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IN VITRO ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF AQUEOUS AND NONAQUEOUS EXTRACTS OF *VERNONIA CINEREA* FLOWERS

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Abstract

The aim of this study was to evaluate the anti-oxidant activity of the flowers of the plant *Vernonia cinerea* (Family: Asteraceae). Flowers extracts of *V. cinerea* in different four organic solvents (methanol, hexane, chloroform and ethyl acetate) and water were assayed for antioxidant activity using FRAP assay. All of these extracts were found to possess significant antioxidant activity at 5mg/ml concentration. The methanol extract of *V. cinerea* flowers showed highest TAC_{FRAP} value (24.5 µmol/ml)) and it was lowest (4.0 µmol/ml) with chloroform extract of flowers. The phytochemical tests indicated the presence of flavonoids in methanol, ethyl acetate and chloroform extracts. Tannins in methanol, Saponins in methanol, ethyl acetate and water, steroids in hexane, triterpenoids and glycosides in methanol and chloroform, vitamin C in methanol, phenolic constituents in methanol and ethyl acetate extracts, respectively. The study shows that *V. cinerea* flowers possess significant antioxidant capacities and may play a role in scavenging the free radical attack on biomembranes.

Key words : *Vernonia cinerea*, anti-oxidant activity, flowers, FRAP assay.

Introduction

Oxidation stress by free radicals is known to be one of the major mechanisms that lead to serious disease including cancer. Consumption of supplements or foods with high antioxidant capacity such as vitamin C, E, glutathione, co-enzyme Q10, red wine and green tea could help to prevent chronic disorders through repair of oxidative damage (Sung *et al.*, 2000). Plants represent a rich source of natural antioxidants (Halliwell *et al*., 1995). Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines (Mann *et al*., 1985). Plants generally produce many secondary metabolites, which constitute an important source of microbicides, anti-oxidants and many pharmaceutical drugs used in traditional medicine. Many natural substances having anti-oxidant activity have been used in health foods for medicinal and preservative purposes (Reynolds *et al*., 1985). *Vernonia cinerea* (Family : Asteraceae) is a terrestrial annual erect herb with ovate leaves and grows up to 80 cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation. It is located especially in different Asian countries such as India, Bangladesh and Nepal and locally known as 'Sahadevi'. *V. cinerea* is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders (Yusuf *et al*., 1994). Species of *Vernonia* (Asteraceae) have been used in traditional medicine. Several stigmastane type sapnonins have been identified in leaves of *Vernonia amygdalina* also known as 'bitter leaf' (Ohigashi *et al*., 1991 and Jisaka *et al*., 1993) having medicinal effect. The flavonioids of *V. amygdalina* leaves have been found to have antioxidant activities better than synthetic antioxidant butylated hydroxyl toluene [(BHT) (Igile *et al.*, 1994)].

Antioxidant can be defined as any substance that when present at low concentration compared to those of an oxidizable substance significantly delay or present a pro-oxidant initiated oxidation of that substrate (Halliwell, 1995). Antioxidant compounds must be present in biological systems in sufficient concentrations to prevent an accumulation of pro-oxidant molecules, a state known as oxidative stress (Wiseman and O' Reilly, 1997; Buettner and Schafer, 2000). Antioxidants have been investigated for the prevention of diseases such as cancer coronary heart disease, for neurogenerative diseases and slowing ageing. Presence of antioxidant activity in various extracts of *Vernonia cinerea* roots and leaves is reported

Table 1 : Amount of plant extract, distilled water and FRAP reagents taken for assay.

Reagents	Samples	Standards	Blank
Plant extract	20 µl	20 µl ($FeSO_4.7H_2O$)	x
Deionized water	30 µl	30 µl	30 µl
FRAP reagents	3000 µl	3000 µl	3000 µl

Table 2 : FRAP activity of different extracts of flowers of *Vernonia cinerea.*

Plant Sample	FRAP Value (mean ± s.d.)
VFM	24.5 ± 1.09
VFH	5.5 ± 1.72
VFC	4.0 ± 0.78
VFE	13.5 ± 0.51
VFW	6.0 ± 0.55

VLM– Vernonia Flower Methanolic extract; VLH- Vernonia Flower Hexane extract; VLC– Vernonia Flower Chloroform extract; VLE– Vernonia Flower Ethyl Acetate extract; VLW– Vernonia Flower Water extract.

Table 3 : Phytochemical analysis of the various flower extracts of *Vernonia cinerea.*

S. no.	Chemical constituent	Methanol	Hexane	Chloroform	Ethyl acetate	Water
1.	Alkaloids	_	-	-	-	-
2.	Flavonoids	+	-	+	+	-
3.	Tannins	+	-	-	-	-
4.	Saponins	+	-	-	+	+
5.	Steroids and sterols	_	+	-	-	-
6.	Triterpenes	+	-	+	-	-
7.	Vitamin C	+	-	-	-	-
8.	Fixed oils and fats	_	-	-	-	-
9.	Glycosides	+	-	+	-	+
10.	Phenolic constituents	+	-	-	+	-

in literature (Lal *et al*., 2014). Therefore, the present study aimed to evaluate the *in vitro* antioxidant activity of different non-aqueous and aqueous extracts of *Vernonia cinerea* flowers test the type of phytochemicals present in different extracts.

Materials and Methods

Collection of the plant sample

Fresh flowers of *Vernonia cinerea* was collected from Medicinal Plants Garden, C.S.J.M. University, Kanpur (U.P.), India and stored in plastic bags. The cut flowers were immediately chopped into small fragments and dried in the shade. The dried samples were ground into coarse powder and were used for extraction.

Chemicals

The chemicals used in the experiment TPTZ (2, 4, 6-tripyridyl-5-triazine), acetate buffer pH 3.6 ferrous sulphate and ferric chloride for antioxidant estimation were purchased from Sigma Solvents. Hexane, methanol, chloroform, ethyl acetate used for extraction were purchased from Merck Solvents. All the chemicals used were of Analytical grade.

Method of extraction

Extraction was done using Soxlet Apparatus using four different organic solvents – hexane, chloroform, ethyl acetate and methanol. Finally water extracts were prepared with flower coarse powder. To this water extract, 5 ml of methanol was added to avoid fungal growth. These extracts were properly labeled and kept in refrigerator at low temperature for further use in estimating *in-vitro* antioxidant activity.

Method for *in-vitro* antioxidant assay

For *in-vitro* antioxidant assays the most widely used assay, Ferric Reducing Antioxidant Power (FRAP) as µ mol ferrous ion equivalents was performed for measuring the total antioxidant activity (Benzie and Strain, 1996).

Reagents for frap assay

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1L with distilled water.

b) TPTZ (2, 4, 6-tripyridyl-s-triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). 0.031 g of TPTZ was added to 10 ml of 40 mM HCl and dissolved at 50ºC.

c) $FeCl_3.6H_2O$: (M.W. 270.30), 20 mM.

0.054 g of $FeCl_3$ was dissolved in 10 ml of distilled water.

The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was $FeSO_4.7H_2O$: 0.1-1.0 mM in methanol.

Frap assay procedure

FRAP solution (3.6 mL) was added to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the flower extract (80 mL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593

nm. For construction of the calibration curve, five concentrations of $FeSO_4$, $7H_2O$ (0.1, 0.2. 0.4, 0.6, 0.8, and 1.0 mM) were used and the absorbance values were measured as for sample solutions. Tables 1 exhibits the amount of plant extract, distilled water and FRAP reagents required for the assay.

Results and Discussion

The results for total antioxidant capacity (TAC) by FRAP methods for different *Vernonia cinerea* flower extracts are presented in table 2. The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1 mM of $FeSO_4$.

After observing results of FRAP assay between different extracts, the highest TAC_{FRAP} value is obtained for Methanol extract of flowers of *Vernonia cinerea*. This was followed by Ethyl Acetate extract of flowers of *Vernonia cinerea* > Water extract of flower of *Vernonia cinerea* > Hexane extract of flowers of *Vernonia cinerea*. Chloroform extract of flowers of *Vernonia cinerea* recorded lowest TAC_{FRAP} value.

The phytochemical tests (table 3) in flower extracts indicated the presence of flavonoids in methanol, ethyl acetate and chloroform extracts. Tannins in methanol, saponins in methanol, ethyl acetate and water, steroids in hexane, triterpenoids and glycosides in methanol and chloroform, vitamin C in methanol, phenolic constituents in methanol and ethyl acetate extracts, respectively.

The current study established that the crude methanol extract of *V. cinerea* flowers had strong anti-oxidant activity. The anti-oxidant activity of plant extracts is generally attributed to the presence of phenolic compounds and flavonoids and there is high correlation between antioxidant activity and phenolics content. Comparison of anti-oxidant activity of root and leaf extracts (Lal *et al*., 2014) with anti-oxidant activity of flower extracts recorded in present study revealed relatively lower magnitude in floral tissue. The anti-oxidant activity of the plant extracts also implicated its neuroprotective effect in Alzheimer Disease (AD) through the scavenging of reactive free radicals and ROS, which otherwise play important role in the formation of neurofibrillary tangles and neurotic plaques (Butterfield *et al*., 2007). Study on AD animal model for finding the role of *V. cinerea* on fibrillar amyloid plaques might give additional information regarding applicability of this plant in AD. This study indicates significant free radical scavenging potential of *V. cinerea*, which can be exploited for the treatment of various free radical-mediated ailments, however, more extensive study is necessary to determine the exact mechanism(s) of action of the extract and its active compound(s).

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