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EVALUATION OF ANTIOXIDANT ACTIVITY OF *ACORUS CALAMUS* LINN. AND *VITEX NEGUNDO* L

Smrity Prabha[1]*, Jyoti Kumar[2] and U. C. Bapat[3]

[1]Assistant Professor, Department of Botany, Ranchi College, Ranchi.
[2]Professor, University Department of Botany, Ranchi University.
[3]Professor, Department of Botany, St. Xavier's College, University of Mumbai.

Corresponding Author: Smrity Prabha
Assistant Professor, Department of Botany, Ranchi College, Ranchi.

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ABSTRACT

This study reports total phenolics and flavonoid content and antioxidant activities of ethanolic extracts of *Acorus calamus* Linn. rhizome and *Vitex. negundo* L. leaves. The DPPH radical scavenging activity was carried out as per the method adopted by Brand-Williams, *et. al.*(1995). Total anti-oxidant capacity was determined using phosphomolybdenum reagent described by Prieto, *et. al.* (1999) and reducing power assay was undertaken using method proposed by Oyaizu, *et. al.*(1986). Total flavonoid and phenolic contents were determined by the standard methods as proposed by Zhishen, *et. al.* (1999) and Eberhardt *et. al.* (2000). The results obtained showed that there is a +ve correlation of anti-oxidant capacities of these plants with their phenolic and flavonoid contents. The IC_{50} value of *V. negundo* leaves indicated higher antioxidant potential as compared to *A. calamus* rhizome, whereas *A. calamus* showed higher reducing power and total antioxidant activities.

KEYWORDS: Antioxidant Activity, *Acorus calamus, Vitex negundo,* phenols, flavonoids, DPPH

1. INTRODUCTION

Free radicals generated, as part of the normal metabolic process, in living beings can cause several pathological conditions such as diabetes miletus, asthma, inflammation, cancer, Parkinson's disease, *etc.* Anti-oxidants protect human body by acting as radical scavengers against the free radicals. Phenolics and flavonoids, which are secondary metabolites, found in various plants are well known scavengers of free radicals. *A. calamus* Linn.[1,2,3] and *V. negundo* L.[4,5] have wide applications as herbal medicines as their roots and leaves exhibit anti-microbial activities. These plants were selected on the basis of their higher anti-fungal activities against Phytopathogenic fungi.[6]

Present study aims at evaluation and comparision of free radical scavenging activity, reducing power activity and the phenolic and flavonoid content of these traditional medicinal plants widely available in wild condition in Ranchi district. Besides, this study also sought to correlate the total antioxidant activity and total phenol and flavonoid content of these plants extracts.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Materials

The plant materials were collected in wild condition from Kanke road, and Bariatu road, Ranchi, Jharkhand. They were identified and authenticated using 'Flora of Bihar and Orissa' and Blatter Herbarium (St. Xavier's College, Mumbai). The above collected plant materials were washed with distilled water (2-3 times), then shade dried and ground separately to fine powder using grinder. Powders were then stored in air tight jars and kept in the dark for experimental use.

2.2. Preparation of Extracts

1 g each of dry plant powder of leaves of V. Negundo and rhizome of A. calamus was soaked overnight separately in 5 ml of ethanol (100%) so as to prepare 20% extracts. The extracts were then filtered through Whatman no. 1 filter paper, the filtrates were collected separately and then transferred into pre-weighed evaporating dishes and the solvent in each was evaporated. The residues, thus, obtained were weighed and diluted with appropriate amount of ethanol so as to obtain the required concentration (Gupta, *et.al*, 2007).[7] Triplicates of the extracts were prepared for experimentation.

3. ASSAYS FOR ANTIOXIDANT ACTIVITY

3.1. Determination of total antioxidant capacity

The method described by **Prieto *et. al* (1999).[8]** was used to determine the total antioxidant capacity of the extracts.[9] Phosphomolybdenum assay was used to find out the total antioxidant capacity. This is based on the reduction of Mo (+6) to Mo (+5) by the sample analyte. The reagent solution contained sodium phosphate (28 mM), ammonium molybdate (4 mM), and sulphuric acid

(0.6 mM) mixed with the extracts (diluted with ethanol to obtain 10 - 50 µg/ ml a range of concentrations). The samples were incubated at 90˚ C for 90 minutes and the subsequent formation of green phosphate/ Mo (+5) complex was measured at 695 nm (Peer Basha, *et. al.*, 2014).[10] For reference, ascorbic acid solution (0.2 - 2 mM) was used. The reducing capacity of the extract was expressed as the ascorbic acid equivalents (milligrams per gram extract).

3.2. Reducing Power Assay
Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity.[11] The method proposed by Oyaizu, *et. al.* (1986).[12] was used for the reducing power assay. Antioxidants which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form Fe^{2+} - Ferrozine complex that has an absorption maximum at 700 nm.[13] The reducing power of the extracts was expressed in terms of absorbance and compared with that of ascorbic acid standard.

3.3. DPPH radical scavenging activity
DPPH (2, 2-diphenyl-1-picryl hydrazyl) is the most generally used stable free radical for testing the potential of compounds as free radical scavengers of hydrogen donors and also to examine the antioxidant activity of plant extracts.[14] The DPPH radical scavenging activity was carried out as per the method adopted by Brand-**Williams, *et al* (1995).[15]** Range of concentrations of plant extracts used for DPPH radical scavenging activity was as follows (**Table 1**):

Table 1: Dilution of Extracts/ Standard for DPPH Radical

S. No.	Plants/ Standard	Concentration (µg/ ml)
1	Standard Ascorbic acid	3 to15
2	*A. calamus* rhizome	200 to 1000
3	*V. negundo* leaves	200 to 1000

Using the following equation, DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{(A_b - A_t)}{A_b} \times 100 \qquad (i)$$

where, A_b is the absorbance of the blank (*i.e.* only DPPH solution, no sample) and A_t is the absorbance in presence of the test compound / ascorbic acid standard.

3.4. Estimation of total phenolics and flavonoids
Modified Folin-Ciocalteau method, as proposed by Eberhardt *et. al.* (2000).[16] was used for estimating the total phenolic content of the plants' extracts. Total phenolic content was expressed as mg of gallic acid equivalents per mg of residue. For estimating the total flavonoid content Aluminium chloride method, as proposed by Zhishen *et* al (1999).[17] was used. Total flavonoid content was expressed as mg of rutin equivalents per mg of residue.

4. RESULTS AND DISCUSSION
4.1. Determination of Total Antioxidant Capacity
With increased concentration of the standard, its antioxidant capacity also got raised suggesting a strong and positive correlation between increased concentration of ascorbic acid and the absorbance (**Figure 1**).

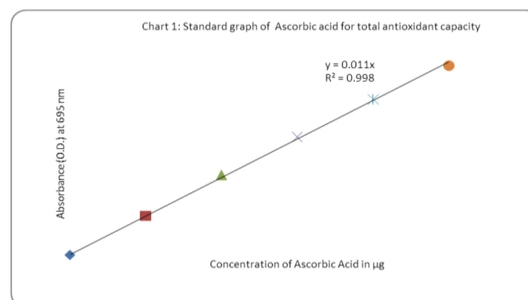

Figure 1: Standard graph of Ascorbic acid for total antioxidant capacity

Amongst the two plant extracts, lower antioxidant capacity was recorded for the ethanolic extract of dry leaves of *V. negundo* (**0.0147±.0004 mg** AAE/ 50 µg of residue). Ethanolic extract of *A. calamus* showed a higher antioxidant capacity (**0.0370±.0003mg** AAE/ 50 µg of residue) (**Figure 2**). Standard Ascorbic acid showed antioxidant capacity of **0.0509± 0.0008** at 50µg/ml of concentration. Thombre, *et. al.* (2013).[18] reported that the ethanolic extract of *V. negundo* leaves showed antioxidant capacity of 233.33 ± 2.95 mg AAE/ gm dry weight of the plant material.

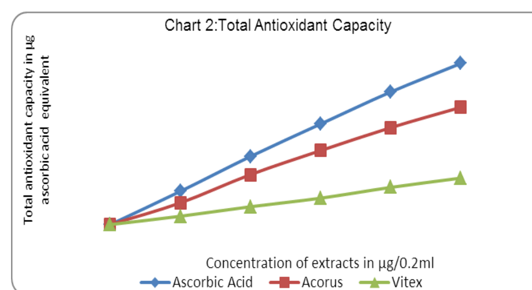

Figure 2: Total Antioxidant Capacity of Extracts

4.2. Reducing Power Assay
The reducing power of the extracts when compared with that of ascorbic acid standard showed that as the concentration of standard / extracts increases, there is an increase in the absorbance indicating the increase in the reduction (Fig.3). Higher reducing power was observed with ethanolic extract of rhizome of *A. calamus* (0.091±0.004 Abs for 50µg), whereas the ethanolic extract of leaves of *V. negundo* showed a lower reducing power (0.034 ± 0.002 Abs for 50µg). Lakshmanashetty *et. al.* (2010).[19] had also reported the reducing power of ethanolic extract of *Vitex negundo* to be (0.918± 0.035) absorbance units at 600 µg/ml. The reducing properties are generally associated with the presence of reductones,

which have been shown to exert antioxidant action by breaking the free radical chain or by donating a hydrogen atom (Pillai, *et. al*., 2014).[13] Subathraa and Poonguzhali (2012).[20] tested reducing power assay of the aqueous extract of *A. calamus* rhizome using various concentrations ranging from 25 - 400µg/ml and reported maximum reduction value of 0.900 ± 0.03 at 400µg/ml.

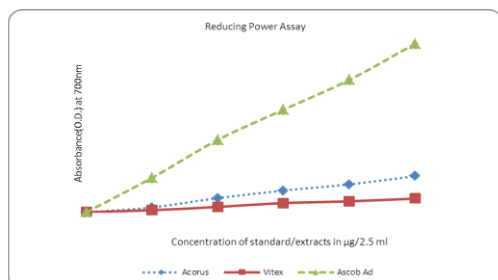

Figure 3: Reducing Power Assay

4.3. DPPH Radical Scavenging Activity

Antioxidant molecules when incubated, react with DPPH and convert it into 2, 2 -diphenyl-1-picryl hydrazine, which is a measure of the scavenging potential of plant extracts and this can be measured at 520 nm.[21] DPPH radical scavenging activity of ascorbic acid (standard) is given below (Figure 4).

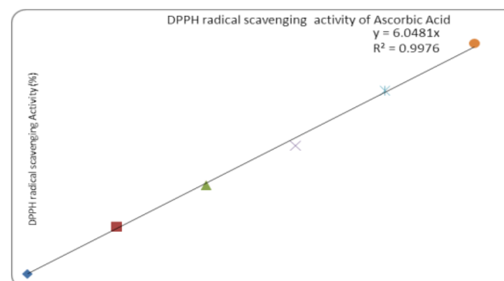

Figure 4: DPPH radical scavenging activity of Ascorbic Acid

DPPH radical scavenging activity of the extracts is given in table 2. It shows that there is a linear relationship between the concentration of extract and the DPPH radical scavenging activity, i.e. as the concentration of extract increases, there is an increase in the DPPH radical scavenging activity. The IC_{50} value for the ethanolic extract of rhizome of ***A. calamus*** was found to be high **1428.57 µg/ ml** indicating that the extract has very low DPPH radical scavenging activity. The lower IC_{50} value was observed for the extract of dry leaves of ***V. negundo*** **(819.67 µg/ ml)** indicating the high DPPH radical scavenging activity. The IC_{50} values of all the extracts were greater as compared to that of **ascorbic acid (8.27 µg/ ml)** indicating that the extracts had lower DPPH radical scavenging activity than ascorbic acid standard

Table 2: Determination of DPPH radical scavenging activity of extracts of *Acorus calamus* and *Vitex negundo*

Extract	Concentration (µg/ml)	DPPH radical scavenging activity** (%)	IC_{50}*** (µg/ml)
Standard Ascorbic Acid	3	18.83 ± 3.43	8.27
	6	35.48 ± 3.36	
	9	51.32 ± 2.47	
	12	73.33 ± 0.57	
	15	**92.21 ± 0.62**	
Acorus calamus rhizome	200	8.45 ± 1.35	1428.57
	400	14.34 ± 0.73	
	600	21.57 ± 0.67	
	800	28.80 ± 2.26	
	1000	**35.08 ± 0.46**	
Vitex negundo leaves	200	15.27 ± 1.50	819.67
	400	25.78 ± 0.67	
	600	37.56 ± 0.51	
	800	49.79 ± 0.99	
	1000	**60.25 ± 1.00**	

Notes: **: % mean values ± Std. Deviation.
***: IC_{50} value for ascorbic acid is 8.27 µg/ml.

4.4. Total Phenolic Estimation

Total phenolic content of the extracts is shown in Figure 4. Total phenolic content of ethanolic extract of rhizome *of A. calamus* was observed to be 0.0045 ± 0.000 mg of gallic acid equivalents per mg of residue at 50 µg/ ml concentration. Singh (2012)[22] also reported the phenolic content in the ethanol extract of *A. calamus* in terms of gallic acid equivalent (GAE) [72.4 ± 0.14 mg/gm at 100 µg/ ml concentration].

Total phenolic content of ethanolic extract of leaves of *V. negundo*, on the other hand, was observed to be 0.00478 ± 0.0001 mg of gallic acid equivalents per mg of residue at 50 µg/ ml concentration. Lakshmanashetty, *et. al.* (2010).[19] found total phenolics content in ethanolic extract of dry leaves of *V. negundo* at 249.96 ± 8.34 GAE/ g.

Total phenolic content was found to be 8.91% and 9.64% (GAE /100 g of plant material) in *A. calamus* and *V. negundo,* respectively (**Figure 5**).

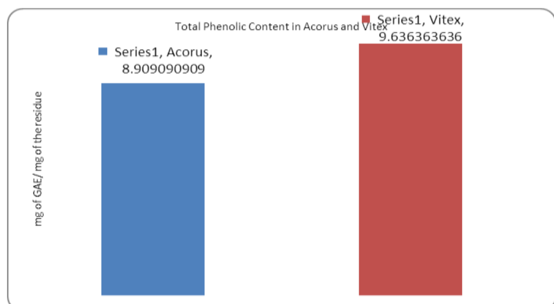

Figure 5: Total Phenolic Content of the Extracts (expressed as mg of gallic acid equivalent mg^{-1} of residue)

4.5. Total Flavonoid Estimation

Total Flavonoid contents of the extracts are shown in Figures 5. Total flavonoid content of ethanolic extract of rhizome of *A. calamus* was observed as 0.0106 ± 0.0002 RE/mg (Rutin equivalent/ mg of residue) at 50 µg/ ml concentration. Singh (2012)[22] also reported the flavonoid content in the ethanol extract of *A. calamus* in terms of Rutin equivalent (RE) [68.54 ± 0.45 mg/g at 100 µg/ ml].

Total flavonoid content in ethanolic extract of leaves of *V. negundo*, on the other hand, was observed to be .0138 ±.0004 RE/ mg of residue at 50 µg/ ml. Lakshmanashetty, *et. al.* (2010)[19] had observed 166.67 ± 9.14 catechin equivalent / g of plant extract of dry leaves of *V. negundo* ().

Total flavonoid contents per 100g of plant material were recorded **27%** and **32% (**RE /100 g of plant material) in *A. calamus* and *V. negundo,* respectively (**Figure 6**).

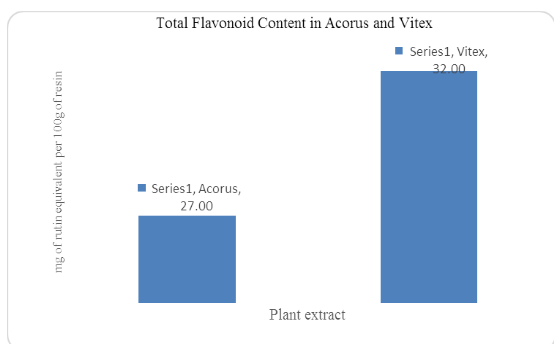

Figure 6: Total Flavonoid Content in Acorus and Vitex (mg of rutin equivalent mg^{-1} of residue)

5. STATISTICAL ANALYSIS

IC_{50} values for DPPH radical scavenging assay was determined by linear regression analysis. Correlation analysis.[1] was undertaken to correlate total antioxidant capacity of the plants (*viz., A. calamus and V. negundo*) with total phenolic and flavonoid contents of these plants. There is a strong +ve correlation between the phenolic and flavonoid contents of *A. calamus* and *V. negundo* and the anti-oxidant capacity. Devi and Ganjewala (2011).[23] had also obtained similar results in case of *A. calamus*.

6. CONCLUSION

Present investigation revealed that phenolic and flavonoid contents in *A. calamus* and *V. negundo* imparted antioxidant properties as there is a +ve correlation between these. The ethanolic extract of *V. negundo* was found to have higher DPPH radical scavenging activity, whereas *A. calamus* showed higher reducing power and total antioxidant activities. Further studies may be conducted to isolate and identify the phenolic and flavonoid compounds from these plants.

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CONFLICT OF INTEREST

There is no conflict of interest amongst the authors for this research manuscript.

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