



# International Journal of Chemistry and Pharmaceutical Sciences

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Research Article

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## Evaluation of Anti Oxidant, Membrane Stabilizing and Anticoagulant Activities of the Whole Plant Extracts of *Vernonia Cinerea* Linn

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### ABSTRACT

*Vernonia cinerea* belongs to the family Asteraceae, is a common weed distributed throughout India. It has many therapeutic uses in traditional medicine of the world. The objective of the study was to evaluate the antioxidant, membrane stabilizing and anticoagulant activities of the whole plant extracts of *Vernonia cinerea* Linn. The whole plant extract of *V. cinerea* were prepared using different solvents which includes acetone, ethyl acetate, ethanol and water. Quantitative phytochemicals were determined in all the extracts. Membrane stabilizing activity was assessed by using erythrocyte in hypotonic solution and heat induced haemolysis method. The aqueous, ethanol, ethyl acetate and acetone extracts of *V. cinerea* at different concentrations (0.2 & 0.4 mg/ml) were tested on plasma by *in-vitro* prothrombin time test. Heparin and saline in distilled water were used as standard and negative control respectively. The time taken for clotting was considered as parameter to assess the anticoagulant activity. Significant amount of non enzymic antioxidants which includes phenol, carotenoids, flavonoids, tannins, -tocopherol and ascorbic acid were present in all the extracts. Ethyl acetate and acetone extracts of *V. cinerea* exhibited potent anticoagulant activity at concentration of 0.4 mg/ml. Aqueous and ethyl acetate extracts of *V. cinerea* possess significant membrane stabilizing activity at concentration of 2mg/ml. The results obtained from the study revealed the fact that aqueous, ethyl acetate and acetone extracts of *V. cinerea* possess significant membrane stabilizing and anticoagulant activities. The protective potential of the extracts may be attributed due to the presence of high concentration of phenols, carotenoids, flavonoids and ascorbic acid.

**Keywords:** *Vernonia cinerea*, Antioxidant, Anticoagulant, Membrane stabilization, Prothrombin time

### ARTICLE INFO

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**Article History:** Received 19 March 2016, Accepted 31 April 2016, Available Online 27 July 2016

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Manuscript ID: IJCPs3035



PAPER-QR CODE

**Citation:** R. Uma, et al. Evaluation of Anti Oxidant, Membrane Stabilizing and Anticoagulant Activities of the Whole Plant Extracts of *Vernonia Cinerea* Linn. *Int. J. Chem, Pharm, Sci.*, 2016, 4(7): 357-361.

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## 1. Introduction

As diseases and health problems are experienced by the human population the need for discovery of potent drugs from plant resources continues to be in demand. Most plant based medicines that are developed by pharmaceutical companies have their beginning in ethno medicine. In recent years, the interest in the plant-based medicine has increased noticeably worldwide. One of such plants belonging to genus *Vernonia* and known to have healing potential is *Vernonia cinerea*. *Vernonia cinerea* Linn (Family- Asteraceae) is an erect annual herb that grows up to 75 cm in height. It is found in tropical or semi-tropical areas as waste land herb [1]. It is commonly known as Sahadevi (Sanskrit), Naichette / Mukuthi poondu (Tamil) and “kurunilla (Malayalam). In folk medicine it is used to treat pitta, vitta, stomach pain, diarrhea and ringworm. The juice of flower is helpful in condition like red eye as an external drop. Traditionally this herb is widely used in tonsillitis, stomach pain, diarrhea, intermittent fever, eczema, herpes, ringworm and elephantiasis [2]. This herb has been used to treat a number of disorders including inflammation, malaria, fever, worms, pain, diuresis, cancer, abortion, brain and various gastro-intestinal disorders [3]. The plants possess antimicrobial [4], antioxidant, and antihelminthic, anti-inflammatory, analgesic and antipyretic, antiflaulent, antispasmodic and antidiuretic properties [5]. Some of the phytochemical compounds present are sterols, flavonoids, sesquiterpene lactones and a terpenoid, ‘leupeol acetate’ which shows anti hyperglycemic and antiulcer properties. Alcoholic and chloroform extracts of the root of *Vernonia cinerea* were found to show a potent antihelminthic activity when compared with the standard drug [6]. Thus, the aim of this study was to evaluate anti oxidant, membrane stabilizing and anticoagulant activities of the whole plant extracts of *Vernonia cinerea* linn. In this paper we report the results of such studies in order to orient future investigations towards new, potent and safe bioactive compounds.

## 2. Experimental Methods

**2.1 Plant materials:** Mature plant of *V.cinerea* was collected from Avadi, Thiruvallur district of Tamilnadu. It was authenticated by Dr.S.Jayaraman, Director Institute of Herbal botany plant anatomy research centre, Tambaram, Chennai-45 (Reg no: PARC/2013/2175)

### 2.1.1 Scientific Description

Kingdom	:	Plantae
Order	:	Asterales
Family	:	Asteraceae
Tribe	:	Vernonieae
Genus	:	<i>Vernonia</i>
Botanical name	:	<i>Vernonia cinerea</i>

**2.2 Preparation of plant extract:** Fresh matured plant was cleaned, washed and shade dried for 10 days was powdered in a blender and kept in air tight container. 50gm of dried plant powder was taken and soaked in 200 ml of water, ethanol, acetone and ethyl acetate solvent. It was kept in

orbital shaker for eight hours at 37°C. The extract was filtered using whatman no. 1 filter paper. Extracted solvent were allowed for evaporation in a drier.

### 2.3 Evaluation of antioxidants

#### 2.3.1 Estimation of total phenol

To estimate the total phenolic content of plant extracts by Folin-Ciocalteu method [7].

##### Procedure:

Total phenols of ethanolic, ethyl acetate, acetone and aqueous extracts of *Vernonia cinerea* was determined according to Folin-Ciocalteu method, 200 microlitres of sample to that 1 ml of (1:2) diluted Folin-Ciocalteu reagent was added and the tubes were made up to 2ml with distilled water. 2ml of saturated sodium carbonate (20%) was added and the tubes were incubated of 5mins in room temperature and absorbance was measured at 700nm using UV- Visible spectrophotometer. Gallic acid was used in standard calibration. The total phenolic content was determined and the result was expressed in mg/g dry weight.

#### 2.3.2 Estimation of Total Flavonoids

To estimate the total flavonoid content of plant extract by spectrophotometer method [8].

##### Procedure:

To 200µl of extract add 1ml of 2% Aluminium chloride and 0.8ml of distilled water. It was left at room temperature for 15minutes after which the absorbance of the reaction mixture was measured at 430nm with a double beam UV-Visible spectrophotometer. The calibration curve was plotted by preparing the Quercetin solutions at concentrations 12.5 to 100mg ml<sup>-1</sup> in methanol. The total flavonoid content was estimated and the results were expressed in mg/g dry weight.

#### 2.3.3 Estimation of Tannin

To estimate the amount of tannin present in the given plant extract by Folin Denis method [9]

##### Procedure:

200 µl of tannin extract for each sample was taken in test tubes and volume was made to 1.0ml with distilled water. Then 0.5ml Folin Denis reagent was added and mixed properly. Then 1.0ml 20% sodium carbonate solution was added and mixed it and kept for 40minutes at room temperature. Optical density was taken at 700nm in spectrophotometer and concentration was estimated from the standard graph. The tannin content was estimated and the results were expressed in mg/g dry weight.

#### 2.3.4 Estimation of Tocopherol

To estimate the amount of Tocopherol present in the given plant extract by Dipyriddy method [10].

##### Procedure:

To 100µl of sample extract add 0.9ml of ethanol. Then 0.2ml of 0.2% Dipyriddy reagent was added and mixed thoroughly. To that add 0.2ml of ferric chloride and 2ml of butanol and incubate at room temperature of 15minutes. Optical density was taken at 520nm in spectrophotometer and the concentration was estimated from the standard

graph. The content Tocopherol was estimated and the results were expressed in mg/g dry weight.

### 2.3.5 Estimation of Ascorbic acid

To estimate the amount of ascorbic acid present in the given sample extract by Di nitro phenyl hydrazine method <sup>[11]</sup>.

#### Procedure:

To 100µl of the sample extract add 0.9ml of distilled water. Then add 0.5ml of Di nitro phenyl hydrazine reagent followed by 2.5ml of 85% H<sub>3</sub>PO<sub>4</sub> and allowed to stand for incubation for 10minutes at room temperature. Optical density was read at 520nm in spectrophotometer and the concentration was estimated from the standard graph. The ascorbic acid content was estimated and the results were expressed in mg/g dry weight.

### 2.3.6 Determination of Carotenoids

Carotenoid pigment was identified using their absorption maxima by spectrophotometry [12].

### 2.4 Membrane stabilizing activity:

The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane <sup>[13]</sup>. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced erythrocyte haemolysis <sup>[14]</sup>. To prepare the erythrocyte suspension, whole blood was obtained from human and was taken in syringes (containing anticoagulant EDTA). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 g.

#### 2.4.1 Hypotonic solution- induced haemolysis:

The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0 mg/mL) or acetyl salicylic acid (0.1 mg/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

$$\% \text{ Inhibition of haemolysis} = 100 \times (\text{OD1}-\text{OD2}/\text{OD1}),$$

Where,

OD1 = optical density of hypotonic-buffered saline solution alone (control) and OD2 = optical density of test sample in hypotonic solution.

#### 2.4.2 Heat- induced haemolysis:

Isotonic buffer containing aliquots (5 ml) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54°C for 20 min in a water bath, while the other pair was maintained at 0-5°C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or acceleration of haemolysis in tests and was calculated

according to the equation: % Inhibition of haemolysis = 100 x [1- (OD2-OD1/OD3-OD1)]

Where,

OD1 = optical density of unheated test sample, OD2 = optical density of heated test sample and OD3 = optical density of heated control sample

### 2.5 Anticoagulant activity

**2.5.1 Study population:** Blood samples obtained from healthy volunteers of age group of 20-25 years.

#### 2.5.2 Collection of blood sample:

The blood sample was obtained in containers containing trisodium citrate to prevent clotting process. Centrifugation was carried out at 3000 rpm for 15minutes to separate the blood cells from plasma to obtain pure platelet plasma for prothrombin test. The freshly prepared plasma was stored at 4°C until its use.

#### 2.5.3 Prothrombin time test:

In a test tube, 0.2ml test plasma, 0.1ml of crude extract of *Vernonia cinerea* of different concentration 0.2 and 0.4mg/ml and 0.3ml of CaCl<sub>2</sub> were added together in a clean fusion tube and incubated at 37°C. A stopwatch was started to record the coagulation time in seconds. The tube was shaken to mix the contents and it was stopped as soon as the clot formation began. The activity is expressed in terms of clotting time in seconds. The steps were repeated three times for each sample and average of the test value was noted. Normal saline was used in place of the extracts for the negative control and 50mg/ml of heparin for the positive control [15,16]

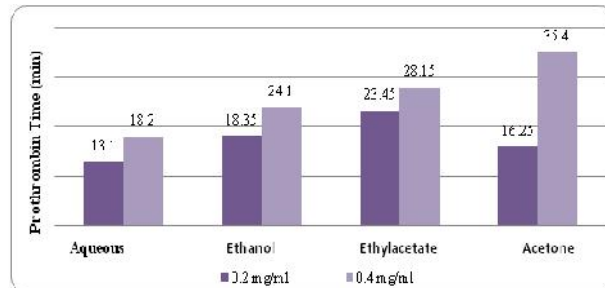
## 3. Results and Discussion

Table I shows the content of total phenols, flavonoids, carotenoids, ascorbic acid and -Tocopherol in various extracts of *Vernonia cinerea*. The total phenol varied from 18.4±1.6 -35.6±2.4 mg/g. The total phenol in aqueous extract of the whole plant extract of *Vernonia cinerea*(35.6 mg/g) and in ethanol extract(28.4mg/g) were higher than that in the ethylactate and acetone extracts of *V.cinerea* (20.4 & 18.4 mg/g) respectively. The flavonoid in ethylacetate extract of the whole plant extract of *Vernonia cinerea* (6.6 mg/g) and in acetone extract (4.82mg/g) were higher than that in the ethanol and aqueous extracts of *V.cinerea* (4.5& 2.92mg/g) respectively. It has been acknowledged that flavonoids show significant antioxidant action on human health and fitness.

The flavonoids act through scavenging or chelating process [17,18]. The high potential of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they possess respectively[19]. The compounds such as flavonoids which hold hydroxyls groups, are responsible for the radical scavenging activity in the plants [20]. Tannins constitute a group of secondary metabolites widely distributed in the plant kingdom. Quantitative screening of tannins (Table I) divulged their presence in all the four extracts with the ethanol extract having 4.4 mg/g of tannin while ethyl acetate and acetone extracts have 3.4mg/g and 2.8mg/g of tannin respectively. Ethanol and aqueous extracts of *V.cinerea* contains higher amount of ascorbic acid (8.4 & 6.5 mg/g) respectively.

Ethanol extract contains higher amount of tocopherol and carotenoids (3.2 & 45.6 mg/g) respectively. It had been demonstrated that these compounds exhibit antioxidant properties and help the body ward off cardiovascular disease, various immune disorders and neurodegenerative diseases [21].

The extracts of *V.cinerea* at concentration 2mg/ml significantly protected the lysis of erythrocyte membrane induced by hypotonic and heat induced method as compared to the standard acetyl salicylic acid (0.10 mg/ml) (Table II).The ethylacetate and aqueous extract demonstrated 64.4 & 62.5 % inhibition of haemolysis of red blood cell as compared to standard 70.4 % produced by acetyl salicylic acid (0.1 mg/ml) in the hypotonic induced haemolysis. The ethanol and acetone extract also exhibited moderate inhibition of haemolysis of red blood cell.In the heat induced haemolysis, the ethyl acetate extract revealed 34.42% inhibition of haemolysis as compared to 40.6% inhibition of haemolysis by the standard acetyl salicylic acid. The acetone extract showed moderate haemolysis inhibition.



**Figure 1:** Anticoagulant activity of various extracts of *Vernonia cinerea*

Values expressed are mean±SD; n=3; Aqueous, methanol, acetone and ethyl acetate extracts of *V.cinerea* were tested for blood coagulation effects in normal human plasma and found to be significantly prolonged the prothrombin time of normal human plasma. In figure 1 aqueous, ethanol, ethyl acetate and acetone extracts of *V.cinerea* showed anticoagulant activity at higher concentration tested, with prolonged clotting time was 18.2, 24.1, 28.5, 35.4 sec at 0.4mg/ml concentrations respectively.

**Table 1:** Quantitative phytochemical analysis of various extracts of *Vernonia cinerea*

Phytochemicals (mg/g)	Aqueous	Ethanol	Ethylacetate	Acetone
Phenols	35.6±2.4	28.4±2.2	20.4±1.8	18.4±1.6
Flavonoid	2.92±0.90	4.5±1.20	6.6±1.4	4.82± 0.96
Tannins	2.1±0.45	4.4±1.2	3.4±0.50	2.8±0.42
Ascorbic acid	6.54±1.2	8.4±0.74	5.6±0.42	4.6±0.60
-Tocopherol	1.80±0.46	3.2±0.84	2.46±0.42	2.1±0.30
Carotenoids	24.6±2.4	45.6±2.62	24±3.20	26.8±2.64

Values expressed are mean ± SD; n=3

**Table 2:** Effect of various extracts of *Vernonia cinerea* on hypotonic solution and Heat induced haemolysis of erythrocyte membrane

S.No	Extracts	Mambrane stabilizing activity Haemolysis Inhibition (%)	
		Hypotonic induced Haemolysis	Heat induced Haemolysis
1	Aqueous	62.5	18.5
2	Ethanol	46.4	19.6
3	Ethyl acetate	64.4	34.42
4	Acetone	44.3	26.2

Values expressed are mean±SD; n=3

#### 4. Conclusion

The present study showed that all the extracts demonstrated different extent of antioxidant activity. It was also shown that ethanol extract showed significantly higher antioxidants which include phenols, flavonoids, ascorbic acid, tocopherol, and carotenoids. It can be concluded that the ethyl acetate and acetone extracts of *V.cinerea* exhibited moderate membrane stabilizing activity and potent anticoagulant activity. This may be due to the presence of sterols, flavonoids, terpenoids, carbohydrate, cardiac glycosides and antioxidants. Further work is needed to isolate the secondary metabolites and study thoroughly for more precise and accurate bioactivities. However isolation and characterization of these compounds and validating

their therapeutic efficacy against different pathologies is required for clinical implementation.

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