AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Anti-oxidant and Cytotoxic Activities of Leaf and Bark Extracts of *Clerodendrum Indicum*.(L.)Kuntze

Rashmi Ranjan Dash[1], Soumya Ranjan Pattanaik[1], Shakti Ketan Prusty[1], Sunita Bhatnagar[1]*

1. Medicinal and Aromatic plants, Regional Plant Resource Centre, Nayapalli, Bhubaneswar-751015. Orissa, India.

ABSTRACT

Clerodendrum indicum.(L.)Kuntze is commonly known as bharangi, roots and leaves are used in skin and digestive disorders by folk people. In Ayurvedic medicine same is used as cough and pitta suppressant. However, very less work is reported from this plant. The aim of the study in this paper was to evaluate its cytotoxic and antioxidant activity of leaf and bark extracts. Cytotoxic potential was explored using brine shrimp mortality assay and antioxidant potential of the extracts was explored using quantitative TLC based DPPH assay and Qualitative Radical scavenging DPPH assay. An attempt was made to isolate the active fraction of the plant from leaf extract. Out of four extracts Methanol extracts of leaf (*C.indicum*) showed significant cytotoxic activity of 90% at the dose of 200µg/ml. Maximum number of antioxidant bands were obtained in chloroform extract of the leaf sample. Methanol extract of leaf gave a flavonoids fraction which showed promising cytotoxic activity. Thus, study has provided lead and needs elaborate experimentation.

Key Words: Brine shrimp, DPPH, Cytotoxic, Antioxidant, *Clerodendrum indicum*, TLC.

*Corresponding Author Email: bhasunita@gmail.com
Received 31 March 2014, Accepted 07 April 2014

Please cite this article in press as: Bhatnagar S. *et al*., Anti-oxidant and Cytotoxic Activities of Leaf and Bark Extracts of *Clerodendrum Indicum*.(L.)Kuntze. American Journal of PharmTech Research 2014.

INTRODUCTION

Medicinal plants are important sources for new chemical compounds with pharmacological effect[1]. The plants are mainly used in medicinal practice for the treatment of various diseases[2]. Clerodendrum genus is rich in medicinal properties; species like C. viscosum, C. phlomidis, C. inerme, C. colebrookianum and C. trichotomum possess significant biological activity such as antioxidant, anthelmintic, cytotoxic and antitumor activity[3, 4]. Keeping in view of medicinal properties of the genus it was considered worthwhile to explore the medicinal potential of *Clerodendrum indicum* which finds a mention in Ayurvedic system of medicine[5] but scientific reports on the species are scanty.

MATERIALS AND METHOD

Collection and Processing of Plant material

***Clerodendrum indicum*:**

Clerodendrum indicum leaves and bark were collected from Medicinal garden of Regional Plant Resource Centre, Bhubaneswar, Odisha (India). Samples were authenticated with the herbarium samples of the Institute. Samples were washed thoroughly under running tap water to remove dust. Further, they were dried in normal environmental condition under shade. The dried leaves were powdered by the help of a grinder and were stored in air tight container.

Extraction of *Clerodendrum indicum*

Successive extraction procedure was carried out by soxhlet apparatus with different solvents of increasing polarity (Hexane, Chloroform, Acetone and Methanol). The extracts were concentrated under vacuum using Rota evaporator and were stored in airtight screw cap vials at 14degree Celsius till further use.

Biological Evaluation:

Antioxidant and cytotoxic activity was conducted using standard protocols[6]

Antioxidant activity

a) TLC based DPPH assay
b) DPPH radical scavenging assay

1. Cytotoxic activity using brine shrimp lethality assay

TLC based DPPH assay:

All the extracts were run in three solvents as per the protocols of Eloff [7]. Solvents used were as follows:

1. Benzene: Ethanol: Ammonium hydroxide(90:10:1) (BEA)

2. Ethyl acetate: methanol: water (40:5.4:4) EMW
3. Chloroform: Ethyl acetate: Formic acid(5:4:1) (CEF)

To detect antioxidant activity, qualitative 2, 2- Diphenyl1-picrylhydrazyl (DPPH) assay was carried out. The TLC sheet was prepared then it was sprayed with 0.2% DPPH in methanol as an indicator. Deby and Margotteaux, [8] .The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol.

$$DPPH + AH \rightarrow DPPH - H + A^{-}$$

(Purple color) (Yellow color)

DPPH radical scavenging assay:

The free radical scavenging test is based [9]on the measurement of the scavenging ability of antioxidants towards the stable free radical 1, 1-diphenyl-2-picrylhydrazil (DPPH).DPPH is reduced to corresponding hydrazine, being inversely related to antioxidant concentration For DPPH free radical scavenging assay 1mM DPPH (2, 2- Diphenyl-1- picryl hydrazyl) solution was prepared. 4mg DPPH was weighed and dissolved in 10ml methanol. The concentration of stock solution should be 2.5mg/ml. DPPH assay was done by serial dilution method starting from concentration 1250µg, 625µg, 312.5µg, 156.25µg, 78.125µg, 39.625µg, 19.812µg, 9.9µg, 4.9 µg. There were duplicate test tube of each concentration was taken.1ml of each sample was taken in the test tube & volume was made up to 4ml by methanol. 500µl DPPH solution was added to each test-tube and stirred thoroughly before incubated for 30min. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration. Then optical density (OD) was measured at λ = 517nm in spectrophotometer. The percentage radical scavenging activity was calculated from the following formula: Percentage radicle scavenging [DPPH] = [(Ao - A1) ÷Ao] × 100

Where Ao was the absorbance of control and A1 was the absorbance of sample.

Cytotoxic activity (Brine shrimp lethality test):

Brine shrimp assay was conducted using the standard protocols[6].

Brine shrimp (*Artemia salina*) eggs were incubated for 48 hrs (3.6 gm of black salt in 200 ml distilled water) to get the desired growth of the larvae for biological evaluation. Stock solution of different extracts were prepared at a concentration of 10 microgm/ml, cytotoxic assay we carried out at 5 doses 25, 50,100, 200microgm/ml for each dose level 3 replicates were used. Motility, readings were taken every hour up to 4 hrs. Motility was graded below.

4+ = high motility

3+ = motile

2+ = sluggish

1+ = slow

Nil = no activity at all

After 24 hrs the final reading was taken & Percentage inhibition was calculated by comparing the treated samples with the controls. Standard deviation was also calculated.

RESULTS AND DISCUSSION

ANTIOXIDANT ACTIVITY

TLC based antioxidant assay

Results are shown in Table I, All the extracts showed good separation in all the solvents, However, leaf extracts showed better antioxidant bands as compared to bark extracts. Acetone extract of the bark of the plant showed a high number of antioxidant molecules which were closely situated hence a streak was obtained. A number of molecules like Combrestatin B5 from *Combretum woodi* [10], a number of sesquiterpenoids from curcuma sp[11] were also isolated by using this very model.

DPPH radical scavenging assay:

Leaf as well as bark extract showed only mild antioxidant activity using DPPH radical scavenging assay(Figure 1). Although prominent bands were obtained in TLC based assay but quantitatively results of this assay were not promising, it could be due to non-synergistic activity of molecules in the extract. Heterocycles in the presence of different molecules either enhance the effect of each other or reduce the effect owing to their structure[12,13]

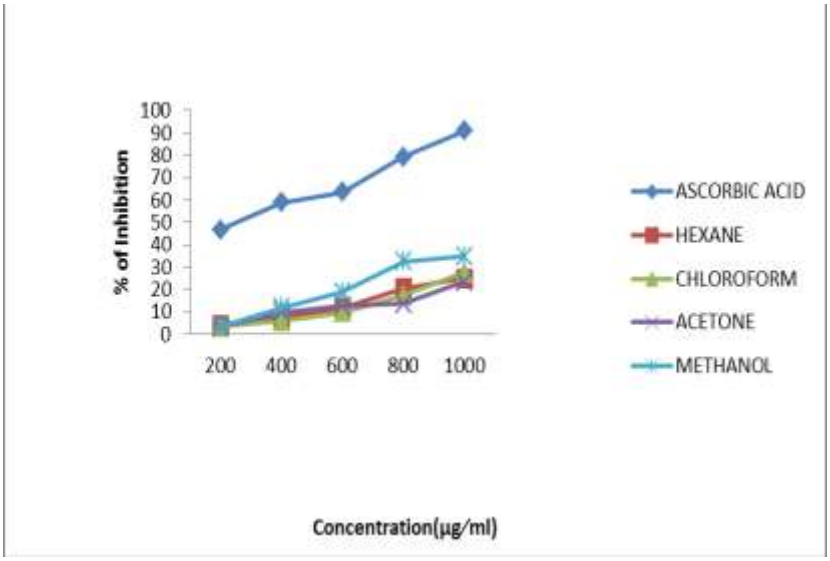

Figure 1: DPPH assay of Clerodendrum indicum leaf extract

Cytotoxic activity of leaf and bark extracts of *Clerodendrum indicum*

Leaf as well as bark extract showed dose dependent cytotoxic activity as can be seen from the Tables II and III. Highest cytotoxic activity was shown by the methanol extract of leaf of *Clerodendrum indicum.* An attempt was made to isolate the active fraction of methanol extract of leaf (Figure. 2). Preparative TLC of the extract was conducted to get 5 fractions namely A, B,C,D and E, All were tested to check for any class of compounds present, only A showed the presence of Tannin and Flavonoids, same was further fractionated and it showed two prominent antioxidant bands. This is in confirmation with earlier studies where tannins and flavonoids have proved to be potential antioxidants [14,15] . Although extract was picked up on the basis of cytotoxic activity but the fraction showed good antioxidant activity as well. This could be due to the fact that same extract had the better antioxidant activity as compared to other solvent extracts. Data is supported by a large number of work where a number of good cytotoxic agents also have antioxidant activities [16,17].

Table I: - *C. indicum* TLC based DPPH assay

Plant Name/ Part	Solvent	Hexane	Chloroform	Acetone	Methanol
C. indicum (leaf)	I	9	6	8	4
	II	7	11	6	6
	III	6	7	7	5
C. indicum (bark)	I	3	4	3	3
	II	0	0	streak	0
	III	0	0	streak	3

I – Benzene: Ethanol: Ammonium hydroxide (90:10:1) **BEA,**

II – Chloroform: Ethyl acetate: Formic acid (5:4:1) **CEF,**

III – Ethyl acetate: methanol: water (40:5.4:4) **EMW**

Table II: - *C. indicum* (leaf) cytotoxic activity using brine shrimp lethality assay

Doses(µg/ml)	Hexane	Chloroform	Acetone	Methanol
25	1.73 ± 3.00	12.08 ± 5.91	7.01 ± 3.03	10.52 ± 5.26
50	3.66 ± 3.17	24.07 ± 3.20	33.32 ± 5.55	38.88 ± 5.55
100	14.76 ± 8.49	35.15 ± 3.15	49.99 ± 5.55	56.50 ± 5.55
200	88.75 ± 3.14	78.33 ± 2.80	83.75 ± 1.08	90.00 ± 11.54

Table III: - *C. indicum* (bark) cytotoxic activity using brine shrimp lethality

Doses(µg/ml)	Hexane	Chloroform	Acetone	Methanol
25	22.22 ± 3.84	9.80 ± 3.39	29.22 ± 3.91	36.20 ± 4.81
50	28.08 ± 0.66	33.33 ± 3.60	43.33 ± 8.16	45.55 ± 5.01
100	35.89 ± 8.88	38.09 ± 4.12	65.37 ± 4.08	67.85 ± 4.04
200	48.48 ± 5.24	51.51 ± 5.24	71.20 ± 6.84	84.08 ± 4.54

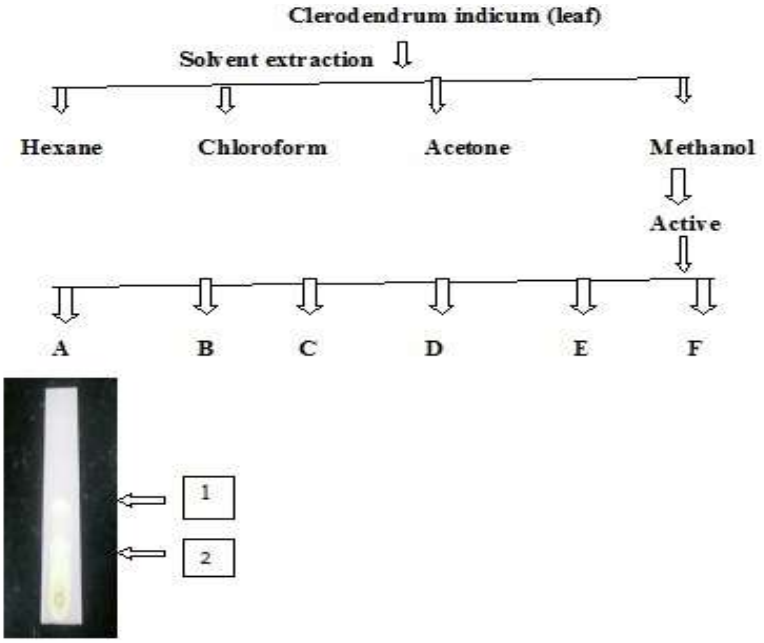

Figure 2: Scheme of isolation of active fraction from methanol extract of *Clerodendrum indicum (Leaf)*

CONCLUSION

Methanol extract of the leaf extract showed good cytotoxic potential and hence was subjected to column chromatographic separation which showed one fraction in which tannin and flavonoids was detected on phytochemical analysis. Thus it can be concluded that study has provided a lead for the isolation of antioxidant and cytotoxic principles from the medicinal plant *Clerodendrum indicum*.

ACKNOWLEDGEMENT

The authors wish to thank Ministry of Environment and Forests for providing laboratory to work and DST for providing fellowship to first three authors.

REFERENCES:

1. Farnsworth NR. Screening plants for new medicines. In Biodiversity part-II, Wilson Eo(Eds).1997;83-97
2. Rashid MA, Hasan CM, Choudhury SAR, Bengum B and Rahman S. Ethnopharmacological investigation of medical plants of Bangladesh. Bangladesh J.physol, Pharmacol. 1997; 12:25-9
3. Sannigrahi S, Mazumder UK, Pal D and Mishra SL. Terpenoids of methanol extract of *Clerodendrum infortunatum* exhibit anticancer activity against Ehrlichi ascites carcinoma (EAC) in mice. Pharm Biol. 2012; 50: 304- 9.

4. Joseph J, Bindhu, AR and Aleykutty NA. *In vitro* and *in vivo* anti-inflammatory activity of *Clerodendrum paniculatum. Linn.* Leaves. Ind. J. Pharm. Sci. 2013:75:376-9.
5. Praveen KA and Nishteswar K. Phytochemical and pharmacological profiles of *Clerodendrum serratum.Linn.* (Bharangi): A review. Int J. Res. Ayur.Pharma.2013: 4:276-8.
6. Bhatnagar S and Pattnaik SR. Comparative analysis of Cytotoxic and antioxidant activities of leaf and bark extracts of Clerodendrum viscosum and Clerodendrum phlomidis. Int. J Biomed Adv. Res 2012; 3(5): 385-390.
7. Eloff JN and Masoko P. Screening of 24 South African combretum and six terminalia Species (Combretaceae). Afr. J. trad. Com. and Alt Med. 2007; 4: 231- 9.
8. 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- 81.
9. Ahmad RH, Khaled A, Shafiq A, Nahla S, Azim A, Ismail SI and Hammouda FM. Chemical investigation of some Capparis sp. Growing in Egypt and their antioxidant activity. Evid based Complement Alt Med.2007:4:25-8.
10. Eloff JN, Famakin JO and Katerere DRP, Isolation of an antibacterial stilbene from Combretum woodii (Combretaceae) leaves. Afr. Jou. Biotech.2005: 4: 1167-71.
11. Zhao, J. Zhang, JS, Yang B. Lv, GP. Li, SP. Free radical scavenging activity and characterization of sesquiterpenoids in four species of curcuma using a TLC bioautography assay and GC-MS analysis. Molecules 2010; 11: 7547–57.
12. Evstigneev MP. Physicochemical Mechanisms of Synergistic Biological Action of Combinations of Aromatic Heterocyclic compounds. Hindawi Publishing Corporation. Organic chemistry International. 2013: 10. ID278143.
13. Sorensen BS, Jensen PB, Sehested M, *et al.* Antagonistic effect of aclarubicin on camptothecin induced cytotoxicity: role of topoisomerase I. Biochem. Pharmacol, 1994: 47: 203-12.
14. Wang J. Yue, YD, Tang F. Sun J. TLC Screening for Antioxidant Activity of Extracts from Fifteen Bamboo Species and Identification of Antioxidant Flavone Glycosides from Leaves of *Bambusa textilis* McClure. *Molecules* 2012; 17: 12297- 311.
15. Zhang SJ, Lin MY, Zhou CH, Wei SD, Lin GH and Ye GF. Antioxidant tannins from stem bark and fine root of Casuarina equisetifolia. Molecules. 2010:15:5658-70.
16. Rumjhum NN, Rahman MM, Kazal MK. Antioxidant and cytotoxic potential of methanol extract of Tabernae montana divaricata leaves. Int Current Pharma

J .2012: 1: 27-31.

17. Firdaus M, Prihanto AA and Nurdiani R. Antioxidant and cytotoxic activity of *Acanthus ilicifolius* Asian Pacific J Trop.Biomed. 2013: 3:17-21.