COMPARATIVE ANALYSIS OF CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF LEAF AND BARK EXTRACTS OF *CLERODENRUM VISCOSUM* AND *CLERODENDRUM PHLOMIDIS*

Sunita Bhatnagar* & Soumya Ranjan Pattanaik
* Medicianl and Aromatic Division, Regional Plant Resource Centre, Nayapalli, Bhubaneswar-751 015, India

E-mail of Corresponding Author: bhasunita@gmail.com

Abstract
Cytotoxic and antioxidant potential of leaf and bark extracts of two Clerodendrum sps namely C. phlomidis and C. viscosum was investigated. Solvent extracts hexane, chloroform, acetone and methanol were tested for their cytotoxic potential using brine shrimp motility assay and antioxidant potential was ascertained using DPPH and FRAP assays. Cytotoxic activity of all the solvent extracts was tested at four doses 25, 50,100 and 200µl/ml. All the extracts showed dose dependent activity. Acetone extract of leaf (C.viscosum) showed significant cytotoxic activity 90.6 % at the dose of 200microgram/ml whereas chloroform extract of C. phlomidis (bark) showed highly significant activity to the tune of 95.6% at the highest dose. Thin layered chromatography based 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay for assessing the antioxidant potential was conducted using three different solvents, Maximum number of antioxidant bands were obtained in non-polar basic solvent that is Benzene: ethanol: ammonium hydroxide (90:10:1) (BEA). Quantitative radical scavenging assay was also conducted and acetone extract of *Clerodendrum phlomidis* leaf exhibited significant results at higher doses.

Keywords: *Clerodendrum viscosum*, *Clerodendrum phlomidis*, antioxidant, cytotoxic and extracts

1. Introduction
Clerodendrum genus consists of at least 20 important medicinal species. *Clerodendrum viscosum* and *Clerodendrum phlomidis* are a part of Indigenous system of medicines in a number of countries such as India, China, Srilanka and Australia[1,2]. *Clerodendrum phlomidis* commonly known as Agnimatha is a part of important Ayurvedic formulation known as Dasmoolarishta[3]. As evident from the name root is the main part from which this medication is derived. Whenever, roots are used as medicinal part, scarcity of the involved plant becomes evident as most of the medicinal plants are collected from the wild[4]. *Clerodendrum viscosum* syn *Clerodendrum infortunatum* has been reported to possess anthelmintic and anti tumor activity[5,6].

A number of other species like *C. inerme*, *C. colebrookianum* and *C. trichotomum* have shown antioxidant activities[7,8,9]. Thus, it was considered worthwhile to explore the antioxidant potential of these Indian medicinal plants. As roots are the most popular part used in the indigenous system of medicine so in this comparitive analysis leaf and bark extracts were compared for the cytotoxic as well as antioxidant was conducted.

2. Experimentals
2.1 Collection and processing of plants: Leaves and bark samples of *Clerodendrum viscosum* and *Clerodendrum phlomidis* were collected from the medicinal germplasm garden of Regional Plant Resource Centre, Bhubaneswar. Samples were deposited in the herbarium of the institute. Accession number of the medicinal plants is 291 and 281respectively.

2.2 Solvent extraction: Plant material collected was dried in shade and made to fine powder using a mechanical grinder. 50 gms each of leaf and bark powder was used for preparing the solvent extracts. Four extracts namely hexane, chloroform, acetone and methanol were prepared for both the species using standard procedures[10.]

Yield of extracts varied from 1% to 8.6% in leaves as well as bark extracts.

2.3 Biological evaluation: Extracts of both the species were subjected to following tests:

1. Cytotoxic active using brine shrimp lethality assay[10]

2. Qualitative antioxidant activity by thin layered chromatography based DPPH assay [11]
3. Quantitative antioxidant activity using radical scavenging DPPH[12] and FRAP Assays[13] For both the tests Ascorbic acid was used as standard antioxidant.

2.3.1 Cytotoxic activity*:* Cytotoxic activity was conducted using brine shrimp lethality assay. Brine shrimp eggs were incubated in 8% saline for 48 hrs. Solvent extracts were tested at the doses of 25 to 200microgram/ml. After 24 hours live larvae were counted and compared with the experimental control.

Statistical analysis of all the tests was conducted using Student's T test and level of significance was ascertained for all the tests.

2.3.2 TLC based antioxidant activity: Qualitative testing of antioxidant activity was done using the standard protocols[11]. TLC was conducted using three solvent systems namely EMW (ethyl acetate: methanol: water in the ratio of 40.5:4:4), CEF (Chloroform: ethyl acetate: formic acid in the ratio of 5:4:1) and BEA (Benzene:ethanol: ammonium hydroxide in the ratio of 90:10:1). After running of the plate up to 8cms they were dried and were sprayed with 0.2% 2,2, diphenyl-1-picryl-hydrazyl. Antioxidant bands were detected as yellow spots against a purple background.

2.3.3 Quantitative antioxidant activity using radical scavenging DPPH assay: Radical scavenging DPPH assay were conducted using the standard protocols[12.] IC50 of the extracts was compared with the standard antioxidant ascorbic acid.

2.3.3Quantitative antioxidant activity using FRAP assay: Antioxidant activity was analyzed by Ferric reducing antioxidant power assay as per the protocol of Ahmed and Beigh[13]. Absorbance was read at 593nm. Three replicates of each sample were taken and data was analyzed and significance was ascertained.

3. Results and Discussions

3.1 Cytotoxic activity: All the leaf extracts of *Clerodendrum viscosum(*CV*)* were more active than the extracts of *Clerodendrum phlomidis* (CP). Acetone leaf extract of CV showed the most significant activity (>90%) at the higher dose of 200microgram/ml whereas its counterpart showed mild activity to the tune of 66.67 % (Table 1). In earlier studies with *Clereodendrum infortunatum* methanol extracts have been reported to have significant anti-inflammatory activities[14]. Brine shrimp assay conducted in this study has earlier been reported as a good biological marker for anti-inflammatory and anticancer activity[15], thus from the results it is clear that CV acetone leaf extract could be a potential lead for the isolation of active molecule for the above two activities. Bark extracts of CP were found to be more potent as compared to the CV, specially the chloroform extract which exhibited 95.65% activity at the higher dose(Table 2). A number of active principles have been isolated from the methanolic extracts of leaves of CP[15], this study has shown for the first time that chloroform extract of the bark of the medicinal plant *Clerodendrum phlomidis* also holds some promise for the presence of active principles.

3.2 Antioxidant activity

3.2.1 TLC based antioxidant activity: Methanol extract of bark of both the species showed a yellow streak in BEA solvent instead of bands suggesting that number of antioxidant molecules is so closely situated that they cannot be counted. Thus in both the species it can be assumed that maximum number of antioxidant activity could be found in the methanol extract. TLC based DPPH antioxidant assay has been used for other medicinal plants like Terminalia and Combretum genus and based on the assay antioxidant molecules like Combretastatin have been isolated[16]. As per Table 3, it is evident that all the extracts of both the species do have antioxidant potential, for supporting the same other two antioxidant assays were also conducted.

3.2.2 Quantitative antioxidant activity using radical scavenging DPPH assay: There was remarkable variation in the antioxidant activity of the two species, In case of *C. phlomidis* leaf extracts acetone extract showed best antioxidant activity slightly at par with the standard antioxidant ascorbic acid, where as in case of *C. viscosum* leaf extracts, methanol extract showed best radical scavenging activity (Fig 1). This study was in confirmation with the earlier study where ethanolic extract showed better radical scavenging activity as compared to ether and chloroform extracts[17] Similar observation was also true for bark extracts as well (Fig 2). Antioxidant activity of Clerodendrum genus has been reported earlier in a number of studies. *Clereodendrum inerme* has been reported to be consisting of phenolic content which is responsible for the antioxidant activity in the species[18]. Same could be true for the two

experimental species studied here. Another species namely *Clerodendrum glandulosam* has shown hepatoprotective activity along with anti-oxidant activity[19], thus this study is in confirmation with the results obtained in this study.

3.2.3 Quantitative antioxidant activity using FRAP assay: In case of FRAP assay acetone extract of leaf as well as bark of both the species showed consistent good results. (Fig 3 and 4). Thus, in all the antioxidant models polar extracts had more antioxidant potential. Although in a number of Clerodendrum species antioxidant activity has been reported but in most of the cases the DPPH radical scavenging assay has been more popular[17,18]. This assay has been successfully used for detecting antioxidant activity in a number of plants such as *Pimpenella tragioides* and *Salvia macrosiphon*[20]. Results of both the assays have shown almost similar results.

Conclusion

Clerodendrum phlomidis and *Clerodendrum viscosum* have very promising cytotoxic and antioxidant potential and thus, there is a valid scientific basis for the use of these plants in the Indigenous system of medicine. Acetone extract of leaf as well as bark has presented itself as a promising lead for the isolation of active principles and need a detailed analysis. Thus, this study has successfully provided lead for the chemists to explore the medicinal plants.

Acknowledgements

Acknowledgements are due to DST, Govt. of Orissa for providing funding for the project fellow Soumya Ranjan Pattanaik. Acknowledgements are also due to the Forest and Environment Department, Government of Orissa for the laboratory facilities.

References

1. Sweeney AP, Wyllie SG, Shalliker RA, Markham JL Xanthine oxidase inhibitory activity of selected Australian native plants. *Journal of Ethnopharmacology* 2001, 75, 273-277.
2. Anynomous 1 *Quality Standards of Indian Medicinal Plants* (Vol 3) (2005) Indian Council of Medical Research, New Delhi, 167.
3. Schippmann U, Leaman DJ, Cunningham AB. *Impact of cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues.* Rome: Inter-Department Working Group on Biology Diversity for Food and Agriculture, FAO, 2002.
4. Anynomous *The Useful Plants of India*, (1992) Publication and Information Directorate, CSIR, New Delhi, pp 132.
5. Sannigrahi S, Mazumder UK, Pal D, Mishra SL. Terpenoids of methanol extract of *Clerodendrum infortunatum* exhibit anticancer activity against Ehrlich's ascites carcinoma (EAC) in mice. Pharm Biol. 2012, 50, 304-9.
6. Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T, Shinohara A, Nakata M (1999) Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. *Journal of Agricultureand Food Chemistry* 1999, 47, 1749-1754.
7. Rajlakshmi D, Banerjee SK, Sood S, Maulik SK. *In-vitro* and *in-vivo* antioxidant activity of different extracts of the leaves of *Clerodendron colebrookianum* Walp in the rat. *Journal of Pharmacy and Pharmacology*, 2003, 55, 1681-1686.
8. Chae S, Kang KA, Kim JS, Hyun JW, Kang SS Trichotomoside: A new antioxidative phenylpropanoid glycoside from *Clerodendron trichotomum*. *Chemistry and Biodiversity*, 2006, 3, 41-48.
9. Mohapatra, R., Bhatnagar, S., and Das, P. Filaricidal activity of a wild herb, *Typhonium trilobatum.* Advances in Platnt Sciences, 2010, 23, 51-52
10. Deby, C., and Margotteaux G. Relationship between essential fatty acids and tissue antioxidant levels in mice. C R *Seances Soc Biol Fil*. 1970, 165: 2675-2681.
11. Sanchez-Moreno, C., Larrauri, J. and Saura-Calixto, F. Free radical scavenging capacity of selected red and white wine, *J. Sci.Food. Agric*. .1999, 79, 1301-1304.
12. Ahmad S and Beigh, SH, Ascorbic acid, Carotenoids, Total Phenolic content and Antioxidant activity of various genotypes of *Brassica Oleracea encephala.* J. Med & Biol Sci, 2009, 3, 1-8.
13. Narayanan N, Thirugnanasambantham P, Viswanathan S, Vijayasekaran V, Sukumar E Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in experimental animals. *Journal of Ethnopharmacology* 1999, 65, 237-241

14. McLauglin, J. L., Chang, C. J., and Smith, D. L., Simple bench-topbioassays (brine shrimp and potato discs)for the discovery of plant antitumour compounds. 1993 In: *Human Medicinal Agents from Plants*. Kinghorn, A. D. and Balandrin, M. F. (Eds.), *ACS Symposium534*, American Chemical Society, Washington, D. C.: 112-137.
15. Masoko P and Eloff, J.N. Screening of 24 south african combretum and six terminalia Species (Combretaceae).Afr. J. trad. Com. And Alt Med. 2007, 4, 231-239.
16. Gouthamchandra K, Mahmood R, Manjunatha H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from *Clerodendrum infortunatum* L. Environ Toxicol Pharmacol. 2010, 30,11-18.
17. Gurudeeban, S., Satyavani, K, Ramanathan, T., Umamaheshwari, G. and Shanmugapriya R. Antioxidant and radical scavenging effect of *Clerodendrum inerme*(L). World Journal of fish and marine sciences, 2010, 2, 66-69.
18. Jadeja RN, Thounaojam MC, Singh TB, Devkar RV, Ramachandran A. Traditional uses, phytochemistry and pharmacology of *Clerodendron glandulosum* Coleb--a review. Asian Pac J Trop Med. 2012, 5, 1-6.
19. Gohari AR, Hajimehdipoor H, Saeidnia S, Ajani Y and Hadjiakhoondi A Antioxidant activity of some medicinal species using FRAP Assay. J of Med Plt. 2011, 10, 54-60.

Table 1. Comparative analysis of cytotoxic activity of leaf extracts of C. *viscosum* and *C. phlomidis*

Extract	Doses(µl)	*C. viscosum*	*C.phlomidis*
Hexane	25	10.36±5.25	14.58±7.22
	50	23.14±3.85	13.72±8.98
	100	49.76±7.45	35.71±18.23
	200	63.28±8.39	37.82±4.68
Chloroform	25	23.80±4.36	18.75±8.84
	50	36.83±5.90	39.21±14.80
	100	48.02±6.14	48.60± 0.00
	200	63.14±7.41	39.17±5.73
Acetone	25	24.33±8.47	25.53±16.06
	50	31.91±9.15	27.58±35.84
	100	42.18±9.33	57.14± 0.00
	200	90.65±12.94*	66.67± 0.00
Methanol	25	14.76±8.82	32.35±13.48
	50	26.08±8.62	25.00±17.68
	100	68.71±5.67	50.00±0.00
	200	72.22±4.81	69.56±7.53

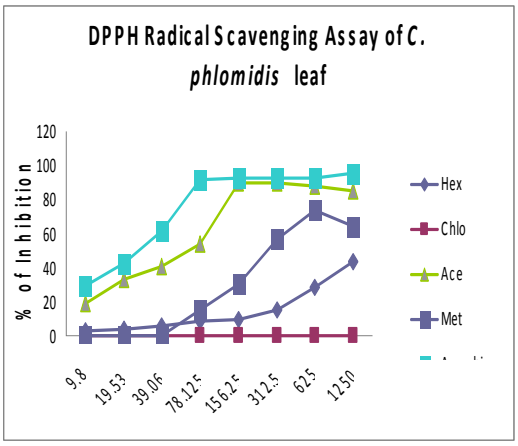
Table 2. Comparative analysis of cytotoxic activity of bark extracts of *C. viscosum* and *C. phlomidis*

Extract	Doses(µl)	*C. viscosum*	*C.phlomidis*
Hexane	25	2.17±0.00	10.00±14.14
	50	8.51±7.38	39.98±24.01
	100	18.18±0.00	78.54±21.46
	200	86.84±12.06	91.29±15.07
Chloroform	25	25.56±8.06	10.00±14.14
	50	21.04±22.38	39.98±12.00
	100	31.39±0.00	78.54±0.00
	200	42.86±20.20	**95.65±7.54****
Acetone	25	12.77±4.93	23.53±10.19
	50	23.66±16.46	41.67±26.02
	100	42.82±9.90	62.50±17.68
	200	61.90±16.49	47.82±18.45
Methanol	25	38.23±8.82	30.43±15.06
	50	44.11±18.37	44.44±9.62
	100	25.00±0.00	64.28±6.19
	200	75.00±35.35	75.00±2.50

Table 3: TLC based antioxidant activity of medicinal plants *Clerodendrum viscosum* and *Clereodendrum phlomidis*

Solvent	Solvent Extract	*C. viscosum*		*C. phlomidis*	
		Leaf	Bark	Leaf	Bark
Benzene:ethanol: ammonium hydroxide 90:10:1 (BEA)	Hexane	2	9	3	6
	Chloroform	5	6	9	4
	Acetone	6	3	4	6
	Methanol	2	Infinite	2	Infinite
Chloroform: ethyl acetate: formic acid 5:4:1 (CEF)	Hexane	1	2	2	3
	Chloroform	4	5	6	8
	Acetone	4	3	1	7
	Methanol	3	4	3	2
Ethyl acetate: methanol: water 40.5:4:4(EMW)	Hexane	1	1	2	1
	Chloroform	1	6	5	3
	Acetone	2	5	1	3
	Methanol	3	1	2	0

Figure 1: Comparative analysis of antioxidant activity of *C. phlomidis* and *C. viscosum* leaf extracts(DPPH radical scavenging assay)



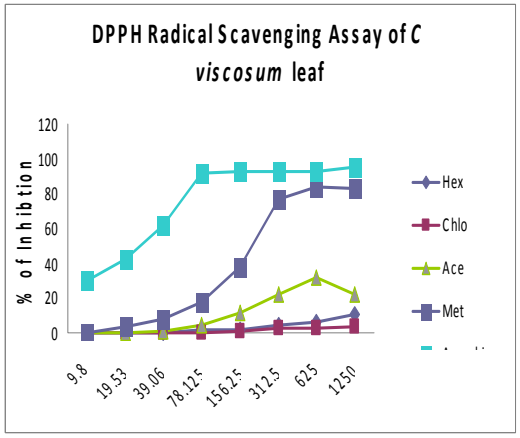
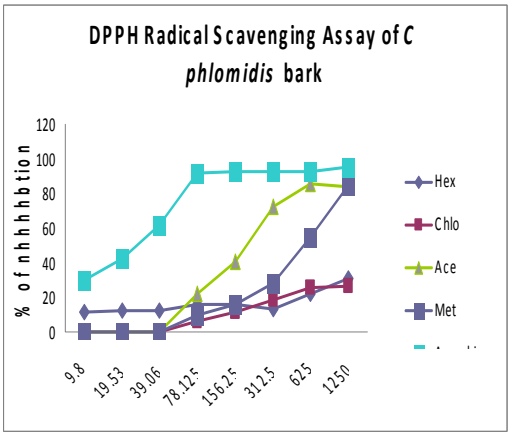


Figure 2: Comparative analysis of antioxidant activity of *C.phlomidis* and *C. viscosum* bark extracts (DPPH radical scavenging assay)



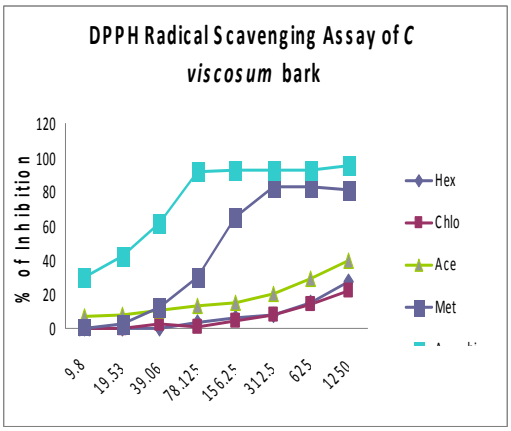
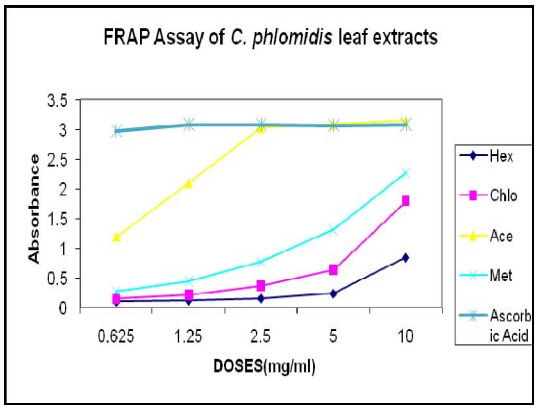


Figure 3. Comparative analysis of antioxidant activity of *C. phlomidis* and *C. viscosum* leaf extracts (FRAP assay)



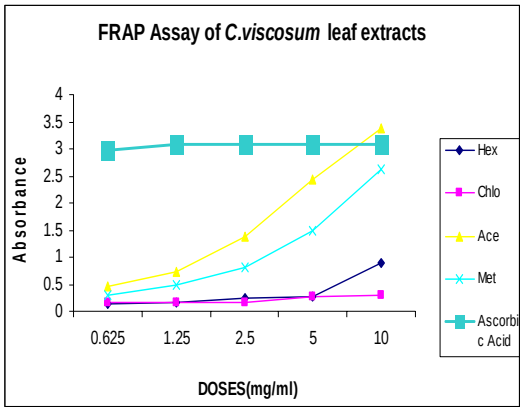


Figure 4: Comparative analysis of antioxidant activity of *C.phlomidis* and *C. viscosum* bark extracts (FRAP assay)