Available online on 15.01.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

In-vitro Antioxidant Activity and Antimicrobial Activity of Hydroalcoholic Extracts of *Vernonia cinerea*

Jaswinder Mehta[1]*, Peenu Mahendra Joshi[1], Priyanka Kushwaha[1], Geeta Parkhe[2]

[1] Career College, Kalibadi Rd Opp to Dussehra ground BHEL, Sector A, Govindpura, Bhopal, MP 462023

[2] Scan Research Laboratories, Sector A H No. 109, J K Road, Indrapuri, Bhopal , MP 462023

ABSTRACT

The aim of present study was to estimate the *in vitro* antioxidant potential and antimicrobial activity of hydroalcoholic extract of *Vernonia cinerea.* Antioxidant activity was assessed by using 2, 2- diphenyl-1-picryl-hydrazyl (DPPH) assay using ascorbic acid as standard antioxidant. The extract was found to scavenge effectively the free radicals. The total flavonoid contents were determined by established methods and were found to be 0.547 mg/100mg in quercitin equivalents. Antimicrobial activity was performed against 2 stains of human pathogenic bacteria by well diffusion method. Hydroalcoholic extract of *Vernonia cinerea* showed good antimicrobial activity against gram positive bacteria. The antioxidant activities may be attributed to the presence of significant amounts of flavonoid compounds. Results indicated that hydroalcoholic extract of *Vernonia cinerea* possess significant antioxidant effect in dose dependent manner, followed by the hydroalcoholic extract of *Vernonia cinerea* possessed good antimicrobial activity.

Keywords: Antioxidant activity, Radical scavenging activity, Free radicals, Antimicrobial activity.

Article Info: Received 03 Dec 2018; Review Completed 09 Jan 2019; Accepted 11 Jan 2019; Available online 15 Jan 2019

Cite this article as:

Mehta J, Joshi PM, Kushwaha P, Parkhe G, *In-vitro* Antioxidant Activity and Antimicrobial Activity of Hydroalcoholic Extracts of *Vernonia cinerea*, Journal of Drug Delivery and Therapeutics. 2019; 9(1):225-228

DOI: http://dx.doi.org/10.22270/jddt.v9i1.2226

***Address for Correspondence:**

Jaswinder Mehta, Career College, Kalibadi Rd, Opp to Dussehra ground BHEL, Sector A, Govindpura, Bhopal, MP 462023

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which react with other molecule by taking or giving electrons, and involved in many pathological conditions[1]. Free radicals can be described as chemical species that have an unpaired electron and play very important role in human health and beneficial in combating against several diseases like cardiovascular disorders, lung damage, inflammation etc. These free radicals are highly unstable and when the amount of these free radicals exceed in the body, it can damage the cells and tissues and may involved in several diseases. Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals[2,3]. The effect of plant extracts on microorganisms has been studied by a very large number of researchers in different parts of the world[4-6]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases[7]. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities[8]. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms[9].This paper deals with the antioxidant and antimicrobial activity of *Vernonia cinerea* which is not scientifically proven. *Vernonia cinerea* (Family: Asteraceae) is a terrestrial annual erect herb. *V. cinerea* is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders[10]. Toxicity study of the plant on mice was carried out but the results were inadequate for definite conclusion[11]. Chloroform extract of stem-bark and leaves of *Vernonia cinerea* showed diuresis property but methanolic extract exhibited antidiuresis[12]. Both polar and non-polar fraction of the plant extract showed analgesic, antipyretic and anti inflammatory effect[13]. Polar extract of *V. cinerea* is found to have antidiarrhoeal activity[14] but there is no study on non-polar fraction. Antibacterial[15] and anti larval activity against filarial vector[16] was reported but no information regarding antifungal and antiprotozoal activity is found. Carbon tetrachloride fraction of methanolic extract possesses significant antioxidant properties[17] but whether this plant extract could affect anticholinesterase and thus finally be used for treating Alzheimer disease because of antioxidant property is not reported.

MATERIALS AND METHODS

Plant material collection

The Roots of *Vernonia cinerea* was collected from local area of Bhopal (M.P.) in the month of Jan, 2018.

Extraction procedure

Vernonia cinerea (Root) was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Dried powdered *Vernonia cinerea* (Root) was extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40 ˚C.

Phytochemical screening

The *Vernonia cinerea* roots extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate and Harborne[18,19]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

Total flavonoid content estimation

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm[20].

TLC (Thin Layer Chromatography) profile

For the separation of different phytochemical compounds in the hydroalcoholic extract of *Vernonia cinerea*, the extract was spotted manually using a capillary tube on pre coated silica gel G TLC plates (15X5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Wagner et al. (1996 & 1984) [21,22]. After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 10% ethanolic sulphuric acid, 10% sulphuric acid, 5% ferric chloride, Kedde reagent, vanillin phosphoric acid reagent and vanillin sulphuric acid reagent were used to identify the respective compounds. The colour of the spots was noted and Rf values were calculated by using the following formula:

$$\text{Retention time (Rf)} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Antimicrobial activity

Pathogenic antimicrobial used

The pathogenic bacteria and fungus used in the current study was obtained from Microbial Culture Collection, National Centre for Cell Science, Pune, Maharashtra, India.

Antibiogram studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37°C for 24-48 hrs. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of the extract prepared from the plant material of *Vernonia cinerea*, using standard procedure[23]. There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37 ºC for 24 hrs and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

In-vitro free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH)

The DPPH radical scavenging activity of all the extracts was evaluated by the method described by Lee JY *et al.,* 2004[24] with slight modification. Ascorbic acid (10-100 µg/ml) was used as the standard. Plant extract (1.5 ml) at different concentrations (10-100 µg/ml) were treated with 1.5 ml of 0.2 mmol DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol solution. The reaction mixture was incubated in the dark at room temperature for 30 min. The absorbance of the sample and standards was measured at 517 nm. The ability of the plant extract and standard to scavenge the DPPH radical was calculated as percentage inhibition of absorbance by using the following formula and IC_{50} values were determined.

$$\text{Calculation of \% reduction} = \frac{\text{Control Abs} - \text{Test Abs}}{\text{Control Abs}} \text{X } 100$$

RESULTS AND DISCUSSION

Phytochemical screening of the plant showed the presence of flavonoids, proteins and amino acids and carbohydrates table 1.

Table 1: Result of phytochemical screening of hydroalcoholic extract of *Vernonia cinerea*

S. No.	Constituents	Root
1.	Alkaloids	-
2.	Flavonoids	+
3.	Diterpenes	-
4.	Phenolics	-
5.	Amino Acids	+
6.	Carbohydrate	+
7.	Proteins	+
8.	Saponins	-

A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned in table 2. After development of plates, they were air-dried and numbers of bands were noted & RF values were calculated.

Table 2: Calculation of R_f Value

Compound	Extract	Rf Value
Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.581

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.040X + 0.009, R^2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance table 3 and fig 1.

Table 3: Preparation of calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

The total flavonoid contents were determined by established methods and were found to be 0.547 mg/100mg in quercitin equivalents table 4. The lawn cultures were prepare with all the microbes used under present study and sensitivity of bacteria towards the various phytochemicals extracts obtained from the *Vernonia cinerea* was studied at the concentration of 25-100 mg/ml using well diffusion method. Antimicrobial activity was performed against 2 stains of human pathogenic bacteria by well diffusion method. Hydroalcoholic extract of *Vernonia cinerea* showed good antimicrobial activity against selective bacteria table 5.

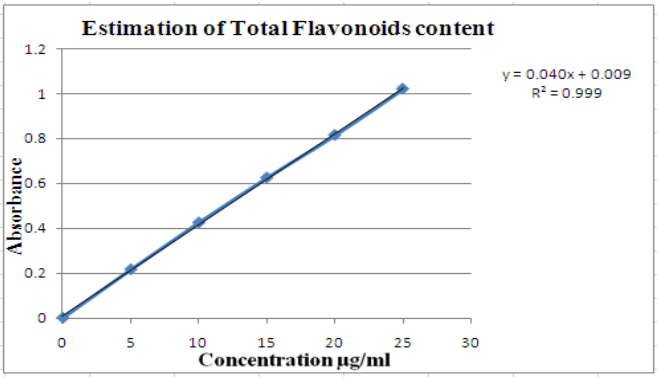

Figure 1: Estimation of total flavonoids content

Table 4: Estimation of total flavonoids content of *Vernonia cinerea*

S. No	Plant material	Total flavonoids Equivalent to Quercetin mg/ 100 mg of extract
1.	*Vernonia cinerea*	0.547

Table 5: Antibacterial activity of *Vernonia cinerea* on different microbes

Extract	Name of microbes	Zone of inhibition		
		100mg/ml	50 mg/ml	25mg/ml
Hydro alcoholic of *Vernonia cinerea*	*E. faecalis*	13	10	7
	Salmonellla bongori	20	18	16

Table 6: Result of *in vitro* free radical scavenging activity

S. No	Ascorbic acid			*Vernonia cinerea*		
	Conc.	Test	% Inhibition	Conc.	Test	% Inhibition
1	10	0.521	35.199	10	28.10945	23.45857
2	20	0.385	52.114	20	50.87065	39.49904
3	40	0.331	58.831	40	54.35323	54.04624
4	60	0.221	72.512	60	67.16418	68.30443
5	80	0.125	84.453	80	74.62687	70.52023
6	100	0.082	89.801	100	77.11443	74.85549
IC_{50} (µg/ml)			25.025	**IC_{50}**		33.979

Absorbance of 0.1mM DPPH (Ao) = 0.804

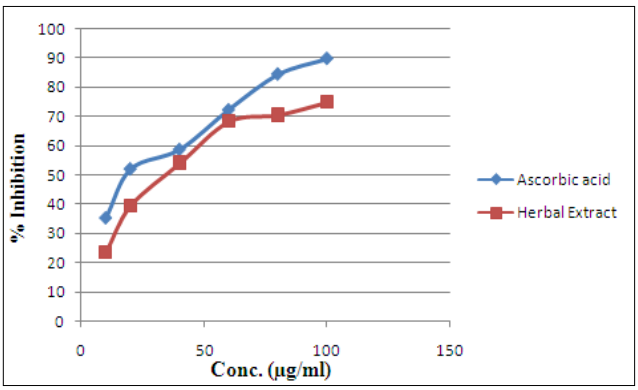

Figure 2: *In vitro* free radical scavenging activity

DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm. It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation. IC_{50} for standard ascorbic acid was found to be 25.025µg/ml and for hydroalcoholic extract of *Vernonia cinerea* was found to be 33.979 µg/ml. Thus the anti-oxidant activity of sample was less than the standard table 6 and fig 2. In order to study the effects of these compounds on biological system needs more studies as these compounds might be responsible for use of this plant in different diseases [25].

CONCLUSION

The hydroalcoholic extract of *Vernonia cinerea* showed antioxidant activity by inhibiting DPPH and total flavonoid content and reducing power activities. The preliminary phytochemical investigation indicates the presence of flavonoids in the plant material. In addition, the hydroalcoholic extract of *Vernonia cinerea* found to contain a noticeable amount of flavonoids, which play a major role in controlling antioxidants. The results of this study show that the hydroalcoholic extract of *Vernonia cinerea* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of hydroalcoholic extract of *Vernonia cinerea* are currently unclear. Therefore, further works have been performed on the isolation and identification of the antioxidant components present in hydroalcoholic extract of *Vernonia cinerea*.

REFERENCES

1. Madhavi D.L., Deshpande S.S. & Sulunkhe D.K., Food antioxidants: technological, toxicological and health perspectives; New york: Marcel dekker., 1996.
2. Upadhye M., Dhiman A., Shriwaikar A., Antioxidant activity of aqueous extract of Holostemma Annulare (Roxb) K Schum. Adv Pharmacol Toxicology., 2009; 10 (1):127–131.
3. Mishra J., Srivastava R.K., Shukla S.V., Raghav C.S., Antioxidants in Aromatic and Medicinal plants. Science tech entrepreneur., 2007, 1–16.
4. Mahansen AM, Abbas, Antimicrobial activity of extracts of extracts of herbal plants used in the traditional medicine of Bahrain, Phytotherapy Research, 1996; 10:251-253.
5. Kývçak BT, Mert HT, Öztürk, Antimicrobial and cytotoxic activities of *Cerratonia siliqua* L. extracts, Turkish Journal of Biology, 2002; 26:197-200.
6. Kumar PV, Chauhan SN, Padh H, Rajani M, Search for antibacterial and antifungal agents from selected Indian medicinal plants, Journal of Ethnopharmacology, 2006; 107:182-188.
7. Neha Shekhawat, Rekha Vijayvergia, Evaluation of Antimicrobial Potential of Some Medicinal Plants against Plant and Human Pathogens, Journal of Pharmacy Research, 2010; 3(4):700-702.
8. Hamburger M, Hostettmann K, Bioactivity in plants: the link between phytochemistry and medicine, Phytochemistry, 1991; 30:3864–3874.
9. Lee CK, Kin H, Moon KH, Shun KH, Screening and isolation of antibiotic resistance inhibitors from herb materials resistance inhibition of volatile components of Korean aromatic herbs, Archives of Pharmaceutical Research, 1998;21(1):62–66
10. Yusuf M, Chowdhury JU, Wahab MA, Begum J. Medicinal plants of Bangladesh. BCSIR, Dhaka (1994) 17–266.
11. Latha LY, Darah I, Jain K, Sasidharan S. Toxicity study of *Vernonia cinerea*. Pharmaceutical Biology. 2010; 48 (1):101-104.
12. Adeboye JO, Asije W, Awe SO. Diuretic and antidiuretic activity of the leaf extracts of *Vernonia cinerea*. Phytotherapy research. 1997; 11(6):454-456.
13. Iwalewa EO, Iwalewa OJ, Adeboye JO. Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* less leaf. Journal of Ethnopharmacology. 2003; 86:229- 234.
14. Ganesh P, Kumar KV, Kumar HS. Antidiarrhoeal activity of methanolic extract of *V. Cinerea* leaves less on female albino rats. International Research Journal of Pharmacy. 2011; 2 (5):211-213.
15. Rizvi SMD, Biswas D, Arif JM, Zeeshan M. In-vitro antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. International Journal of Pharmaceutical Sciences Review and Research. 2011; 9 (2):164-169
16. Arivoli S, Tennyson S, Martin JJ. Larvicidal efficacy of Vernonia cinerea (L.) (Asteraceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Biopesticides. 2011; 4 (1):37-42
17. Kumar PP, 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.
18. Kokate CK. Practical Pharmacognosy. 4th edition. Delhi: Vallabh Prakashan; 1994.
19. Harborne JB. Phytochemical methods. London: Chapman and Hall; 1973.
20. Mervat M. M. El Far, Hanan A. A. Taie. “Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol” Australian J Basic Applied Sc. 2009; 3:3609-3616.
21. Wagner H, Baldt S and Zgainski EM. Plant drug analysis. New York, Berlin, Springer, 1996.
22. Wagner H, Bladt S and Zgainski EM. Plant drug analysis – A Thin Layer Chromatography Atlas. Springer – Verlag, Berlin, 1984.
23. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1966; 45(4):493-496.
24. Lee JY, Yoon JW, Kim CT, Lim ST. Antioxidant activity of phenylpropanoid esters isolated and identified from *Platycodon grandiflorum* A. DC. Phytochemistry 2004; 65(22):3033-3039.
25. Panda S, Jafri M, Kara A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia. 2009; 80 (2):123-126.