IJPSR (2010), Vol. 1, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 May, 2010; received in revised form 21 September, 2010; accepted 03 October, 2010

COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY BETWEEN ETHANOLIC AND AQUEOUS EXTRACT OF *CLEOME RUTIDOSPERMA*

Anup K. Chakraborty*[1], Manoj S. Charde [2], Harekrishna Roy [1], Satyabrata Bhanja [1] and M. Behera [1]

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jeypore College of Pharmacy [1], Rondapalli, Jeypore, Koraput, Odisha, India

Dean Academics and Head of PG Departments, NRI group of Institutions [2], Bhopal, MP, India

Keywords:

Cleome rutidosperma,

Extraction,

Antioxidant

Correspondence to Author:

Anup Kumar Chakraborty

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jeypore College of Pharmacy, Rondapalli, Jeypore, Koraput, Odisha, India

ABSTRACT

Cleome rutidosperma is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, pain and skin disease. Extraction of the aerial parts of the plant *Cleome rutidosperma* using water and ethanol and evaluation of their antioxidant activity has been envisaged in this present research work. Both the extracts exhibits significant antioxidant activity in DPPH, Nitric oxide and hydroxyl radical induced *In-vitro* assay methods. The DPPH radical inhibition (%) was 53.13, 57.13 and 79.12 for CRA, CRE and ascorbic acid respectively. The *Cleome rutidosperma* extracts (CRA and CRE) showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentrations 250µg/ml, showing 29.22% and 63.32% of NO inhibition, respectively. The CRA and CRE extracts (25-400µg/ml) significantly scavenged the hydroxyl radical generated by the EDTA/H_2O_2 system, when compared to that of ascorbic acid.

INTRODUCTION: *Cleome rutidosperma* (Capparidaceae) is a low-growing herb, up to 70 cm tall, found in waste herb, grounds and grassy places with trifoliate leaves and small, violet-blue flowers, which turn pink as to West Africa, although it has become naturalized in various parts of tropical America as well as Southeast Asia [1, 2]. According to traditional use, the different parts of the plants of Cleome genus are used as stimulant, antiscorbutic, antihelmintic, vesicant, rubefacient and carminative [3]. The anti-plasmodial, analgesic, locomotor, antimicrobial, diuretic, laxative [4, 5] activities of *Cleome rutidosperma* were reported earlier.

Cleome rutidosperma is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, pain and skin disease. The popular use of the roots, however, refers mainly to its analgesic, anti-inflammatory and antihelmintic activity [6]. However, there are no scientific reports on the antioxidant activity of this plant. Therefore, in the light its use in traditional medicine, the present study was undertaken to investigate free radical scavenging activity of the ethanolic and aqueous extract of *Cleome rutidosperma* using *In-vitro* assay methods.

MATERIALS AND METHODS:

Plant materials: The Plant material (whole plant) was collected from the forests of Ganjam district of Odisha, India during September 2008 and was authenticated at Botanical Survey of India, Shibpur, Howrah and West Bengal, India. The fresh aerial parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse-powder.

Preparation of extracts: The aerial parts of the plant were powdered. 150g of powder was subjected to extraction using Soxhlet apparatus with various solvents like water and ethanol. The solvent was then removed under reduced pressure which will give a greenish-black colored sticky residue. The prepared extracts were then subjected to antioxidant activity studies.

Evaluation of Antioxidant activity of the *Cleome rutidosperma*:

Scavenging of DPPH radical [7, 8]: This assay is based on the measurement of the scavenging ability of the antioxidant test substances towards the stable radical. The free radical scavenging activity (Yokazawa *et al*., 1998) of the extracts (CRA and CRE) was examined in vitro using DPPH radical. The test extracts were treated with different concentrations from a maximum of 250µg/ml to minimum of 4µg/ml. The reaction mixture consisted of 1 ml of 0.1mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl buffer (pH 7.4), 1 ml of ethanol and 0.05 ml of the extract. The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding the extract. The experiment was performed in triplicate and the % of scavenging activity was calculated using the formula;

100 - [100/ blank absorbance × sample absorbance]

Scavenging of nitric oxide [9, 10]: Sodium nitroprusside (Sreejavan Rao, 1997) (5M) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25^0 C for 5 hrs. After 5 h, 0.5 ml of incubated solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of the chromophore formed was measured at 546 nm. The control was also carried out in similar manner using

distilled water instead of extracts. The experiment was performed in triplicate and % scavenging activity was calculated using the formula;

100 - [100/ blank absorbance × sample absorbance]

The activity was compared with ascorbic acid, which was considered as standard antioxidant.

Hydroxyl Radical Scavenging activity [11]: The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The reaction mixture contained deoxyribose (2-8mM), $FeCl_3$ (0.1mM), EDTA (0.1 mM), ascorbate (0.1 mM), H_2O_2 (1mM), KH_2PO_4-KOH buffer(20mM, pH 7.4) and various concentrations (25-400µg/ml of extracts and standard 10-80µg/ml) of standard drug in a final volume of 1 ml. The reaction mixture was incubated for 1 hr at 37°C, deoxyribose degradation was measured at 532 nm (Mary *et al.,* 2002).

Statistical Analysis [12]: The data on *in-vitro* studies were reported as mean ± Standard deviation (n=5). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5% level significance was employed. The P values<0.05 were considered as significant [14].

RESULTS:

DPPH Scavenging: The aqueous (CRA) and ethanolic extracts (CRE) of the *Cleome rutidosperma* showed promising free radical scavenging effect of DPPH in a concentration dependent manner upto a concentration of 250µg/ml. The CRE showed more scavenging activity than CRA. The reference standard ascorbic acid also shows a significant radical scavenging potential in the concentration of 1 µg/ml. The DPPH radical inhibition (%) was 53.13, 57.13 and 79.12 for CRA, CRE and ascorbic acid respectively in **table 1**.

Nitric oxide Scavenging: The *Cleome rutidosperma* extracts (CRA and CRE) showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentrations 250 µg/ml, showing 29.22% and 63.32% of NO inhibition, respectively. Ascorbic acid was used as reference standard. The % inhibition is shown in **Table 2**.

OH Radical Scavenging: The CRA and CRE extracts (25-400 µg/ml) significantly scavenged the hydroxyl radical generated by the EDTA/H_2O_2 system, when compared to that of ascorbic acid. The percentage scavenging of OH radicals by CRA and CRE was increased in a dose dependent manner. The standard ascorbic acid (10-80 µg/ml), also showed scavenging effect (**Table 3**).

TABLE 1: *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CLEOME RUTIDOSPERMA* BY DPPH METHOD

Drug	% Scavenging (Mean ± SEM) of triplicates						
	4 µg/ml	8µg/ml	15 µg/ml	30 µg/ml	60µg/ml	150µg/ml	250µg/ml
CRA	21.46* ±0.002	25.64*± 0.001	27.44*±0.001	33.87*± 0.001	39.4*± 0.001	48.22*± 0.002	51.13* ±0.002
CRE	29.02*±0.002	31.86*±0.002	35.85* ± 0.001	39.3* ±0.001	46.44*±0.002	52.03* ±0.002	57.13*±0.002
	0.1 µg/ml	0.2 µg/ml	0.4 µg/ml	0.6 µg/ml	0.8 µg/ml	1 µg/ml	---
Vit-C	6.2 ±0.002	15.54*±0.001	31.51* ±0.001	48.18*± 0.003	64.15*±0.001	79.1*2±0.001	---

CRA= Aqueous extract, CRE= Ethanolic extract, *P<0.05

TABLE 2: *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CLEOME RUTIDOSPERMA* BY NITRIC OXIDE SCAVENGING METHOD

Drug	% Scavenging (Mean ± SEM) of triplicates						
	4 µg/ml	8µg/ml	15 µg/ml	30 µg/ml	60µg/ml	150µg/ml	250µg/ml
CRA	6.24 ±0.002	10.02± 0.001	12.29±0.001	15.53± 0.001	19.33*± 0.001	24.46*± 0.002	29.22* ±0.002
CRE	49.41±0.002	55.71*.±0.002	55.9*± 0.001	56.19 *±0.001		58.32*±0.002	
					56.34*±0.002		61.32 *±0.002
	0.1 µg/ml	0.2 µg/ml	0.4 µg/ml	0.6 µg/ml	0.8 µg/ml	1 µg/ml	---
Vit-C	6.2 ±0.002	15.54*±0.001	31.51* ±0.001	48.18*± 0.003	64.15*±0.001	79.12*±0.001	---

CRA= Aqueous extract, CRE= Ethanolic extract, *P<0.05

TABLE 2: *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CLEOME RUTIDOSPERMA* BY HYDROXYL RADICAL SCAVENGING METHOD

Drug	% Scavenging (Mean ± SEM) of triplicates				
	25 µg/ml	50µg/ml	100µg/ml	200 µg/ml	400µg/ml
CRA	13.13 ±0.002	28..02± 0.001	35.49±0.001	42.53± 0.001	57.03± 0.001
CRE	19.61±0.002	32.71.±0.002	41.9± 0.001	59.19 ±0.001	64.77±0.002
	10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml
Vit-C	26.82 ±0.002	41.54±0.001	52.51 ±0.001	68.18± 0.003	79.15±0.001

CRA= Aqueous extract, CRE= Ethanolic extract

DISCUSSION: The ethanolic extract of the aerial parts of *Cleome rutidosperma* possesses significant anti inflammatory activity. Reactive oxygen species (ROS) generated endogenously or exogenously are associated with the various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process. ROS plays an important role in the pathogenesis of inflammatory diseases. Thus antioxidants which can improve these disorders, the free radical scavenging activity of the extracts were evaluated based on the ability to scavenge the DPPH. This assay is highly important to provide information about the reactivity of organic compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge the free radicals independently. Hydroxyl radical is the principal contributor for tissue injury. The formation of hydroxyl radical from fenton reaction was quantified using 2, D-deoxyribose degradation. The extracts CRA and CRE inhibited hydroxyl scavenging activity.

Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as a marker for NO scavenging activity (Mukherjee *et al*., 1989). The chromophore formation was not complete in the presence of

extracts of *Cleome rutidosperma* (CRA and CRE), which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extracts (CRA and CRE) increases in the dose dependent manner.

ACKNOWLEDGEMENT: Authors are thankful to the all the members of department of Pharmacognosy as well as Pharmaceutical Chemistry, Jeypore College of Pharmacy and also to NRI group of institutions for their co-operation during the whole research work. Authors are also thankful to the Botanical Survey of India, Shibpur, Howrah and West Bengal, India for authentication and identification of this plant.

REFERENCES:

1. J. M. Widespread, Flora Malesiana, Series I, Vol. 6, 1972, 61.
2. B. Waterhouse, A. Mitchell. Northern Australia Quarantine Strategy Weeds Target List, AQIS Miscellaneous Publication, Canberra, 1998, 29.
3. K. R. Kirtikar, B. D. Basu. Indian Medicinal Plants, Lalit Mohan Basu, Dehradun, 1991,181
4. A. Bose, S. Mondal, J. K. Gupta, G. K. Dash, T. Ghosh, Si S, Studies on diuretic and laxative activity of ethanolic extract and its fractions of *Cleome rutidosperma* aerial parts, Pharmacognosy Magazine, 2(7), 2006, 178-182.
5. A. Bose, J. K. Gupta, G. K. Dash, T. Ghosh, Si S, D. S. Panda, Diuretic and antibacterial activity of aqueous extract of *Cleome rutidosperma.* D.C. Indian Journal of Pharmaceutical Sciences, 69(2), 2007, 292.
6. Anonymous: Orissa Review, Biju Pattnaik Medicinal Plants Garden Research Centre, Jeypore, 2005, 51-54.
7. T. Yokozawa, C. P. Chen, E. Dong, T. Tanaka, G. I. Nonaka, I. Nishioka. Study on the inhibitory effect of tannins and flavonoids against the 1, 1- diphenyl-2-picrylhydrazyl radical. Biochemical Pharmacology 1998, 56: 213-222.
8. E. M. Conner, M. B. Grisham. Inflammation, free radicals and antioxidants nutrition; 1996, 12:274.
9. M. Comporti. Three models of free radical induced cell injury, Chem biol interact; 1989, 72:1-56.
10. J. M. C. Gutteridge, Age pigments and free radicals; Fluorescent lipid complexes formed by iron and copper containing proteins, Biochem Biophys Acta; 1985, 834:144.
11. K. Z. Guyton, M. Gorospe, N. J. Holbrook. Oxidative stress and the molecular biology of antioxidant defenses, Cold Spring Harbor laboratory Press, New York; 1997, 242-272.
12. S. Bolton. In Pharmaceutical Statistics-Practical and Clinical Applications. New York: Marcel Dekker; 1997

****