ejpmr, 2015,2(4), 856-863

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

IN VITRO RADICAL SCAVENGING ACTIVITY OF *CEPHALANDRA INDICA*

M. Duraisankar* and V. Ravichandiran

School of Pharmaceutical sciences, Vels University (VISTAS), Pallavaram, Chennai.

Article Received on 08/06/2015 **Article Revised on 29/06/2015** **Article Accepted on 20/07/2015**

***Correspondence for Author**

M. Duraisankar

School of Pharmaceuticals Sciences, Vels University (VISTAS), Pallavaram, Chennai.

ABSTRACT

Cephalandra indica (Bimba, kanduri, Cucurbitaceae) is famous for its hypoglycemic and anti-diabetic Properties in Ayurvedic system of medicine. Other applications include the therapy of various conditions such as skin diseases and gonorrhoea. There are many patented formulations derived from *Cephalandra indica.* Which are now distributed increasingly all over the world this has given rise to a concomitant increase in research on the phytochemical constituents and biological activity of *C. indica*. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. In the present study, we examined the anti-oxidant effects of root of *Cephalandra indica*. Dried and powdered root of C.indica were extracted with Ethyl acetate, n-butanol and methanol. Antioxidant assays were carried out by using different *in vitro* models such as, DPPH radical scavenging activity. The EC_{50} values of different extract for DPPH radical scavenging activity was found. In DPPH radical scavenging assay the IC_{50} value of the ethyl acetate extract was found to be 330µg/mL while β - carotene had the IC_{50} value 220 µg/ml. Moreover *C.indica* Extract showed strong reducing power and total antioxidant capacity. The anti-oxidant activities of other solvent extracts were poor when compared to the Ethyl acetate extract. These results suggest that, the active antioxidant compounds are better extracted in Ethyl acetate and there is a direct correlation between the flavonoid glycoside and its anti-oxidant activity.

KEYWORDS: Medicinal plant; *Cephalandra indica*, solvent extracts; anti-oxidant activity.

INTRODUCTION

Medicinal plants have occupied a vital place in the socio-cultural, development of rural people of India. *Cephalandra indica* (family- Cucurbitaceae) is perennial; scandent or

prostrate plant which is commonly known as bimbu in Bengali, kova in Malayalam, kundru in Punjabi, kundaru in Urdu and bhimb in Hindi.[24] Leaves are 5- 10 cm, long and broad, bright green above, paler beneath, studded and sometimes rough with papillae. Flowers are peduncles and sub-fusiform. The fresh tab root is thick, tuberous, long tapering, more or less tortuous with a few fibrous rootlets attached to it.[6] Roots are flexible, soft and break with a fibrous fracture.[4,5] The cork is composed of rows of cells. Fruits are fusiform-ellipsoid, slightly beaked, 2.5-5 by 1.3-2.5 cm marked when immature with white streaks, bright scarlet when fully ripped.[15,18] Seeds are obovoid, rounded at the apex, slightly papillose, much compressed and yellowish grey. This plant is traditionally used in various diseases like psoriasis, ringworm, itching, small pox, skin diseases, ulcer, scabies, diabetes, asthma, bronchitis, dysentery, vomiting, cough and cold. Pharmacological studies on *C.indica* demonstrated antimicrobial, antilitihic and antioxidant activities.[1,2] The purpose of the present study is to in-vitro methods **DPPH radical scavenging activity** of different solvent extract of *Cephalandra indica.*[4]

MATERIALS AND METHODS

Chemicals: 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma chemicals, Co., Chennai. All other chemicals used were of analytical grade.[16]

Phytochemical screening: The freshly prepared extract of *C.indica* was qualitatively tested for the presence of chemical constituents. Phyto-chemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorff reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and Saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulfuric acid. These were identified by characteristic color changes using standard procedures.[22, 23]

Preparation of crude plant extracts: Test plants were collected locally or obtained from the local market. Plant material consisting of mature plant ***Cephalandra indica*** root were collected and dried. The dried root materials were powdered using a grinder. The extraction was done at room temperature.[7, 8] About 100 g of dried, Ground plant materials were soaked in methanol (1 L of 98%) for 5-7 days separately.[9] The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40^{o}C and stored at 4^{0} C for further use. The stock solution takes different fraction n- butanol, Ethyl

acetate and methanol. The crude extracts (5 mg/ml) were prepared by dissolving a known amount of dry extract in different fraction.[19, 20] The Working solutions (1, 2, 4, 6, 8, 10, 15, 25, 50, 75, 100, 250, 500 and 750 µg /ml) of the extracts was Prepared from the stock solution using suitable dilution.

Assay of DPPH test in-vitro radicals scavenging and antioxidant

Sample preparation

1) Ethyl acetate fraction

2) Butanol fraction

3) Methanol fraction

The effects of extract on DPPH radical were estimated by with miner modification. In brief, 2Ml of DPPH in methanol ($3.6x10^{-5}$) is added to 50µl of various concentrations of extract / fraction. The mixture is vortexes for 15sec and left to stand at 37^0c for 30 min[10]. The decrease in the absorbance at 515nm was continuously recorded in a spectrophotometer for 15 min at room temp. All determination was performed in triplicate. The DPPH scavenging activity (decrease of absorbance at 515nm) of Extracts and compound were plotted against time and the (%) percentage of DPPH radicals scavenging ability of the sample were calculated from the absorbance value and 15min duration as follows (Table-2).

$$\% \text{ Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

Reducing power: The reducing power of *Cephalandra indica was* determined according to the method previously described.[11,12] Different concentrations of *Cephalandra indica* extract (100 µg – 1,000 µg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. β - Carotene was used as a reference standard. Phosphate buffer (pH 6.6) was used as blank solution.[13] The absorbance of the final reaction mixture of two parallel experiments were taken was expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extract of *C. indica* revealed the presence of various bioactive components of which flavonoids and tannins were the most prominent and the result of phyto-chemical test has been summarized in the Table 1. Phenolic compounds and flavonoids have been reported to be associated with anti-oxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. Literature reviews about the plant also confirm the presence of terpinoids group. Many naturally occurring triterpinoids exhibited a good anti-inflammatory activity have been isolated from various plants. Triterpinoids have a wide spectrum of biological activities and some of them may be useful in medicine.[14,21] There is growing interest in natural triterpinoids caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of biological activities, they are bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and anti-allergic.[24] In this present study the antioxidant activity of the methanol extracts of the root of *C. indica* were investigated by using DPPH scavenging assay, reducing power of the extract and by determining total antioxidant capacity of the extract. The two methods have proven the effectiveness of the Ethyl acetate extract compared to the reference standard antioxidant β-Carotene.[25] The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.[30] Comparison of the antioxidant activity of the extract and β – carotene, the Ethyl acetate extract of *C. indica* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 330 µg/ml(Table-3). The IC_{50} value of the extract was found to be comparable to reference standard β- carotene (IC_{50} 220 µg/ ml).The reducing ability of a compound generally depends on the presence of reductants, which have been exhibited anti-oxidative potential by breaking the free radical chain, donating a hydrogen atom.[26,27] The presence of reduction (i.e. antioxidants) in *C. indica* extract causes the reduction of the Fe3+/ ferric-cyanide complex to the ferrous form.[3] Therefore, the Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.[28] The reductive capabilities of the plant extract compared to β- carotene.[4] The reducing power of extract of *Cephalantra indica* was very potent and the power of the extract was increased with quantity of sample.

PHYTOCHEMICAL STUDIES OF ROOT

Table - 1

Chemical constituents *Cephalandra indica*					
Alkaloids	**Flavonoids**	**Glycosides**	**Tannin**	**Carbohydrates**	**triterpinoids**
+	+ + +	+ + +	+ + +	+ + +	+ +

Table: 2: DPPH radical scavenging activities of *Cephalandra indica*

Concentration	Optical density			
	β-Carotene	**Ethyl acetate fraction**	**Butanol fraction**	**methanolic fraction**
50	0.751±0.022	0.844±0.011	0.875±0.022	0.890±0.010
100	0.591±0.004	0.715±0.045	0.785±0.010	0.835±0.007
250	0.363±0.011	0.606±0.048	0.630±0.021	0.687±0.009
500	0.291±0.005	0.420±0.038	0.462±0.034	0.490±0.005
1000	0.202±0.007	0.341±0.003	0.349±0.007	0.365±0.007

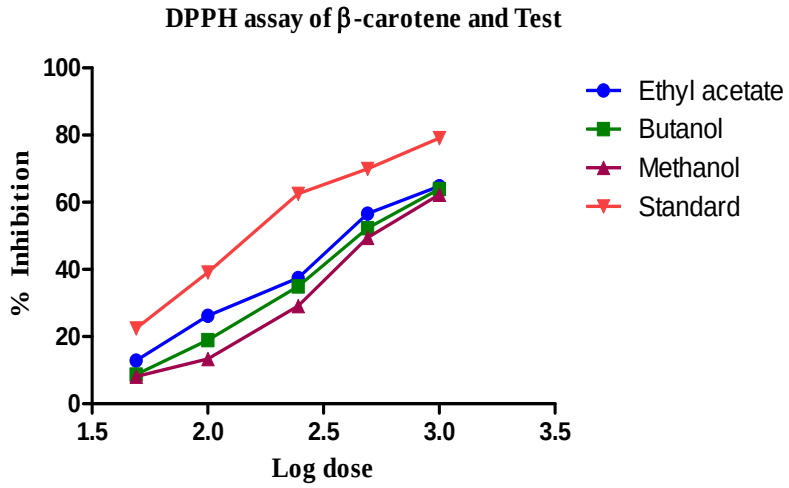

Figure: 1: DPPH free radical scavenging activity of standard β – Carotene

Table: 3: DPPH radical scavenging activities of *Cephalandra indica in* IC_{50} value

Concentration	% of Inhibition			
	β- Carotene	**Ethyl acetate fraction**	**Butanol fraction**	**50% methanol fraction**
50	22.497 ± 0.2782	12.899 ± 0.3472	9.700 ± 0.6438	8.152 ± 0.3592
100	39.112 ± 0.3852	26.212 ± 0.3485	18.988 ± 0.5644	13.400 ± 0.9266
250	62.583 ± 0.1877	37.461 ± 0.3078	34.984 ± 0.5977	29.102 ± 0.5258
500	69.969 ± 0.4381	56.656 ± 0.5891	52.321 ± 0.4936	49.432 ± 0.3874
1000	79.153 ± 0.4223	64.809 ± 0.3241	63.983 ± 0.3464	62.332 ± 0.5858
IC50 Value	220µg/ml	330µg/ml	510µm/ml	530µg/ml

CONCLUSION

C. indica is a famous plant for its safe anti-diabetic property. It proved the insulin stimulatory effect of *C indica* leaves from existing b-cells in diabetic rats. It possesses hypoglycemic, anti-diabetic, hypolipidemic, hepatoprotective, larvvicidal, anti-inflammatory, analgesic and

antipyretic activities (Figure-1). It is found to be devoid of anti-tuberculosis properties. Various phytoconstituents reported in *C. indica* are cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3- one, taraxerone and taraxerol. Terpenoids are found to be responsible for anti-diabetic activity (Table-5). Despite the broad use of *C. indica* in traditional medicine, very few systematic pharmacological and phytochemical studies are reported till date assessing its therapeutic properties.

REFERENCES

1. Akter R, Hasan SMR, Siddiqua SA, majumder MM, Hossain MM, AlamMA, Haque S, Ghani A. Evaluation of analgesic and antioxidant potential of the leaves of Curcuma S. J.Pharm. Sci. 2008; 1(1&2): 3-9.
2. Atawodi SE. Antioxidant potential of African medicinal plants. Af.J. Biotech. 2005; 4(2): 128-133.
3. Azad KAK, Akhtar S, Mahtab H. Cephalandra indica fruits in the treatment of patients with diabetes mellitus. Bangladesh Med. Res. Council Bull., 1979; 5(2): 60-66.
4. Ajay. SS, Bhaskar VH, Teotia D.Quality standardization of Cephalantra indica. Asian J. Chem., 2009; 21(7): 5351-5354.
5. Aruoma OI. Methodological considerations for characterizing potential antioxidants action of bio-active components in food plants. Mutat. Res. 2003; 523 – 524:9-20.
6. Basu K, Ghosh BK. Chemical investigation of Cephalandra indica. Transactions of the Bose Res. Inst. (Calcutta). 1972; 35(2): 43-44.
7. Bhakuni DS, Srivastava SN, Sharma VN, Kaul KN.Chemical examination of the fruits of Coccinia indica. J. Sci. Ind. Res., Section B: Phys. Sci., 1962; 21B: 237-238.
8. Brahmachari HD, Augusti KT. Orally effective hypoglycemic principles from Coccinia indica. J. Pharm. Pharmacol., 1963; 15(6): 411- 412.
9. Chandrasekar B, Mukherjee B, Mukherjee SK. Blood sugar lowering potentiality of selected Cucurbitaceae plants of Indian origin. Indian J. Med. Res., 1989; 90: 300-305.
10. Dhargalkar IM, Guha SK. Nutritional values of Indian vegetables. J. Proc. Inst. Chemists, 1959; (31): 109-112.
11. Gupta SS, Variyar MC. Experimental studies on pituitary diabetes. Iv. Effect of Gymnemasylvestre and Coccinia indica against the hyperglycaemic response of somatotropin and corticotropin hormones. Indian J. Med. Res., 1964; 52: 200-207.

12. Hossain MZ, Shibib BA, Rahman R. Hypoglycemic effects ofCoccinia indica: Inhibition of key gluconeogenic enzyme, glucose-6-phosphatase. Indian J. Exp. Biol., 1992; 30(5): 418-420.
13. Kumar GP, Sudheesh S, Vijayalakshmi NR. Hypoglycemic effect of Coccinia indica: Mechanism of action. Planta Med., 1993; 59(4): 330-332.
14. Kamble SM, Kamlakar PL, Vaidya S, Bambole VD. Influence ofCoccinia indica on certain enzymes in glycolytic and lipolytic pathway in human diabetes. Indian J. Med. Sci., 1998; 52(4): 143-146.
15. Khan AK, AKhtar S, Mahtab H. Treatment of diabetes mellitus with Coccinia indica. Brit. Med. J., 1980; 280(6220): 1044.
16. Khastgir HN, Choudhuri SN, Gupta PS. Roots of Coccinia indica.J. Indian Chem. Soc., 1958; 35: 905-906.
17. Kundu S, Ray AB. Chemical examination of Coccinia indica fruits. J. Indian Chem. Soc., 1987; 64(12): 776-777.
18. Kuriyan R, Rajendran R, Bantwal G, Kurpad AV. Effect of supplementation of Coccinia cordifolia extract on newly detected diabetic patients. Diabetes Care, 2008; 31(2): 216-220.
19. Mallick C, De Debasis, Ghosh D. Correction of protein metabolic disorders by composite extract of Musa paradisiacal and Coccinia indica in streptozotocin-induced diabetic albino rat: An approachthrough the pancreas. Pancreas, 2009; 38(3): 322-329.
20. Mukerji B. Effect of Coccinia indica on alloxan diabetes inrabbits. Indian J. Med. Sci., 1953; 7: 665-672.
21. Mukherjee K, Ghosh NC, Datta T.Coccinia indica as a potential hypoglycemic agent. Ind. J. Exp. Biol., 1972; 10(5): 347-349.
22. Nahar N, Mosihuzzaman M, Khan MD Shahinul H.Determination of free sugars in plant materials having antidiabeticactivity. Dhaka Univ. J. Sci., 1998; 46(1): 167-170.
23. Niazi J, Singh P, Bansal Y, Goel RK. Anti-inflammatory,analgesic and antipyretic activity of aqueous extract of fresh leaves ofDeokate and Khadabadi 159Cocciniaindica.Inflammopharmacol., 2009; 17(4): 239-244.
24. Pari L, Venkateswaran S. Protective effect of Coccinia indica onchanges in the fatty acid composition in streptozotocin induced diabetic rats. Pharmazie, 2003; 58(6): 409-412.
25. Platel K, Srinivasan K. Nahrung Plant foods in the management of diabetes mellitus. Vegetables as potential hypoglycemic agents, 1997; 41(2): 68-74.

26. Qudrat-i-Khuda M, Khaleque KA, Miah MAW. Chemical investigations on Cephalandraindica. I. Constituents of dry aerialparts. Dacca Sci. Res., (Dacca, Pakistan), 1965; 2(1/2): 27-231.
27. Rahman M, Mahbubur C, Tofail A, Mosihuzzaman M. Analysis ofwater- and alkali-soluble polysaccharides of Cocciniaindica(Telakucha) plant. J. Bangladesh Chem. Soc., 1990; 3(2): 199-204.
28. Rao GM, Rao V, Sudhakara M, Pandey MM, Rawat AKS, Sirwaikar A,Joshi AB. Anti-inflammatory and antinociceptive activities of"CocciniaindicaWA." fruit juice powder in animals. Nat. Prod. Sci., 2004; 10(1): 20-23.
29. Rao GM, Mohana VM, Rao CV, Rawat AKS, Mehrotra S. Hepatoprotective effect of Coccinia indica against CCl4 inducedhepatotoxicity. Nat. Prod. Sci., 2003; 9(1): 13-17.
30. Shakya VK. Antidiabetic activity of Coccinia in dicain streptozotocin induced diabetic rats. Asian J. Chem., 2008; 20(8): 6479- 6482.