ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.31615-31618, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF PHYTOCHEMICAL, PHYTONUTRIENT AND THIN LAYER CHROMATOGRAPHY PROFILING OF SEQUENTIAL EXTRACTS OF *VERNONIA CINEREA*

***Uma Ramaswamy and Vicky Mani**

Department of Biochemistry, Dwaraka Doss Goverdhan DossVaishnav College, Arumbakkam, Chennai, Tamilnadu, India

ARTICLE INFO

Article History:
Received 23rd February, 2016
Received in revised form
08th March, 2016
Accepted 24th April, 2016
Published online 31st May, 2016

Key words:

Vernonia cinerea,
Phytonutrient,
Phytochemicals,
Thin layer chromatography.

ABSTRACT

Vernonia cinerea is a annual herb which is commonly found in India especially in South India. The aim of the study was to evaluate the phytochemical, phytonutrient (carbohydrates, protein, lipid, vitamin,minerals, ash, moisture) and thin layer chromatography profiling of the sequential extracts of whole plant *V.cinerea*. The sequential extract of acetone, ethanol, ethylacetate and aqueous extract of *V.cinerea* for analyzing phytochemical and confirmed by thin layer chromatography (TLC). The phytochemical result depicts the presence of various bioactive compounds which includes phenolics, saponins, steroids, alkaloids, flavonoids, terpenoids, tannins and cardiac glycosides. The phytonutrient report shows total protein content was found to be higher (42.6 mg/g) than lipid(11.6 mg/g) and carbohydrate(1.62 mg/g) respectively. Among the minerals quantified calcium content was higher than phosphorus and iron. Riboflavin was found to be present in lesser amount when compared to vitamin thiamine,tocopherol and ascorbic acid.TLC profiling of all the extracts of *V.cinerea* confirms the presence of phytochemicals and different Rf values of compound reflects an idea about polarity. The results obtained in the present study indicated the whole plant *V.cinerea* as a rich source of phytonutrients, phytochemicals and minerals has medicinal property and it can be used as dietary source.

Citation: Uma Ramaswamy and Vicky Mani, 2016. "Evaluation Of Phytochemical, Phytonutrient And Thin Layer Chromatography Profiling Of Sequential Extracts Of *Vernonia Cinerea*", *International Journal of Current Research*, 8, (05), 31615-31618.

INTRODUCTION

Present day people come to realize the importance of the folk medicine because it has less side effect than other synthetic medicines. Therefore research is looking for new compound to develop drug and focused their attention towards plants. Plant based antimicrobials have been proned to be effective in the treatment of infectious diseases and it has less side effect than synthetic antibiotics. Vernonia cinerea belong to Asteraceae family is well distributed in India. It is considered one of the most advanced family from all the dicotyledonous .It is commonly known as "Sahadevi" (Sanskrit), "Naichette" or "Mukuthi poondu" (Tamil), "kurunilla"(Malayalam). In folk medicine it is used to treat pitta, veta, stomach pain, diarrhea and ringworm. The juice of flower is helpful in condition like red eye as an external drop. Root and leaves of Vernonia species are used in phytomedicine to treat fever, kidney disease and stomach discomfort (Gill, 1992). The leaf juice extract is used to treat skin diseases and the leafy extract for treating dysentery in children (Maruthapandian *et al.,* 2010).

****Corresponding author: Uma Ramaswamy,***
Department of Biochemistry, Dwaraka Doss Goverdhan DossVaishnav College, ArLumbakkam, Chennai, Tamilnadu, India.

The water soluble fraction of the methanol extract of the defatted dried ground whole plant of V.cinerea showed significant diuretic activity in rats comparable to lasin as known diuretic(Varghese,2010). Ayurvedia pharmacopoeia of India recommends the plant to be used for treating intermittent fever, filariasis, blisters and vaginal discharge. The present study was to indentify the phytochemical, phytonutrient, and TLC profiling of sequential extract of V.cinerea.

MATERIALS AND METHODS

Plant materials: Matured 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).

Scientific Discription

Kingdom : Plantae
Order :Asterales
Family:Asteraceae
Tribe :Vernonieae

Genus:*Vernonia*
Botanical name : *Vernonia cinerea*

Preparation of plant extract: Fresh matured plant was cleaned, washed and shade dried for 10 days. After drying it 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 five hours at 37ºC. The extract was filtered using whattman. no. 1 filter paper. Extracted solvent were allowed for evaporation in a drier.

Phytochemical analysis: Qualitative phytochemical analysis was done based on Harbone (1994), Trease and Evans(1996).

Qualitative analysis of phytonutrients

Moisture and ash content of the plant material was determined by standard method (AOAC Method, 1997).

Estimation of Carbohydrates: Carbohydrate was estimated by Dubois method (1954). To 50 mg of plant sample, 2.0 ml of 5% trichloro aceticacid was added and centrifuged at 2000 rpm. To the supernatant add 100 ml of 5% ethanol and kept overnight in cold condition. Again the tubes were allowed to centrifuge at 4000 rpm for 10 minutes. To the dried precipitate, 2 ml of 1N sodium hydroxide was added. To the extracted sample 1ml of distilled water, 1ml of 5% phenol followed by the addition of 5ml concentrated sulphuric acid and allowed to incubate at room temperature for 10 minutes. Optical density was read at 490 nm using spectrophoto meter.

Estimation of protein: Protein was estimated by Lowry's method (1951). Different dilution of bovine serum albumin (BSA) solutions are prepared by mixing stock BSA solution (1mg/ml) with water. The BSA concentration range is 0.05 to 1mg/ml. From these different dilutions, pipette out 0.02ml extract solution to different test tubes and add 2ml of alkaline copper reagent. This solution is incubated at room temperature for 10 minutes. Then add 0.2 ml of Folin-ciocalteau reagent solution to each tubes and incubate for 30minute. Adjust the colorimeter with blank and the OD was measured at 660nm.

Estimation of lipid: Lipid was estimated by Bligh and dyer method (1959)

Estimation of pigments: Chlorophyll a, b and total carotenoid was determined by Lichenthaler method (1987)

Estimation of vitamins: Vitamin E and Vitamin C were determined by (Jayashree *et al*, 1985: Sarojini *et al.,* 1999) respectively, vitamin thiamin and Riboflavin determined by spectro fluorimetric method.

Estimation of minerals: Minerals which includes iron, calcium, phosphorus were determined from plant ash. Iron was determined by Ramsay method (1958), calcium and phosphorus were determined by Titrimetric and Fiske subbarow method, 1928.

Bioactive compound detection using TLC: TLC was carried out to isolate the principle components that were present in extract of plant. TLC studies were carried out for different extracts on commercially available precoated TLC sheet SIL G/UV254(Machery-Nagel). The different solvent system of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

Solvent phase: The different solvent system used were Chloroform: Methanol: Water (7:3:1) and Acetone: n Hexane: Ethylacetate (20:79.5:0.5) and Toluene: nHexane: Diethylamine (7:2:1).

The above prepared plates, extract were applied on pre coated TLC plates using capillary tubes and developed in TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under ultraviolet light at both 254nm and 366nm. They were sprayed with spraying agent and some were placed in hot air oven for 1minute for development of color in separated bands. The movement of analyte were expressed by its retention factor (Rf) values were calculated for different sample.

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front.}}$$

RESULTS AND DISCUSSION

Estimation of carbohydrates, total protein and lipids

The result of carbohydrate ,total protein, lipid, moisture, ash was shown in Table 1 . Total protein content was found to be higher (42.6mg/g) than lipid (11.6mg/g) and carbohydrate (1.62mg/g) in *V.cinerea*. Ash and moisture content was found to be 70.5 and 9.2 % respectively.

Table 1. Proximate Composition of Whole Plant *Vernonia cinerea L.*

S.NO	BIOCHEMICALS	VALUES
1	Moisture content (%)	9.2 ± 0.5
2	Ash content (%)	70.5 ± 5.2
3	Carbohydrates (mg/g)	1.62 ± 0.40
4	Protein (mg/g)	42.6 ±0.82
5	Lipid (%)	11.6 ±0.50

Values were expressed in mean + SD (n=3 determination)

Estimation of Pigments

Chlorophyll a and b was found to be 0.32 and 0.86 mg/g respectively where as the total carotenoid content was found to be 2.64 mg/g respectively (Figure- 1). In plants and algae carotenoids have both photosynthetic and photoprotective role (Taiz and Zeiger, 2006; Holick 2002 and Rock ,2003) reported that naturally occurring carotenoids other than β- carotene have exhibited anticancer activity are being concluded further as potential chemo preventive agent.

Estimation of Vitamins and Minerals

In *Vernonia cinerea* on quantifying the minerals shows Calcium was found to be maximum than Iron and Phosphorous. The values are tabulated in Figure 2. These

minerals are essential for more enzymes system in carrying out biochemical function like energy production, protein metabolism and bone formation etc. Figure 3 depicts the levels of vitamins. Riboflavin was found to be present in lesser amount when compared to thiamine, vitamin E and vitamin C (Non enzymic antioxidants). Vitamin thiamine, α tocopherol and ascorbic acid has been determined to the extent of 4.2 µg/mg, 1.86 µg/mg and 2.86 µg / mg of *V.cinerea*. Antioxidants possess diverse biological activities such as anti inflammatory, anti artherosclerotic and anti carcinogenic activities.

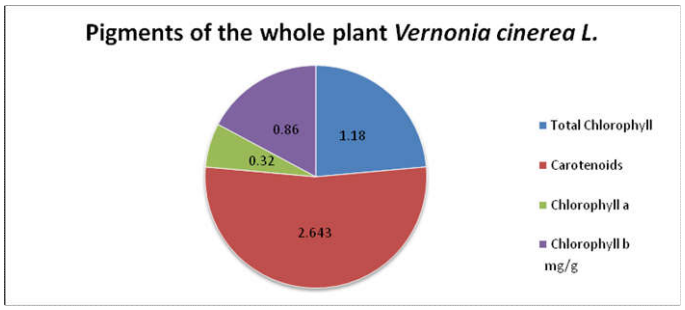

Figure 1. Pigments Of The Whole Plant *Vernonia cinerea*

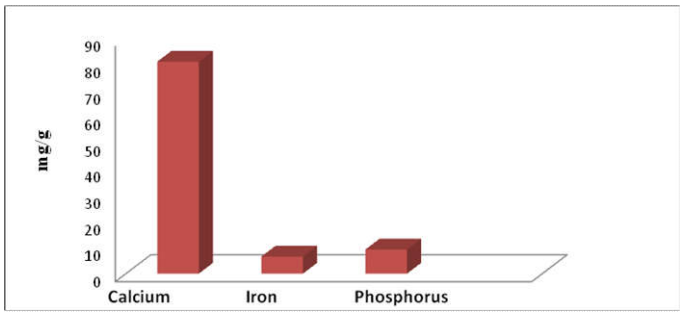

Figure 2. Analysis of minerals of *Vernonia cinerea L.*

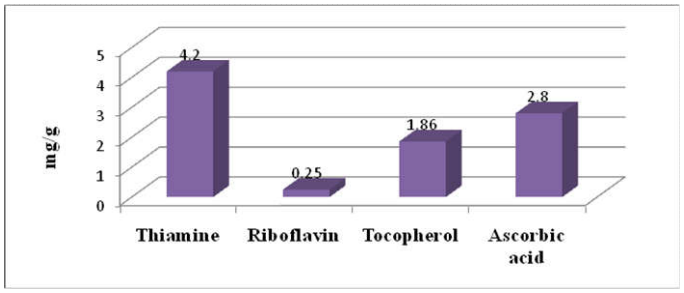

Figure 3. Analysis of vitamins in the whole plant of *Vernonia cinerea L.*

Phytochemical analysis of whole plant *Vernonia cinerea*

Vernonia cinerea revealed the presence of phytochemicals which includes phenols, saponins, steroids, alkaloids, flavonoids, terpenoids, tannins and cardiac glycosides it is presented in Table 2. Comparing the four solvent extract ethanol and ethylacetate extract was found to contain maximum flavanoid, saponin and steroid content along with plant phenolic such as alkaloids or free radical scavengers shows the presence in the extract of *Vernonia cinerea*. The major phytonutrients identified to have nutraceutical properties including terpenes, phytosterol, phenol and thiols (Srilakshmi, 2003).

Table 2 - Qualitative Phytochemical analysis of the whole plant *Vernonia cinerea.*

S.no	Phytochemicals	Aqueous	Ethanol	Ethylacetate	Acetone
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	-
3	Terpenoids	++	++	++	++
4	Saponins	+	+	+	+
5	Steroids	+	++	++	++
6	Aminoacids	+	+	+	+
7	Carbohydrates	+	+	+	+
8	Tanins	+	++	+	+
9	Cardiacglycosides	+	+	+	+
10	Phenols	++	++	+	+

Where (-) Absent, (+) Moderate, (++) Strong.

Table 3. TLC profiling of sequential extracts of *Vernonia cinerea*

Sequential extracts	Solvent Phase	Solvent run	Peak obtained(cm)	Rf values
	Chloroform:Methanol: Water	6	2.5	0.41
			4.5	0.75
	Acetone:Hexane: Ethylacetate	6.5	2.0	0.30
			2.9	0.44
			4.4	0.67
			5	0.76
Acetone			5.5	0.89
	Toluene:Ethylacetate:Diethylether	9.3	2.3	0.24
			4.0	0.41
			5.5	0.59
			7.8	0.84
			8.5	0.91
	Chloroform:Methanol: Water	6	2.0	0.33
			2.5	0.41
			4.0	0.66
			4.5	0.75
	Acetone:Hexane: Ethylacetate	6.5	2.5	0.38
Ethylacetate			2.9	0.44
			4.3	0.66
			5.0	0.76
	Toluene:ethylacetate:Diethyl ether	9.3	4.0	0.41
			5.4	0.60
			6.2	0.66
			6.9	0.73
			7.8	0.84
	Chloroform:Methanol: Water	6.0	1.6	0.30
			2.5	0.41
			4.0	0.66
			4.5	0.75
	Acetone:Hexane: Ethylacetate	6.5	2.5	0.38
Ethanol			4.4	0.49
			5.5	0.89
	Toluene:ethylacetate:Diethyl ether	9.3	2.0	0.24
			4.8	0.41
			5.5	0.48
			6.2	0.66
			8.0	0.84

Table 4. Rf values of sequential extracts of *Vernonia cinerea*

S.no.	Phytochemicals	Rf value
1	Flavonoids	0.46
2	Saponin	0.42,0.61
3	Terpenes	0.28,0.66
4	Phenol	0.44,0.67
5	Aminoacids	0.65

TLC: TLC of all sequential extracts of *V.cinerea* obtained by sequential extract method was carried out to confirm its nature by analyzing TLC chromatogram and to isolate the ingredients from the extracts (Table 3).TLC of acetone extracts of *V.cinerea* revealed the presence of two compounds having Rf values of 0.41 and 0.75 respectively, when a solvent phase of

chloroform; methanol: water was used. Acetone: Hexane: Ethylacetate solvent phase revealed the presence of common compounds having Rf values of 0.30, 0.44, 0.67, 0.76 and 0.89 cm respectively. In another solvent phase of Toluene: Ethyl acetate: Diethyl ether, maximum number of spots were obtained. Spots were clear and prominent with Rf value of 0.24, 0.41, 0.59, 0.84 and 0.91cm respectively. TLC of ethyl acetate extract of *V .cinerea* revealed the presence of 4, 6 and 7 spots in solvent1, II and III respectively.TLC profiling of ethyl acetate extract of *V. cinerea* revealed the presence of compounds having Rf value of 0.30,0.44,0.46,0.61,0.67 indicating the presence of flavonoid, terpenes, saponin and phenol. TLC of ethanol extract of *V.cinerea* revealed the presence of 4 spots having Rf value 0.33, 0.41, 0.66 and 0.75 respectively, when solvent phase I was used. Whereas maximum number of clear and prominent spots were obtained in solvent phase II and III .TLC profiling of ethanol extract of *V.cinerea* revealed the presence of compounds having Rf value of 0.24,0.42,0.48,0.66,0.84 indicating the presence of flavonoid, terpenes, saponin ,amino acids and phenol(Table 4).

Conclusion

The green chemistry of *Vernonia cinerea* reveals the presence of phytochemical, and phytonutrient. Among the phytochemical and phytonutrient flavanoid, saponin, steroid, total protein, calcium content was more in whole plant. TLC studies give the idea about the polarity of compound. This result contributes its effectiveness as a traditional medicine so it can be used for the synthesis of drugs and other medicinal product.

Acknowledgement

We thank the Secretary and Principal of Dwaraka Doss Goverdhan Doss Vaishnav College, Chennai -106, India for providing us with the necessary infrastructure and facilities required for the study.

REFERENCES

AOAC Official methods of Analysis 16th Edition. Association of official Analytical Chemists, Washington, DC, 1997.

Bligh, E. G., Dyer, W. J. 1959. A Rapid method for total lipid extraction and purification . *CanJ biochem. Physiol.,* 7:3-15

Dubois, M., Giles, M.I.L., Hamilton, J. K., Reber, P. A., Smith R. 1954. Colorimetric methods for the determination of sugar and related substances: *Analytical chemistry,* 28, 1954,350-356

Fiske and Subbarow, Y. 1928. *J. Biol. Chem.,* 66; 375-400

Gill, L. S. 1992. Ethnomedical uses of plants in Nigeria. Unibea press, Benincity, Nigeria, P.243

Harborne, J. B. 1994 . Phytochemical method, 2nd Chapman and Hall, Newyork, 3, 100-117.

Holick, C. N. 2002. American Diet Association, 156: 536.

Jayashree, V. S., Kamat, S. Y. 1985. Distribution of tocopherol (vitamin E) in marine Algae from Goa, West coast of India, *Indian. J. Mar, Sci.,* 14: 2281 v 229)

Lichenthaler, H. K. 1927. Cholorophyll and carotenoid pigments of photosynthetic biomembrane; Packer L and Doucer eds, Methods in enzymology. Washington academy press,; volume 148 :350-382.

Lowry, O. H., Rosenbrough, N. J., Farr, A.L. and Randall, R.J. 1951. *J.Biol.chem.,* 193:265 (the orginal method.)

Maruthapandian, A. and Mohan, V. R. 2010. *J of Her Med & Toxi*, 4(2) :89 -92.

Ramsay, W. H. 1958. Advances in clinical chemistry, Academic Press, Newyork.

Rock, C. L. 2003. American Diet Association, 103: 423.

Sarojini, Y., Nittala, S. S. 1999.Vitamin C content of some macroalgae of Vishakapatnam East cost of India; *Indain J. Mar. Sci.,* 28, 4081 V 412.

Srilakshmi, B. 2003. Food science, New Age International (p) Ltd, Publisher, New delhi, P 401

Taiz, L. and E. Zeiger, 2006. Plant Physiology, 4th ed. Bailliere Tindall ltd, London 832.

Trease, G. E. and Evans, W. C. 1996. A textbook of Pharmacognosy, 14th ed. Baillie Tindall ltd, London, 832.

Varghese, K. J., Anila, J., Nagalakshmi, R., Resiya, S. and Sonu, J. 2010. *Inter J of Pharm Sci and res*., 1(10):50- 59

****