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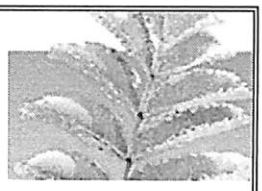

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# Quantification of secondary metabolites and anti-oxidant potential of selected members of the tribe Heliantheae

**Neethi C Nair and D Sheela**

**Abstract**
The development of multi drug resistance organisms has become major global issue in the treatment of infectious diseases. So we have to develop new therapeutic compounds with novel action spectrum and diverse chemical structure which could work effectively against emerging pathogens. The plants *Eclipta prostrata* L., *Wedelia chinensis*, (Osbeck) Merr and *Xanthium strumarium* L., which belong to the tribe Heliantheae of the family Asteraceae has large amount of secondary metabolites such as phenol, flavanoids and tannin. The study revealed that due to the presence of these secondary metabolites, the plants show good anti-oxidant activity. The flavonoid content is highest that range from 22.07-27.95mg/gm than total phenol and tannin content. Thus the selected plants show therapeutic properties and can be used for the preparation of natural drugs.

**Keywords:** therapeutic, emerging, pathogens, flavonoid, therapeutic

## Introduction
The Asteraceae is a well-known family in Indian flora and is considered as one of the most advanced family from all the dicotyledonous. The group includes about 25,000 species, spread across 1,620 genera and 12 subfamilies. (Maria *et al.*, 2007) [7]. A large number of plants belonging to the family Asteraceae contained chemical compounds exhibiting antimicrobial, anticancer and antioxidant properties. The Heliantheae is the third-largest tribe in this family. This tribe is found to have good antioxidant potential due to the presence of secondary metabolites such as phenol, flavonoids and tannins. The plants selected for the study are *Eclipta prostrata* L. *Wedelia chinensis*, (Osbeck) Merr and *Xanthium strumarium* L.
In ayurvedic medicines, *Eclipta prostrata* L. is considered as powerful liver tonic, rejuvenative and used for dyeing hair. In decoction, the plant is used in uterine haemorrhage and menorrhagia. According to Meena *et al.* (2011) [9] *Wedelia chinensis* (Osbeck) Merr is a powerful liver tonic, rejuvenates and especially good for the hair and is considered tonic, alternative and useful in coughs, cephalalgic skin diseases and alopecia. Xanthatin and xanthinos in from the burs of *Xanthium strumarium* L. are potential anticancer agents. The roots and leaves are used as anodyne, anti-rheumatic, appetizer, diaphoretic diuretic, emollient, laxative and sedative (Ginesta Peris *et al.*1994) [3].
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) [1, 8]. As it is proved that these plants are rich in antioxidants, the pharmacological properties could be used as therapeutic agents. In the present study, the anti-oxidant potential and concentration of secondary metabolites among the plants, *Eclipta prostrata* L., *Wedelia chinensis-s* (Osbeck) Merr and *Xanthium strumarium* L. of the tribe Heliantheae were studied.

## Materials and Methods
### Collection of plant materials.
Fresh plants of *Eclipta prostrata* L. *Wedelia chinensis* (Osbeck) Merr and *Xanthium strumarium* L. are collected from various parts of Thrissur and Ernakulam District of Kerala, India. The plants were authenticated by comparing the herbarium specimens of KFRI, Peechi,

Thrissur 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 vortexed (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 result were expressed as phloroglucinol equivalent (PGF) in mg/g.

**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 is taken in boiling tube. Keep this in boiling water bath at 60 $^{0}$C for 11/2 hours. Then allow 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. Keep this for 30 minutes. Then read absorbance at 760nm.The total tannin content was expressed as mg/g tannic acid equivalence.

**Antioxidant Activity**

**DPPH Radical Scavenging Assay**

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assays was carried out spectrophotometrically 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.

**Table 1:** Total Phenol content among the plant samples.

| SL. No. | Sample | Total phenol mg/gm |
|---|---|---|
| 1. | *Eclipta prostrate* L. | 20.29 ±0.15 |
| 2. | *Wedelia chinensis* (Osbeck) Merr | 17.80 ±0.11 |
| 3. | *Xanthium strumarium* L. | 19.53 ±0.33 |

Mean ± SD of the triplicates expressed in mg/g

**Table 2:** Total Flavanoid content among the plant samples.

| SL. No. | Sample | Total flavanoid mg/gm |
|---|---|---|
| 1. | *Eclipta prostrate* L | 27.95 ±0.09 |
| 2. | *Wedelia chinensis* (Osbeck) Merr | 22.07 ±0.12 |
| 3. | *Xanthium strumarium*, L. | 23.40 ±0.26 |

Mean ± SD of the triplicates expressed in mg/g

**Table 3:** Total Tannin content among the plant samples.

| SL. No. | Sample | Total tannin mg/gm |
|---|---|---|
| 1. | *Eclipta prostrate*, L. | 6.63 ± 0.11 |
| 2. | *Wedelia chinensis* (Osbeck) Merr | 9.12 ± 0.15 |
| 3. | *Xanthium strumarium* L. | 4.94 ±0.11 |

Mean ± SD of the triplicates expressed in mg/g

**Table 4:** % of Antioxidant activity among the plant samples.

| Sl. No | Samples | 1ml | 3ml | 5ml | 7ml | 9ml |
|---|---|---|---|---|---|---|
| 1. | *Eclipta prostrata* L. | 24 | 40 | 64 | 78 | 82 |
| 2. | *Wedelia chinensis* (Osbeck) Merr | 17 | 34 | 60 | 72 | 82 |
| 3. | *Xanthium strumarium*, L. | 23 | 27 | 35 | 48 | 79 |

**Table 5:** Anova Single factor for Secondary Metabolites

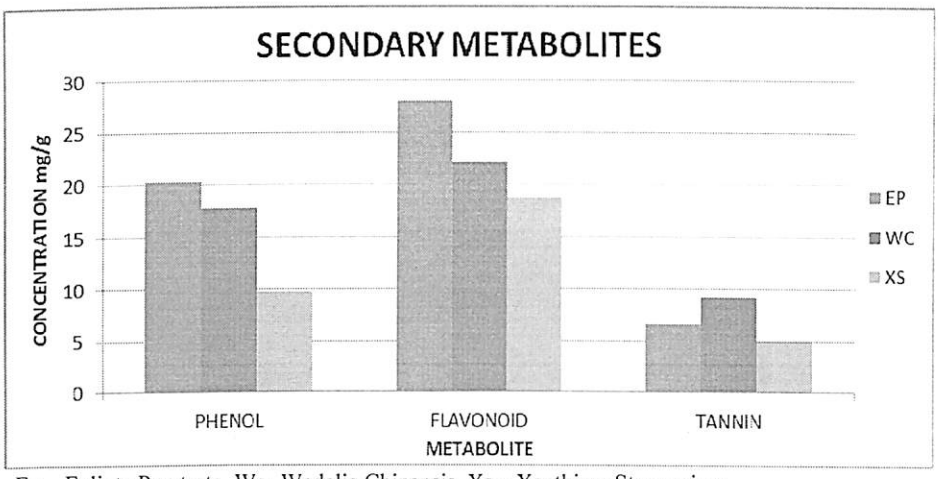
| Summary | | | | |
|---|---|---|---|---|
| Groups | Count | Sum | Average | Variance |
| Phenol | 3 | 57.69 | 19.20 | 1.63 |
| Flavonoid | 3 | 73.42 | 24.47 | 9.50 |
| Tannin | 3 | 20.69 | 6.90 | 4.42 |

| Anova | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 488.21 | 2 | 244.11 | 47.70 | 0.0002 | 5.14 |
| Within Groups | 31.12 | 6 | 5.19 | | | |
| Total | 519.33 | 8 | | | | |

**Table 5:** Anova Single factor for Antioxidants

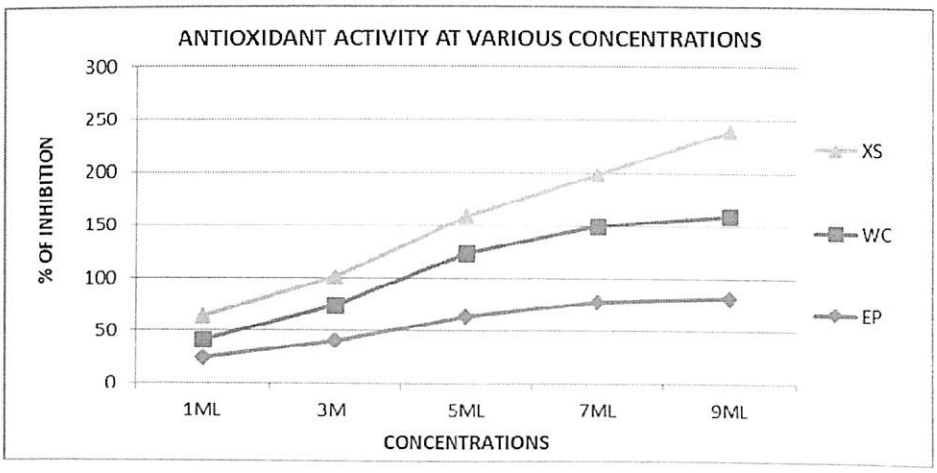
| Summary | | | | |
|---|---|---|---|---|
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
| 1ml | 3 | 64 | 21.33 | 14.33 |
| 3ml | 3 | 101 | 33.67 | 42.33 |
| 5ml | 3 | 159 | 53 | 247 |
| 7ml | 3 | 198 | 66 | 252 |
| 9ml | 3 | 239 | 79.67 | 4.33 |

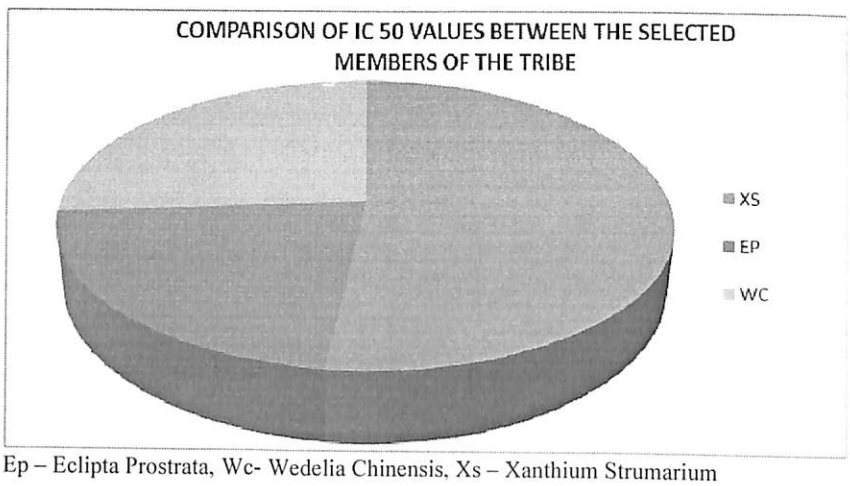
| Anova | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 6692.93 | 4 | 1673.23 | 14.94 | 0.0003 | 3.48 |
| Within Groups | 1120 | 10 | 112 | | | |
| Total | 7812.93 | 14 | | | | |




Ep – Eclipta Prostrata, Wc- Wedelia Chinensis, Xs – Xanthium Strumarium

**Fig 1:** Graph showing secondary metabolites




Ep – Eclipta Prostrata, Wc- Wedelia Chinensis, Xs – Xanthium Strumarium

**Fig 2:** Antioxidant activity among the selected plants

## Results and Discussion

Most of the members of the Asteraceae are rich in secondary metabolites with biological activities (Gupta, 1995; Okunade, 2002) [4, 10]. A large number of plants belonging to the family Asteraceae contained chemical compounds exhibiting antimicrobial, anticancer and antioxidant properties. Phenolic compounds, flavonoids and tannins are a major group of compounds that act as primary antioxidants or free radical scavengers (Potterat, 1997) [13]. The presence of phenol, flavonoid and tannin in tha family Asteraceae was also reported by many workers and they are considered as a source of many biologically active compounds like polyphenolic compounds (Ivanescu *et al.*, 2010) [6], flavonoids (Ferracane *et al.*, 2010) [2] and Tannins (Hassanpour *et al.*, 2011) [5]. Tannins are useful in the treatment of inflamed or ulcerated tissues and they have potential in cancer prevention and anticancer (Ruch *et al.*, 1989; Motar *et al.*, 1985) [14, 11]. According. The present study carried out on the plant samples revealed the presence of phenols, flavonoids and tannins and this is in accordance with the work done by Li *et al.*, 2003 [7] that the presence of flavonoids exhibit antioxidative action in plants. *Eclipta prostrata* L., shows highest antioxidant activity compared to the other two plant samples. By analysing the Anova result, we can conclude that there is no significant difference in the concentration of secondary metabolites among the three plant samples. The $IC_{50}$ value of the tribe ranges from 2.37-5.61µg/ml. It is also evident that there is a significant difference in the antioxidant potential with respect to the concentration and percentage of inhibition among the species.

## References


1. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavo-noids from Licania licaniaeflora. J Eth-nopharmacol. 2002; 79:379-81.
2. Ferracane R, Graziani g, Gallo M, Fogliano V, Ritieni. Journal of Pharmaceutical and Biomedical Analysis. 2010; 51(2):399-404.
3. Ginesta-Peris E, Garcia-Breijo FJ, Primo-Yúfera E. Antimicrobial activity of xanthatin from Xanthium spinosum, L. Lett. Appl. Microbiol. 1994; 18:206-208.
4. Gupta MP. 270 Plant as medicinales iberoamericanas. Bogota. CYTED-SECAB, 1995, 324.
5. Hassanpour S, Sadaghian M, MaheriSis N, Esharatkhah Chaichi Semsari M. Effect of condensed tannin on contolling faecal protein excretion in nematode-infected sheep: *In vivo* study. J Ami Sci. 2011; 7:896-900.
6. Ivanescu B,Vlase L, Corciova A, Lazar M. HPLC-DAD-MS study of polyphenols from Artemisia absinthium, A. annua and A. vulgaris, Chem Nat Compd. 2010; 46:3-30
7. Li H, Wang Z, Liu Y. Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yao-Cai. 2003; 26:444-448.
8. Maria JA, Paulina B. Baccharis (Compositae): a review update. Marigold petals. Journal of Food Science. 2007; 41:163-164.
9. Maxwell SR. Prospects for the use of anti-oxidant therapies. Drugs. 1995; 49:345-61.
10. Meena AK, Rao MM, Meena RP, Renu PP. Pharmacological and phytochemical evidences for the plants of Wedelia Genus – A Review. Asian J Pharm Res. 2011; 1:7-12.
11. Motar MLR, Thomas G, Barbosa Fillo J M. Effects of Anacardium occidentale stem bark extract on *in vivo* inflamatory models. J Ethnopharmacol. 1985; 95:139-142
12. Okunade AL. Review Ageratum co-nyzoides L. (Asteraceae). Fitoterapia. 2002; 73:1-16.
13. Potterat O. ntioxidant and free radical scavenger of natural origin. Current Organic Chemistry. 1997; 1(4):415-440.
14. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, Carcinogenesis. 1989; 10:1003.