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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY AMONG SELECTED MEMBERS OF THE TRIBE EUPATORIEAE

***Neethi.C.Nair and Sheela, D.**

Department of Botany St.Teresa's College, Ernakulam, Kerala, India

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ABSTRACT

Free radicals are the causes of a large number of human degenerative diseases affecting a wide variety of physiological functions. Antioxidants, even at relatively small concentrations inhibit oxidation by acting as free radical scavengers and thus converting these radicals into less reactive oxygen species. The present study is on the plants such as *Ageratum conyzoides*, L., *Eupatorium ayapana*, Vent., and *Chromolaena odorata*, L. which belong to the tribe Eupatorieae of the family Asteraceae. These members are rich in secondary metabolites such as phenol, flavanoids and tannin. The presence of these phenolics makes them a good antioxidant. The study reveals the quantity of secondary metabolites in these plants and there by proving their antioxidant potential and so these can be used for the preparation of natural drugs.

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INTRODUCTION

The functional properties such as antimicrobial and antioxidant, of plant extracts are widely used in processed food preservation, pharmaceuticals, cosmetics, alternative medicine and natural therapies (Bakkali *et al.*, 2008). The Asteraceae is a large family of about 25,000 species which are rich in secondary metabolites with biological activity (Okunade, 2002). As a part of our continued interest in invasive plants and weeds as potential sources of pharmacologically important compounds, and as previously reported (Mihigo *et al.*, 2015), we undertook the antioxidant investigations of *Ageratum conyzoides* L., *Eupatorium ayapana*, Vent., and *Chromolaena odorata* L., the invasive weeds which belong to the tribe Eupatorieae of the family Asteraceae. *Ageratum conyzoides* L., is used for the treatment of conjunctivitis, liver pain, opthalmia, pheumonia, sterility, skin diseases, wounds and cuts; and the root is used against harmful effects of perceived evil spirits in children (Kohli *et al.*, 2006; Roy *et al.*, 2008; Rahman *et al.*, 2012; Motaleb *et al.*, 2013). Antioxidants from natural sources help the endogenous antioxidants to neutralize oxidative stress. They inhibit or prevent oxidation of substrates and evolve to protect cells from the damage of Reactive Oxygen Species (ROS).An imbalance between antioxidants and ROS causes the damage of cells (Gulcin, 2010). Oxidative stress due to the overabundance of reactive oxygen species (ROS) is an unavoidable consequence of normal aerobic respiration. Because of this, our normal balance is shifted in favor of pro-oxidants, which is one of the major cause of today's diseases such as cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Braca *et al.*, 2002) Maxwell, (1995). As it is proved that these plants are rich in antioxidants, the pharmacological properties could be used as therapeutic agents. Currently the anti oxidant potential and concentration of secondary metabolites among the plants, *Ageratum conyzoides*, L., *Eupatorium ayapana*, Vent., and *Chromolaena odorata*,L. which belong to the tribe Eupatorieae of the family Asteraceae were studied.

**Corresponding author: Neethi.C.Nair*
Department of Botany St.Teresa's College, Ernakulam, Kerala, India.

MATERIALS AND METHODS

Collection of plant materials

Fresh plants of *Ageratum conyzoides*, L., *Eupatorium ayapana*, Vent., and *Chromolaena odorata*, L. are collected from various parts of Thrissur and Ernakulam District of Kerala, India. The plants were authenticated by comparing the herbarium specimens of BSI, Coimbatore, India and the voucher specimens were stored in the herbarium. The plants were washed thoroughly in running water followed by distilled water .Then the plants are shade dried for two weeks .The

dried specimens were stored in airtight covers at low temperature for further investigation.

Preparation of plant extracts

Nearly 20 gm of the powered sample is weighed and extracted with 200 ml hot methanol in a Soxhlet apparatus at 55^0 C for 10 hours. The extract is then concentrated in a boiling water bath to a volume of 30 ml and is stored in airtight brown bottles.

Phytochemical screening

Estimation of total phenol

The sample extract (1m) was mixed with 5ml of Folin-Ciocalter reagent (10% in DW). After 5 minutes, 4ml of 7.5% (W/V) Na_2CO_3 was added to each tube, the test tubes were cap-screwed and vortexes (20 sec).After incubates at room temperature for 2 hours in the dark, the absorbance of the reaction mixture was measured at 740nm using UV visible spectrophotometer against blank sample which contained the same mixture without the sample extract. Using a six point calibration curve (20-120 mgL) the total phenolics were determined by comparison of the values obtained, within the calibration curve of phloroglucinol. The results were expressed as phloroglucinol equivalent (PGF) in mg/gm.

Estimation of total Flavonoids

Total flavonoids content was measured by aluminium chloride chlorimetric assay. An aliquot (1ml) of extract or standard solution of catechin (20,40,60,80,100 mg/l) was added to 10ml volumetric flask containing 4ml of distilled water to the flask was added 0.3 ml 5% $NaNO_2$ (Sodium nitrate). After 5 minutes 0.3 of 10% Al_2Cl_3 (Aluminium chloride) was added. At the 6^{th} minute, 2ml 1M NaOH was added (40gms in 1000ml) and the total volume was made upto 10ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The total flavanoid content was expressed as mg catechin equivalence (CE/100 gm) fresh mass.

Estimation of total tannin

1gm of sample and 10ml 0.05 M NaOH was taken in boiling tube. Keep this in boiling water bath at 60^0 C for 11/2 hours. Then allow it to cool and centrifuge the solution.5ml of the supernatant is pipetted and 1ml of citrare buffer, 1ml of folin reagent and 10ml carbonate tartarate reagent are added. This was kept for 30 minutes. Then read absorbance at 760nm.The total tannin content was expressed as mg/gm tannic acid equivalence.

Antioxidant Activity

DPPH Radical Scavenging Assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assays was carried out spectrophotomerically at 517nm. The percent inhibition was calculated as:

%Inhibition =100 x (Ablank – Asample/Ablank)

where A blank the absorbance of the control reaction (containing all reagents except the test sample), and A sample is the absorbance of test samples.

RESULTS AND DISCUSSION

Most of the plants in Asteraceae are considered as a source of many biologically active compounds like polyphenolics (Ivanescu *et al.*, 2010), Flavonoids (2010) and tannin (Hassanpour *et al.*, 2011). These three are the major group of compounds that act as primary antioxidants or free radical scavengers (Potterat *et al.*, 1997). The present study conducted in the plants showed that they are rich in these secondary metabolites. In these Flavonoids shows high activity ranges from 1.7- 3.2 %. Phenol ranges from 1.8 – 3 % and that of tannin is 1 – 3.1 %. *Chromolaena odorata*, L. has highest activity for all the three metabolites. The IC_{50} value ranges from 3.46 – 5.1 µg/ml. Again *Chromolaena odorata*, L. shows lowest IC_{50} value which infers that it has the highest antioxidant activity. On the basis of the results, the antioxidant properties is beneficial to the antioxidant protection system against oxidative damage and thus *Chromolaena odorata*, L. is proved to be a source of natural antioxidant and an alternative to synthetic ones. Thus this plant can be used for making drugs with other formulations.

Table 1. Total Phenol content among the plant samples

S.No.	Sample	Total phenol %
1.	*Ageratum conyzoides*, L.	1.75 ± 0.17
2.	*Chromolaena odorata*, L.	2.96 ± 0.15
3.	*Eupatorium ayapana*, Vent.	1.86 ± 0.21

Mean ± SD of the triplicates expressed in %

Table 2. Total Flavanoid content among the plant samples

S.No.	Sample	Total flavanoid %
1.	*Ageratum conyzoides*, L.	2.28 ± 0.24
2.	*Chromolaena odorata*, L.	3.17 ± 0.14
3.	*Eupatorium ayapana*, Vent.	1.70 ± 0.06

Mean ± SD of the triplicates expressed in %

Table 3. Total Tannin content among the plant samples

S.No.	Sample	Total tannin %
1.	*Ageratum conyzoides*, L.	0.64 ± 0.14
2.	*Chromolaena odorata*, L.	1.07 ± 0.15
3.	*Eupatorium ayapana*, Vent.	0.83 ± 0.12

Mean ± SD of the triplicates expressed in

Table 4. % of Antioxidant activity among the plant samples

S.No	Samples	IC_{50} Value µg/ml
1.	*Ageratum conyzoides*,L.	5.1
2.	*Chromolaena odorata*,L.	3.46
3.	*Eupatorium ayapana*,Vent.	9.8

Table 5. Anova single factor for secondary metabolites

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
PHENOL	3	65.8575	21.9525	45.04832
FLAVANOID	3	71.583	23.861	54.59518
TANNIN	3	25.388	8.462667	4.879609
DPPH	3	18.36	6.12	10.8292

ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	745.2194	3	248.4065	8.613836	0.006919	4.066181
Within Groups	230.7046	8	28.83808			
Total	975.924	11				

Table 6. Anova single factor for antioxidants

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
1ml	3	60	20	111
3ml	3	87	29	133
5ml	3	139	46.33333	481.3333
7ml	3	189	63	469
9ml	3	221	73.66667	389.3333

ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6056.267	4	1514.067	4.780257	0.020451	3.47805
Within Groups	3167.333	10	316.7333			
Total	9223.6	14				

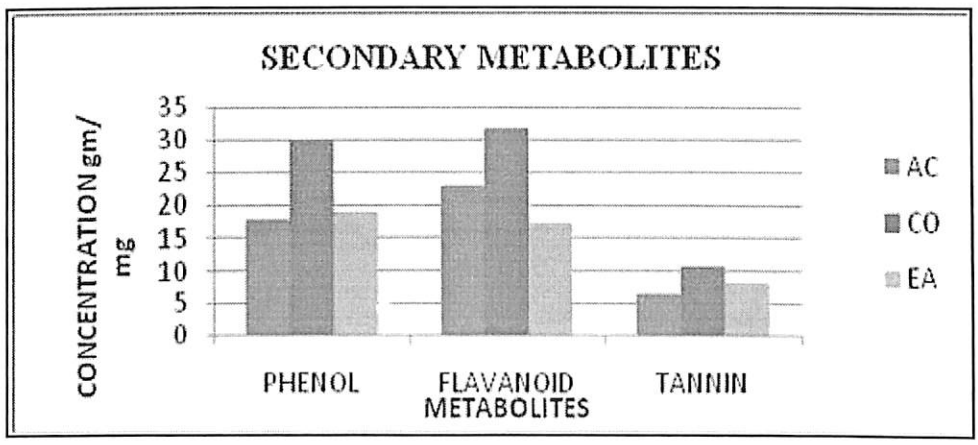

Fig. 1. Graph showing secondary metabolites

AC - *Ageratum conyzoides*, L., CO - *Chromolaena odorata*, L., EA - *Eupatorium ayapana*, Vent.

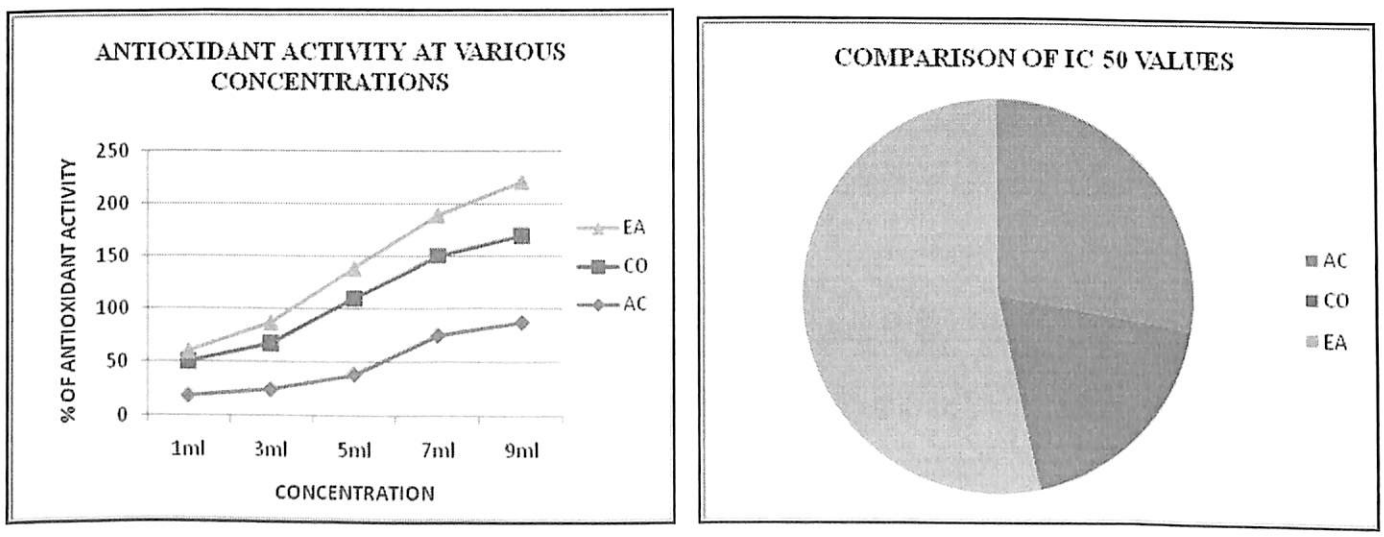

Fig. 2. Graph showing Antioxidant Activity

AC - *Ageratum conyzoides*, L., CO - *Chromolaena odorata*, L., EA - *Eupatorium ayapana*, Vent.

Conclusion

The present investigation showed that, the selected plants in the tribe possess high anti oxidant capacity with respect to the secondary metabolites, phenol, flavonoid and tannin. Thus these plants show good percentage of inhibition, revealed to be a better dietary anti oxidant. So the therapeutic potential of the selected plants should be explored for the preparation of new formulations.

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