Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

INVESTIGATION ON REGIONAL VARIATION IN TOTAL PHENOLIC CONTENT, ALKALOID CONTENT AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF *CLEOME CHELIDONII L*.

GANGA RAO.B*, RAJESWARARAO.P, PRAYAGA MURTY.P, SAMBASIVA RAO.E, MADHUKIRAN.P, MALLIKARJUNA RAO.T, V.S.PRANEETH.D

NAIP/ICAR Sub-Project, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India 530003
Email: ganga.battu@gmail.com

Received: 13 Aug 2011, Revised and Accepted: 16 Sep 2011

ABSTRACT

In this study we investigated and compared the total phenolic, alkaloid content and *In-vitro* antioxidant activity of aerial and root part alcoholic extracts of *Cleome chelidonii* collected from two different regions Marteru (Godavari river region) and Lam (Krishna river region) of Andhra Pradesh, India. Quantitative regional variation was observed in alkaloid content for alcoholic aerial and root part extracts of *Cleome chelidonii* from Marteru and Lam regions of Andhra Pradesh. Concentration dependent antioxidant activity was observed for all these extracts and observed regional variation in two regions of extracts for scavenging of Superoxide, Hydroxyl and DPPH radicals. Among the two regions, Marteru region and considering part wise root part extract of *Cleome chelidonii* showed good antioxidant and alkaloid content.

Keywords: Alkaloid content, Antioxidant activity, *Cleome chelidonii*, Regional variation, Total phenolic content.

INTRODUCTION

Weeds are treated as unwanted material in the crop fields as they are sharing the nutrients, water and other essentials ultimately affecting the main crop and they are taken out by the farmers as a waste. Many Crop weeds are known to possess medicinal values. However, these weeds of medicinal value are not subjected to systematic phyto-pharmacological evaluation. If a systematic scientific approach is adopted, its acceptability enhances and promotes them as herbal drugs. Because herbal products can have differences in their composition depending on the soil where they grow[1] and agro-climatic conditions. The Chemotypic and biological activity variation of medicinal value of weed flora at different climatic zones has not been studied much so far. Hence, the present investigation provides an opportunity to profile chemical and *in vitro* antioxidant variations due to different climatic zones.

Cleome chelidonii Linn (Latin name is *Polanisia chelidonii*) is a traditional plant belonging to the family Capparaceae. It is grown as perennials throughout dry seasons. It has a pink rose or white colour flowers. *Cleome chelidonii* is generally known to be used for the treatment of colic, dysentery, headache, otitis, and rheumatism[2]. It has also been found to possess multiple therapeutic properties such as its use a vermifuge, the treatment of skin diseases[3] and its anti-inflammatory, antinociceptive and antipyretic properties[4].*C.chelidonii* contains glucocapparin and glucocleomin[5]. This weed is distributed throughout India from Himalayas down to Ceylon. One of the aims of this work was to investigate and compare the total phenolic and alkaloid content and *In-vitro* antioxidant activity of aerial and root parts of *Cleome chelidonii* collected from two different regions (Marteru and Lam regions) of Andhra Pradesh, India.

MATERIAL AND METHODS

Drug and Chemicals

Folin-Ciocalteau (FC) reagent, Bromocresol green (BCG),Riboflavin, Deoxy ribose, Nitroblue tetrazolium, 2,2-Diphenyl -1-picrylhydrazyl (DPPH),were purchased from Sigma chemicals, USA. All other chemicals used were of analytical grade.

Plant Material and Preparation of extracts

The plant material was collected from two different regions of Andhra Pradesh i.e., Marteru , Lam. The authenticity of the plant was confirmed by Taxonomist Dr. Prayaga Murty Pragada, Department of Botany, Andhra University,Visakhapatnam. The Voucher specimens were deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University. Freshly collected *cleome chelidonii plant was* separated as aerial and root parts and dried under shade. The coarse powdered aerial and root parts were macerated in 70% v/v ethanol. The liquid extract was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and used for investigation.

Quantification of Total Phenols

Total phenolic content was determined using the Folin-Ciocalteau reagent[6]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

Total Alkaloid Content

Total alkaloid content was determined by the Fazel et al.,2008 method[7].The plant extract (1mg/ml) was dissolved in 2 N Hcl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± S.E.M.

In-vitro antioxidant activity

The alcoholic aerial and root part extract of *C.chelidonii* from two regions were screened and compared for free radical scavenging activity.

Superoxide radical Scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich, 1969 method[8] , which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the $Fe^{2+}/EDTA/H_2O_2$ system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS) [9].

DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca et al., 2003[10]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine.

Lower the absorbance higher the free radical scavenging activity.

RESULTS AND DISCUSSION

Total Phenolic Content

The phenolic content was found absent in alcoholic leaf and root extracts of *Cleome chelidonii* from two regions.

Total alkaloid content

The determined total alkaloid content in alcoholic aerial and root part extracts of *C.chelidonii* from Marteru and Lam regions ranged 5±0.3-33.4±0.3 mg/gm. By comparing two regions Marteru region showed more alkaloid content than Lam region.

***In-vitro* antioxidant activity**

Superoxide radical scavenging activity

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA[12]. Therefore, studying the scavenging activity of plant extracts/compounds on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity. In the present study, the alcoholic leaf and root extracts of *Cleome chelidonii* was found to possess concentration dependent scavenging activity on superoxide radicals. The standard drug Ascorbic acid showed better percentage of inhibition of superoxide radical's alcoholic extract of *Cleome chelidonii*. The 50% Inhibition concentrations (IC_{50} values) for superoxide radical by alcoholic extract of *Cleome chelidonii* and Ascorbic acid were given in Table 2.

Hydroxyl radical scavenging activity

Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism[13]. Due to the high reactivity, the radicals have a very short biological half-life. The generated hydroxyl radicals initiate the lipid peroxidation process and/or propagate the chain process via decomposition of lipid hydroperoxides. A single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupts its function, and lead to cell death.

In this study alcoholic extracts of *Cleome chelidonii* from two regions was found to possess concentration dependent scavenging activity on hydroxyl radicals. Among the extracts root extract of *Cleome chelidonii* from Marteru region showed better percentage of inhibition of hydroxyl radical. The 50% Inhibition concentrations (IC_{50} values) for superoxide radical by alcoholic extract of *Cleome chelidonii* and Ascorbic acid were given in Table 3.

DPPH radical Scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxid ant on interaction with DPPH both transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1-diphenyl -2 -picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug[14]. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment. The data are displayed with mean ± Standard error mean of triplicate. The mean IC_{50} values for DPPH radical by alcoholic aerial and root extracts of *Cleome chelidonii* from Marteru and Lam regions and Ascorbic acid were found to be 1525.00 µg, 850.50, 750.00 µg , 600.25 µg and 60.24 µg respectively (Table 4) .

Table 1: Regional Variation in Total alkaloid content (mg/gm) of aerial and root part extracts of *Cleome chelidonii* from Marteru and Lam regions

Marteru region		Lam region	
Aerial part ext.*C.chelidonii*	root ext.*C.chelidonii*	Aerial part ext.*C.chelidonii*	Root ext.*C.chelidonii*
26.4±1.0	33.4±0.3	10.6±0.15	5±0.3

Table 2: *In-vitro* concentration dependent percentage inhibition of Superoxide radical by alcoholic aerial and root extracts of *Cleome chelidonii* from Marteru and Lam regions.

Conc (µg/0.1 ml)	Marteru region		Lam region		Ascorbic acid
	Aerial part ext.*C.chelidonii*	root ext.*C.chelidonii*	Aerial part ext.*C.chelidonii*	Root ext.*C.chelidonii*	
50	37.21±1.4	36.15±1.6	30.35±1.3	32.35±2.4	43.17±0.7
100	47.36±2.2	46.30±2.0	44.61±1.3	47.36±2.5	52.41±0.2
250	55.60±2.1	59.20±1.5	48.84±2.2	57.29±3.2	61.10±0.2
500	57.72±1.3	63.21±2.4	60.25±1.2	63.21±1.5	75.31±1.3
750	60.68±2.5	68.29±2.3	68.50±1.7	68.29±2.5	81.52±1.6
1000	74.63±3.2	78.44±1.5	75.26±2.3	73.36±2.1	86.48±0.5
2000	78.86±2.5	81.40±3.2	80.34±2.2	81.61±1.4	87.17±1.4
IC_{50} value	140.25	130.25	180.35	140.25	80.24

Table 3: *In-vitro* concentration dependent percentage inhibition of hydroxyl radical by alcoholic aerial and root extracts of *Cleome chelidonii* from Marteru and Lam regions

Conc (µg/0.1 ml)	Marteru region Aerial part ext.*C.chelidonii*	Root ext. *C. chelidonii*	Lam region Aerial part ext.*C.chelidonii*	Root ext.*C.chelidonii*	Ascorbic acid
50	31.29±1.82	38.27±2.32	24.74±2.13	22.41±1.62	31.67±1.2
100	43.34±2.3	44.40±3.21	40.17±2.13	43.55±2.32	40.30±1.2
250	54.55±2.32	60.25±2.42	55.60±1.32	56.45±3.2	55.61±1.1
500	58.35±2.62	66.38±1.52	64.48±3.32	61.31±2.34	72.27±2.1
750	71.25±2.64	75.90±3.5	67.44±3.62	69.56±2.31	81.52±1.6
1000	75.48±2.6	79.70±3.22	76.32±4.21	75.69±1.24	84.70±1.6
2000	76.32±2.5	84.57±3.62	80.34±3.24	83.51±2.42	84.85±3.2
IC_{50} value	185.50	152.24	200.50	180.25	190.20

Table 4: *In-vitro* concentration dependent percentage inhibition of DPPH radical by alcoholic aerial and root extracts of *Cleome chelidonii* from Marteru and Lam regions

Conc (µg/0.1 ml)	Marteru region		Lam region		Ascorbic acid
	Aerial part ext.*C.chelidonii*	root ext.*C.chelidonii*	Aerial part ext.*C.chelidonii*	Root ext.*C.chelidonii*	
50	13.05±1.1	20.42±1.6	21.26±1.3	22.32±1.1	45.30±2.1
100	14.21±1.2	24.63±2.2	26.42±2.1	28.11 ±2.3	75.61±2.1
250	25.05±2.1	31.47±2.4	35.68±2.4	35.16±1.3	81.82±2.4
500	33.47±2.5	35.16±4.2	41.26±1.8	42.63±2.2	86.52±2.2
750	36.11±1.1	40.21±2.5	50.00±2.3	59.58±1.5	88.18±2.1
1000	38.53±2.3	65.37±3.2	72.32±1.5	80.42±4.5	90.15±2.2
2000	84.62±2.4	59.79±3.5	84.32±5.1	85.26±5.2	90.45±3.4
IC_{50} value	1525.00	850.50	750.00	600.25	60.24

CONCLUSION

In present study we found that clear regional variability in alkaloid content and free radical scavenging activity against Superoxide, Hydroxyl and DPPH radicals. Marteru region showed better alkaloid content and antioxidant activity than Lam region. This probably due to good soil and weather conditions of Marteru region. When compared to aerial and root part, root part extract from two regions showed better *in vitro* antioxidant activity. Investigation on regional variation of biological activities for these extracts is in progress.

ACKNOWLEDGMENT

The authors are thankful to World Bank funded NAIP/ICAR Sub-Project.

REFERENCES

1. Salamon I. Production of *chamomile*, *Chamomilla recutita* (L.) Rauschert, in Slovakia. J Herbs Spices Med Plants 1992; 1:37-45.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, 2nd ed., Periodical Experts Book Agency, Delhi; 1991.
3. Chopra RN. Indigenous Drugs of India, Calcutta; 1958.
4. Parimalakrishnan S, Dey A, Smith A, Manavalan R. Evaluation of anti-inflammatory, antinociceptive and antipyretic effects of methanol extract of *Cleome chelidonii*. Int. J. Biol. Chem. Sci. 2007;1(3): 223-228.
5. Songsak T, Lockwood GB. Production of two volatile glucosinolate hydrolysis compounds in *Nasturtium montanum* and *Cleome chelidonii* plant cell cultures. Fitoterapia 2004;75: 296-301.
6. Singleton V L, Rossi JA. Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. Amer J Enology Viticulture 1965; 16: 144-58.
7. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi ,Mohammadreza Verdian-rizi. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J. Pharm. Sci. 2008; 32: 17-20.
8. Mc Cord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprien). J Biol Chem 1969; 244: 6049-6055.
9. Elizabeth Kunchandy, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990;58: 237-240.
10. Braca A, Fico G, Morelli I, De Simone F, Tome F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species.J Ethnopharmacol 2003; 86:63-67.
11. Anita Murali, Purnima Ashok, Madhavan V. In vitro antioxidant activity and HPTLC Studies on the roots and rhizomes of *Smilax zeylanica l.* (smilacaceae). Int J Pharm Pharm Sci 2011;3(1):192-195.
12. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000; 63:1035-42.
13. Walling C. Fenton's reagent revisited. Acc Chem Res 1975; 8:125-31.
14. Rumit Shah, Heena Kathad, Rajal sheth, Naveev sheth. *In Vitro* Antioxidant Activity of roots of *Tephrosia Purpurea* Linn. Int J Pharm Pharm Sci 2010;2(3):30-33.