2, 2- Azinobis (3 ethylbenzothiazoline 6 sulfonic acid) (ABTS) were purchased from Sigma Aldrich USA. Other chemicals and solvents used were of analytical grade and purchased from local distributor.

Collection of Plant material

Plants of *Vernonia cinerea* were uprooted from different regions of Pune ($18^0$32'17.6"N73^{0}48'20.4"E and $18^0$31'35.0"N 73^{0}50'43.5"E). Plant was authenticated from BSI and Voucher specimen was submitted with ARGVEC3 code. The parts of plant, root, stem and leaves were separated and dried in shade.

Preparation of plant extracts

Air dried plant parts were powdered and stored in air tight containers. The powder was extracted by cold maceration technique at room temperature for 24 hours with constant shaking. The extracts were prepared in range of polar and nonpolar solvents as water, ethanol, methanol, acetone, ethyl acetate, petroleum ether and chloroform for extraction of different types of phytoconstituents. The proportion of plant material to solvent was 1:10(w/v).The extracts were filtered, centrifuged and dried by evaporation. The dried extract were suspended in DMSO to obtain yield of 10mg/ml of extract and stored at -20°C until they were used. All 21 extracts were evaluated for their potential for antioxidant activity using three assays.

DPPH radical scavenging assay

The assay was carried out according to Blois (1958).[14] DPPH (0.1 mM) was prepared in 100% methanol. To 1 ml of DPPH solution, 200 µl of

extract was added in a range of concentration (50 μg to 250 μg/ml). The mixture was incubated for 30 minutes in dark. After dilution, the absorption of reduced DPPH was measured at 517 nm (UV-VIS spectrophotometry). Methanolic DPPH was used as control. The Radical scavenging activity (RSA) was calculated in percentage by following formula.

RSA (%) = (Abs control - Abs sample /Abs control)* 100

The results were also reported as EC_{50} which is amount of antioxidant required to decrease the DPPH· concentration by 50%.EC_{50} values were calculated from regression analysis and expressed as μg dry weight equivalents per ml sample. The experiments were performed in triplicates. The results are expressed in average ± standard deviation.

ABTS radical scavenging assay

The assay was carried out according to the procedure given in review article.[15] Reagent was prepared by mixing 10 ml of ABTS (14 mM) and 10 ml potassium persulphate (4.9 mM). The mixture was kept in the dark for 16 h. The absorbance of the reagent was adjusted with distilled water to 0.700 ± 0.02 at 734 nm and then used for the assay. A stable ABTS radical cation is produced by oxidation of ABTS by potassium persulphate. 200 μl of plant extract in a range of concentration (50 μg to 250 μg) was added to 1 ml reagent mixture. After incubation for 10 minutes the reduction of radical cation was measured at 734 nm.

The results were expressed in terms of RSA in percentage. The results are also reported as EC_{50} which is amount of antioxidant required to reduce the ABTS radicals by 50%. ABTS reagent without plant extract was used as control whereas Ascorbic acid as positive control.

RSA (%) = (Abs control - Abs sample /Abs control)* 100

Phosphomolybdenum assay

Phosphomolybdenum reduction potential of plant extracts were determined according to Prieto *et al.*[16] Reagent was prepared by mixing ammonium molybdate (4 mM), sulphuric acid (0.6 M) and monosodium phosphate (28 mM) and final volume was made as per requirement.

Assay mixture contained 200 μl of plant extract in a range of concentration (50 μg to 250 μg), and 1ml reagent solution. The mixture was incubated at 95^0C for 90 minutes. The absorbance of green complex was measured at 695 nm. The reagent itself was used as blank and Ascorbic acid as positive control.

The result is represented in terms of the number of microgram equivalents of ascorbic acid per milligram of plant extract.

LC-MS profiling

LC- MS profiling of crude extracts showing maximum antioxidant activity was carried out.LC- MS analysis was performed on Agilent 1260 binary LC system and separated on Agilent Zorbax Extend C 18 RRHT column (50x2.1 mm, 1.8 μm) using aqueous 0.1% formic acid (mobile phase A) and Acetonitrile (mobile phase B). A stepwise gradient of solvent A and solvent B was applied at flow rate of 0.3 ml/min for 30 minutes. The capillary voltage, cone voltage, fragmentor voltage were 4kV, 45V and 170 V, respectively. The gas temperature was set at 325^0. Electroscopy mass spectra data were recorded on a positive ionization mode. The data was acquired at scan rate of 3Hz in mass range 100- 1000 m/z and analyzed with Mass hunter qualitative software and METLIN database.

Statistical analysis

The antioxidant activity of various extracts using different methods was analysed by two Factor Analysis of variance (ANOVA) followed by Tukeys post hock test using SPSS version 24. All the experiments were performed in triplicates.

RESULT

DPPH radical scavenging assay

DPPH radical scavenging activity was measured spectrophotometrically after incubation of plant extracts with DPPH for 30 minutes in dark. In the chosen plant extracts the highest DPPH radical scavenging activity was observed in methanolic extract (45.12% EC 50 312.06 μg/ml) among various leaf extracts; ethanolic (54 % EC 50 164.53 μg/ml) and methanolic (52.67% EC 50 232.33 μg/ml) among various stem extracts; methanolic (37.04% EC 50 442.27 μg/ml) among root extracts. As compared to root extracts, the antioxidant activity of stem and leaf extracts were significantly different (p 0.05) (Figure 1).

ABTS radical scavenging activity

ABTS radical scavenging potential of leaf methanolic extract (45.56 % EC 50 355.06 μg/ml) was highest among other extracts. Whereas in stem, it was highest in methanolic extract (56.13% EC 50 264.12 μg/ml) compared to other extracts. Methanol extract (52.12 % EC 50 257.28 μg/ml) of root has shown maximum antioxidant activity. There was significant difference in antioxidant activity among extracts of different plant parts ($p< 0.05$) (Figure 1).

Phosphomolybdenum assay

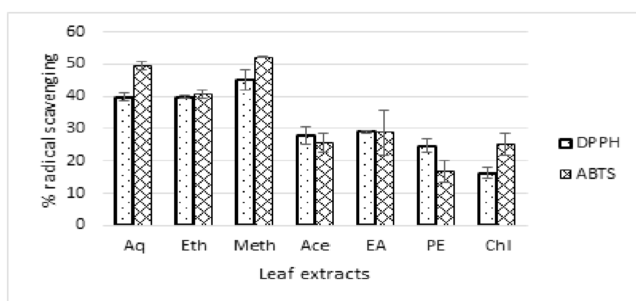
The reduction of Mo was highest with methanolic leaf extract (129.56 μmolAAE/mg of extract), methanolic stem extract (307.37 μmolAAE/mg of extract) and aqueous root extract (368.29 μmolAAE/mg of extract). Phosphomolybdenum reduction potential of different extracts from root, stem and leaf are shown in Figure 2.

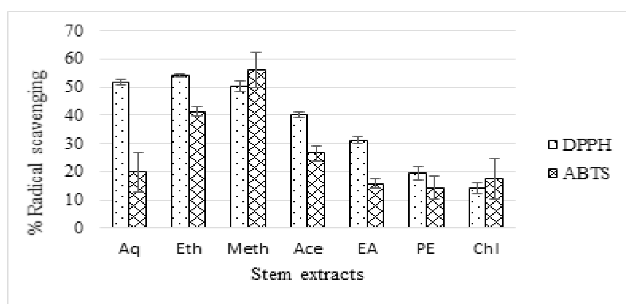
LC- MS profiling

MS data analysis using Mass Hunter and METLIN database search reveals presence of various primary and secondary metabolites and intermediate compounds. The identified secondary metabolites predominantly belongs to sesquiterpenes, diterpenes, triterpenes, steroids, glucuronides, quinones and phenols. The comparison of the phytochemicals in three different extracts along with molecular formula, molecular mass and m/z is shown in Table 2.

Table 1: ANOVA for antioxidant activity by DPPH, ABTS and Phosphomolybdenum assays using different extracts from root, stem and leaf

Source	Df	MS	P
DPPH			
Extracts	6	1026.522	< 0.05
Organs	2	938.114	< 0.05
Extracts x Organs	12	105.721	< 0.05
Error	42	3.539	
ABTS			
Extracts	6	930.941	< 0.05
Organs	2	488.777	< 0.05
Extracts x Organs	12	245.922	< 0.05
Error	42	19.859	
Phosphomolybdenum			
Extracts	6	.012	< 0.05
Organs	2	.022	< 0.05
Extracts x Organs	12	.008	< 0.05
Error	42	.000	





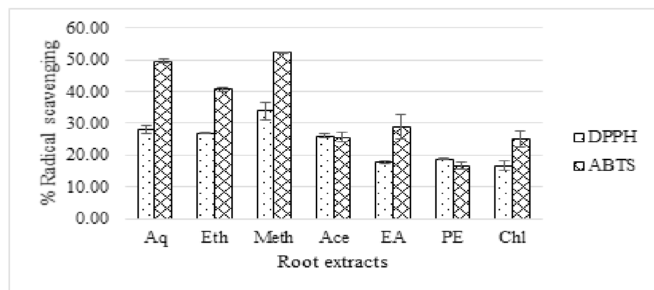


Figure 1: Comparison of DPPH and ABTS radical scavenging activity (%) of different leaf extracts (A), stem extract (B) and root extract (C). Values are mean of three replicates ± Std. dev. Aq = Aqueous, Eth = Ethanol, Meth = Methanol, Ace = Acetone, EA = Ethyl acetate, PE = Petroleum ether, Chl = Chloroform. The error bars represent standard deviation. The activity is significant among the extracts and organs ($p < 0.05$)

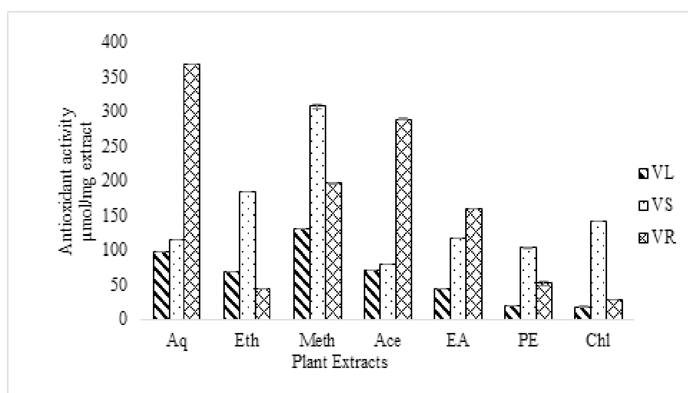


Figure 2: Phosphomolybdenum assay using extracts from different plant parts. Values are mean of three replicates ± Std. dev. VL = Leaf Extract, VS = Stem Extract, VR = Root Extract, Aq = Aqueous, Eth = Ethanol, Meth = Methanol, Ace = Acetone, EA = Ethyl acetate, PE = Petroleum ether, Chl = Chloroform. The reduction of phosphomolybdenum complex is significant among organs and extracts ($p < 0.05$)

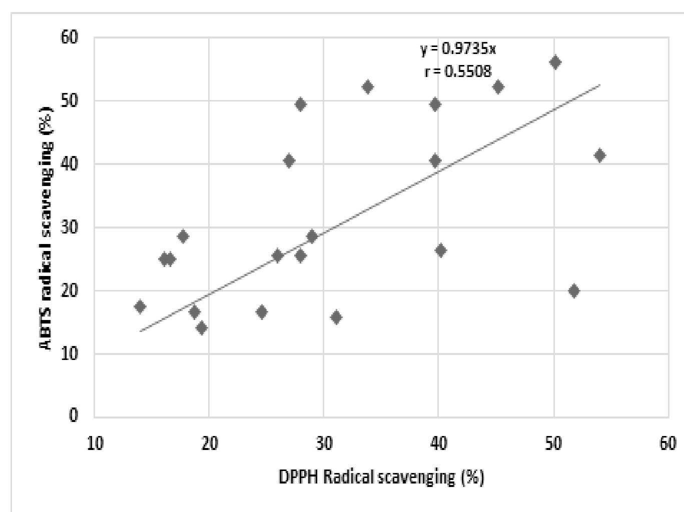


Figure 3: Correlation between % radical scavenging activity by DPPH and ABTS assays.

DISCUSSION

The aim of the present study was to determine and compare the antioxidant activity of different organs of *V. cinerea* which is an ethnomedicinal plant and identification of probable bioactive phytoconstituents with antioxidant properties. We have carried out comprehensive study by choosing polar and nonpolar extracts of root, stem and leaves of a plant body.

The antioxidant assays carried out using DPPH and ABTS radicals. They are Single Electron Transfer (SET) reactions where reaction is either electron transfer or through hydrogen atom transfer.[17] $ABTS^{\cdot +}$ method is considered to be more sensitive since they react rapidly with lipophilic and hydrophilic antioxidants. The reduction of DPPH is quicker or slower process as it depends on the conformation of antioxidant compounds.[18] We observed that the reaction of antioxidants with $DPPH^{\cdot}$ was slower than that with $ABTS^{\cdot +}$ suggesting use of ABTS radicals is more suitable for determination of radical scavenging potential of crude extracts which contain mixture of compounds.

There was difference in the antioxidant activity in DPPH and ABTS methods but similarity was observed in the type of extract showing maximum radical scavenging activity. There is no strong correlation (Figure 3) between % scavenging measured by both tests (Pearson's correlation coefficient $r=0.558$) but is significant at $p<0.01$. These observations indicate that the behavior of antioxidant compounds towards DPPH and ABTS is different or the difference in the result is due to requirement of different incubation time by two different assays.

Results of phosphomolybdenum assay differ from DPPH and ABTS methods. The formation of green complex was less in extracts of nonpolar solvent compared to extracts of polar solvent. Aqueous and acetone extracts of root have exhibited significant antioxidant activity compared to other extracts. In phosphomolybdenum assay the antioxidant activity was higher in root extracts whereas in DPPH and ABTS assays radical scavenging activity was high in stem and leaf extracts. This result indicates that the reduction of Mo depends on the types and amount of antioxidants.

In three different assays there was significant difference in antioxidant activities by extracts (Table 1) prepared in polar and nonpolar solvents ($p<0.05$) indicating difference in the types and amounts of compounds extracted by different types of solvents.

Table 2: Comparative account of chemical composition of crude extracts from LC MS

Molecules	Formula	Mass	m/z	VS Me*	VR Me*	VL Me*
Orsellinic acid, Ethyl ester	$C_{10}H_{12}O_4$	196.07	179.06	+	-	+
Geranial	$C_{10}H_{16}O$	152.11	135.11	-	-	+
Valeryl Salicylate	$C_{12}H_{14}O_4$	222.08	205.08	-	+	+
Eupatoriochromene	$C_{13}H_{14}O_3$	218.09	201.09	-	-	+
Peucenin	$C_{15}H_{16}O_4$	260.104	261.11	+	-	+
Dihydrocaryophyllen-5-one	$C_{15}H_{24}O$	220.182	203.178	+	-	+
Punctaporin B	$C_{15}H_{24}O_3$	252.171	235.168	+	+	+
N-Histidyl-2-Aminonaphthalene	$C_{16}H_{16}N_4O$	280.132	303.121	+	-	+
Tetrahydrotrimethylhispidin	$C_{16}H_{20}O_5$	292.13	275.126	-	-	+
Mycophenolic acid	$C_{17}H_{20}O_6$	320.125	303.121	+	-	+
Emedastine	$C_{17}H_{26}N_4O$	302.21	307.18	+	+	+
Embelin	$C_{17}H_{26}O_4$	294.182	277.17	-	-	+
10-Deoxymethynolide	$C_{17}H_{28}O_4$	296.19	279.19	-	-	+
Zearalenone	$C_{18}H_{22}O_5$	318.145	319.152	-	-	+
DL-7-hydroxy stearic acid	$C_{18}H_{36}O_3$	300.26	283.26	-	+	-
1-Monopalmitin	$C_{19}H_{38}O_4$	330.27	313.27	-	+	-
Ginkgolide J	$C_{20}H_{24}O_{10}$	424.13	447.126	-	-	+
Ginkgolide C	$C_{20}H_{24}O_{11}$	440.13	463.12	-	-	+
Duartin, Dimethyl ether	$C_{20}H_{24}O_6$	360.156	361.163	-	-	+
Emodin 8-glucoside	$C_{21}H_{20}O_{10}$	432.10	433.11	-	-	+
b-D-Glucopyranosiduronic acid	$C_{21}H_{26}O_8$	406.16	309.15	+	-	+
Allopregnanolone	$C_{21}H_{34}O_2$	318.25	341.24	-	-	+
Mitoxantrone	$C_{22}H_{28}N_4O_6$	444.20	445.21	+	+	-
Anandamide (20:2, n-6)	$C_{22}H_{41}NO_2$	351.312	334.309	-	-	+
6beta-Hydroxytriamcinolone	$C_{24}H_{31}FO_7$	450.20	468.24	-	-	+
4'-Hydroxypenbutolol glucuronide	$C_{24}H_{37}NO_9$	483.24	466.24	-	-	+
Sulindac sulfide glucuronide	$C_{26}H_{25}FO_8S$	516.12	517.13	+	+	+
Propafenone glucuronide	$C_{27}H_{35}NO_9$	517.22	518.23	-	-	+
L-Oleandrosyl-oleandolide	$C_{27}H_{46}O_{10}$	530.30	513.30	-	-	+
6-Deoxotyphasterol	$C_{28}H_{50}O_3$	434.37	439.35	-	-	+
7-Deacetoxy-7-OxoKhivorin	$C_{30}H_{38}O_9$	542.25	565.24	-	-	+
Acetoxydeoxydihydrogedunin	$C_{30}H_{40}O_7$	512.278	535.267	+	+	+
Cucurbitacin J	$C_{30}H_{44}O_8$	532.30	555.29	-	-	+
3S-squalene-2,3-epoxide	$C_{30}H_{50}O$	426.38	409.38	+	-	-
Khivorin	$C_{32}H_{42}O_{10}$	586.27	609.26	+	+	+
Khayanthone	$C_{32}H_{42}O_9$	570.284	593.273	+	+	+
Cucurbitacin A	$C_{32}H_{46}O_9$	574.311	579.290	-	-	+
11alpha-AcetoxyKhivorin	$C_{34}H_{44}O_{12}$	644.28	627.27	-	-	+
Methoxyneurosporene	$C_{41}H_{62}O$	570.48	609.44	-	-	+
Allose	$C_6H_{12}O_6$	180.06	219.02	-	+	-
Pyrocatechol	$C_6H_6O_2$	110.036	93.033	-	+	+
Carnitine	$C_7H_{16}NO_3$	162.11	144.101	-	-	+
m-Salicylic acid	$C_7H_6O_3$	138.03	121.02	+	+	+
Dihydro-3-coumaric acid	$C_9H_{10}O_3$	166.06	166.08	-	-	+
Oleananoic acid acetate	$C_{32}H_{52}O_4$	500.38	483.38	-	+	-

*VR Me – Root methanol, VS Me – Stem methanol, VL Me – Leaf methanol

The importance of use of combination of methods for determination of antioxidant activity has been suggested in several studies[19,20] and it is clear from the above discussion that antioxidant activity differs with the method used and also depends upon nature of antioxidants.

In view of potential antioxidant activity of methanolic extracts of root, stem and leaves; they were subjected to LC-MS screening for identification of phytoconstituents. MS analysis reveals the presence of different classes of secondary metabolites along with primary metabolites. The metabolic diversity was observed and most prominent compounds were types of isoprenoids, steroids and phenols. In our study stem and leaf methanol extracts exhibited more radical scavenging activity compared to root methanol extracts. It can be correlated with diversity of different classes of compounds identified in stem and leaf methanol extracts. Most of the antioxidant studies are focused on isolation, identification and quantification of phenolic compounds which have proved to be having strong antioxidant property.[21,22] Different hydroxybenzoic acid and hydroxycinnamic acid derivatives are identified in methanol extracts of *V. cinerea*. It is most likely that these could be bioactive components. But the antioxidant potential of terpenoids,[23,24] quinones,[25] steroids,[26] and Chromone derivatives[27] has also been demonstrated in various studies and these classes of compounds are identified in the screened extracts. There are several reports where plants rich in terpenes and phenolics are used as food additives owing to their antioxidant and antimicrobial properties.[28] In our study the compounds identified are predominantly terpenes along with other classes. Sesquiterpenes and triterpenoids have been reported and isolated from *V. cinerea* by different investigators[9,10] and also been reported to have anti-inflammatory and cytotoxic properties.[8,11] These findings suggest that terpenes, phenolic compounds could be alone or by synergistic process responsible for antioxidant activity.

CONCLUSION

The antioxidant activity of different extracts from root, stem and leaves was comparable and significant. Use of different methods in determination of antioxidant activity suggested that the behavior of phytoconstituents is different with different oxidants. The root, stem and leaves of *V. cinerea* are source of antioxidant compounds and can be used in herbal preparations required for the treatment of ROS mediated diseases.

ACKNOWLEDGEMENT

We are grateful to Dr.R.S.Zunjarrao, Principal Modern College of Arts, Science and Commerce, Pune 411005 for providing instrumentation facility.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

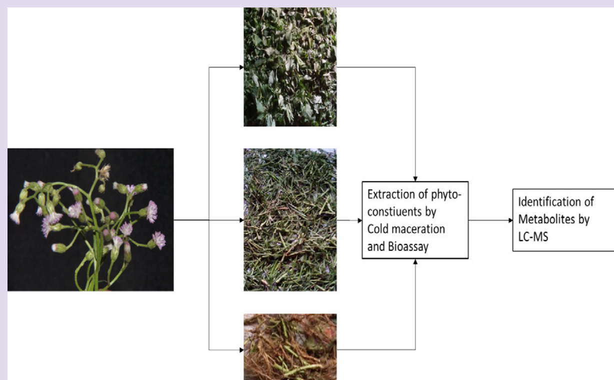
ABBREVIATION USED

DPPH: 2, 2 - Diphenyl- 1- Picrylhydrazyl; **ABTS:** 3 ethylbenzo¬thiazoline 6 sulfonic acid; **LC-MS**: Liquid Chromatography Mass Spectrometry; **RRHT column:** Rapid Resolution High Throughput.

REFERENCES

1. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. Journal of Clinical Investigation. 2004;114(12):1752-61.
2. Moreira PI, Smith MA , Zhu X, Honda K, Lee HG, Aliev G, *et al.* Oxidative damage and Alzheimer's disease: are antioxidant therapies useful? Drug News Perspect. 2005;18(1):13-9.
3. Androne L, Gavan NA, Veresiu IA, Orasan R. *In vivo* effect of lipoic acid on lipid peroxidation in patients with diabetic neuropathy. *In vivo.* 2000;14(2):327-30.
4. Petruzzellis V, Troccoli T, Candiani C, Guarisco R, Lospalluti M, Belcaro G, *et al.* Oxerutins (Venoruton): efficacy in chronic venous insufficiency- a double-blind, randomized, controlled study. Angiology. 2002;53(3):257-63.
5. Lapchak PA. Critical assessment of edaravone acute ischemic stroke efficacy trials: is edaravone an effective neuroprotective therapy? Expert. Opin Pharmacotherapy. 2010;11(10):1753-63.
6. Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. American Journal of Clinical Nutrition. 2003;78(suppl):570S-8S.
7. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary Springer Verlag Berlin. 2007;699–700.
8. Kuo YH, Kuo YJ, Yu AS, Wu MD, Ong CW, Yang Kuo LM, *et al.* Two novel sesquiterpene lactones, cytotoxic vernolide-A and -B, from Vernonia cinerea. Chem Pharm Bull (Tokyo). 2003;51(4):425-6.
9. Misra TN, Singh RS, Srivastava R, Pandey HS, Prasad C, Singh S. A New Triterpenoid from Vernonia cinerea. Planta Med. 1993;59(5):458-60.
10. Chen X, Zhan ZJ, Yue JM. Sesquiterpenoids from Vernonia cinerea. Nat Prod Res. 2006;20(2):125-9.
11. Pratheesh kP, Kuttan G. Vernonia cinerea L. scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in carrageenan induced paw edema model. Immunopharmacology and Immunotoxicology. 2009;31(1):94-102.
12. Rajamurugan R, Selvaganabathy N, Kumaravel S, Ramamurthy CH, Sujatha V, Suresh Kumar M, *et al.* Identification, quantification of bioactive constituents, evaluation of antioxidant and *in vivo* acute toxicity property from the methanol extract of Vernonia cinerea leaf. Pharmaceutical Biology. 2011;49(12):1311-20.
13. Moon JK, Shibamoto T. Antioxidant activity for Plant and Food components. J Agric Food Chem. 2009;57(5):1655-66.
14. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;29:1199-200.
15. Selvakumar K, Madhan R, Srinivasan G, Bhaskar V. Antioxidant assays in Pharmacological research. Asian J Pharm Tech. 2011;1(4):99-103.
16. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of Vitamin E. Analytical Biochemistry. 1999;269(2): 337-41.
17. Prior RL, Wu X, Schaich K. Standardized methods for the determination of Antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290-302.
18. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Food Science and Technology. 1995;28(1):25-30.
19. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis. 2002;13(1):8-17.
20. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry. 2004;85(4):633-40.
21. Ciz Milan, Cizova H, Denev P, Kratchanova M, Slavov A, Lojek A. Different methods for control and comparison of the antioxidant properties of vegetables. 2010. Food Control. 21. 518-523.
22. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry. 2004;84(4):551-62.
23. Vagi E, rapavi E, Hadolin M, Vasarhelyine PK, Balazs A, Blazovics A *et al.* Phenolics and Triterpenoid antioxidants from Origanum majorina L herb and extracts obtained with different solvents. J Agric Food Chem. 2005;53:17-21.
24. Matkowski A. Plant *in vitro* culture for the production of antioxidants. Biotechnology Advances. 2008;26(6):548–60.
25. Weng XC, Gorden MH. Antioxidant activity of Quinones extracted from Tanshen (Salvia militorrhiza Bunge). J Agric Food Chem. 1992;40:1331-8.
26. Karan SK, Mishra SK, Pal D, Mondal A. Isolation of β sitosterol and evaluation of antidiabetic activity of Aristolochia indica in alloxan induced diabetic mice with reference to *in vitro* antioxidant activity. Journal of Medicinal Plant Research. 2012;6(7):1219-23.
27. Narumol P, Weerasak S, Patcharawee N, Ungwitayatorn. *In vitro* Antioxidant activity study of Novel Chromone derivatives. Chemical Biology and Drug Design. 2012;79(6):981-9.
28. Ortega RLA, Rodriguez-Garcia I, Leyva JM, Cruz-Valenzuela MR, Silva-Espinoza BA, Gonzalez AGA, *et al.* Potential of Medicinal Plants as Antimicrobial and Antioxidant Agents in Food Industry: A Hypothesis. Journal of Food Science. 2014;79(2):129-37

PICTORIAL ABSTRACT



SUMMARY

- The study provides comprehensive analysis of antioxidant activities of V. cinerea using different organs and extraction of different phytoconstituents. The untargeted metabolic analysis using LC-MS could reveal presence of different classes of therapeutically active secondary metabolites involved in antioxidation. Methanolic extracts of plant organs are potent in antioxidant activities. Determination of antioxidant activities using different methods is useful to understand the mechanism of antioxidant activities and nature of antioxidants.

AUTHOR PROFILE

Dr. Nutan Padmanabh Malpathak: She is currently working as Professor and Head in Department of Botany, Savitribai Phule Pune University. Author actively works on Plant tissue culture, Secondary Metabolites, Metabolic Fingerprinting, Bioactivity assessment and Bio-prospecting. Author has many Publications to her name.

Mrs. Arti Praveen Goggi: She is currently working as an assistant professor at PES Modern College, Shivajinagar, Pune 411005. Mrs Goggi is pursuing the PhD at Savitribai Phule, Pune University under the guidance of Dr. Nutan Malpathak.