INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 January, 2014; received in revised form, 20 March, 2014; accepted, 03 May, 2014; published 01 July, 2014

ANTIOXIDANT ACTIVITY OF TAXIFOLIN OBTAINED FROM METHANOLIC EXTRACTS OF *CORDIA DICHOTOMA LINN*. SEEDS

Mahasweta Roy*, Bhupendra Kumar, Nishant Kumar, Amrit Patel and Babita Kumar

College of Pharmacy, Shree Ganpati Institute of Technology, Ghaziabad, Uttar Pradesh, India

Keywords:
Cordia dichotoma forst, Antioxidant activity, Taxifolin, DPPH, seeds extracts.

Correspondence to Author:

Mahasweta Roy

College of Pharmacy, Shree Ganpati Institute of Technology, Shree Ganpati Knowledge Park Opp- Jindal Pipes Ltd., NH-24 Ghaziabad UP India 201302.

E-mail: shweta.chunchun@gmail.com

ABSTRACT: *Cordia dichotoma* L. family Boraginaceae is a medium sized tree, is taken as food. The immature fruits are pickled and are also used as vegetable. The various extracts and fractions of *Cordia dichotoma* were screened for their free radical scavenging properties using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and comparing it with butylated hydroxyl toluene (BHT) as standard antioxidant. The methanolic extract of seeds of *Cordia dichotoma* Linn and its fraction containing Taxifolin showed promising (P<0.001) DPPH free radical scavenging activity at a concentration of 100 µg/ml.

INTRODUCTION: Free radicals i.e. chemical species possessing an unpaired electron are produced continuously in cells either as accidental byproducts of metabolism or as deliberates. The most important free radicals in biological systems are radical derivatives of oxygen are antioxidant in nature.

The Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies show beneficial therapeutic potentials.Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum, Piper cubeba Linn., Allium sativum Linn., Terminalia bellerica ,Camellia sinensis Linn., Zingiber officinale* Roscoe and several Indian and Chinese plants .

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(7).2896-01

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(7).2896-01

The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [1]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [2].

Cordia dichotoma L. family Boraginaceae is a medium sized tree with short crooked trunk; leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with a round and cordate base; flower white, small in lax terminal or axillary cyme; fruits drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part. The plant part used is bark, leaves and fruits [3].

It is also known by various other names; for example Bird Lime Tree (common name), Sebesten Plum, Indian cherry (English), Lasora, Lasura (Hindi) and Slesmatakah (Sanskrit) It is taken as food. The immature fruits are pickled and are also used as vegetable [4].

Cordia dichotoma seeds have disclosed the presence of α-amyrins, betulin, octacosanol, lupeol-3-rhamnoside, β-sitosterol, β-sitosterol-3-glucoside, hentricontanol, hentricontane, taxifolin-3, 5-dirhamnoside and hesperitin-7- rhamnoside. *Cordia dichotoma* is already reported to have anti-inflammatory, Antihelminthic and Wound healing activity [5, 6, 7].

MATERIAL AND METHODS:

Collection of Plant Material: The fruits of *Cordia dichotoma* were collected from the local market of Lucknow and were authenticated as *C. dichotoma* (Boraginaceae) by pharmacognostic evaluation and a voucher specimen was deposited at National Botanical Research Institute Lucknow, for future reference (voucher no. NBRI/CIF/Re./08/2008/32)

Animals: Healthy male or female albino Wister rats each weighing 150-200 g were used for study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 ±3^{o}C and 35-60% humidity). Standard palletized feed and tap water were provided *ad libitum*.

Successive Extraction with Various Solvents: gms of air-dried seed powder of *Cordia dichotoma* was extracted by successive extraction (exhaustive) with various solvents of different polarity like Petroleum ether (60-80^{o}C), Diethyl ether, Chloroform, Acetone and Methanol in a soxhlet. Each extract was concentrated to a small volume and allowed to dry. After drying, the respective extracts were weighed and percentage extractive values were determined.

Isolation of Constituent Fractions: The methanolic extract was subjected to column chromatography. For this, the extract was chromatographed on a silica gel column (60-80 mesh) with methanol as a stationary phase. Proper time was allowed for seggregation and stabilization of fractions on the column. First elution was done by using chloroform (50 ml) after leaving the solvent for 10 minutes in the column for sufficient partitioning and then collection of 10 ml fractions in 5 test tubes at the rate of 20-25 drops per minute. All the fractions in the test tubes were subjected to chemical test and chromatography (TLC) for identification of components.

All fractions showing single spot TLC were analyzed by UV spectroscopy scanning consequent fractions with single spot TLC with similar R_f values and UV scan were combined and subjected to precipitation of components by using various solvents of opposite polarity. There after the IR spectroscopy of these isolated components were done to identify the type of components. After chloroform second elution was done by adding chloroform: methanol 70: 30 and fractions were collected, subjected to TLC, UV scanning and precipitation as above

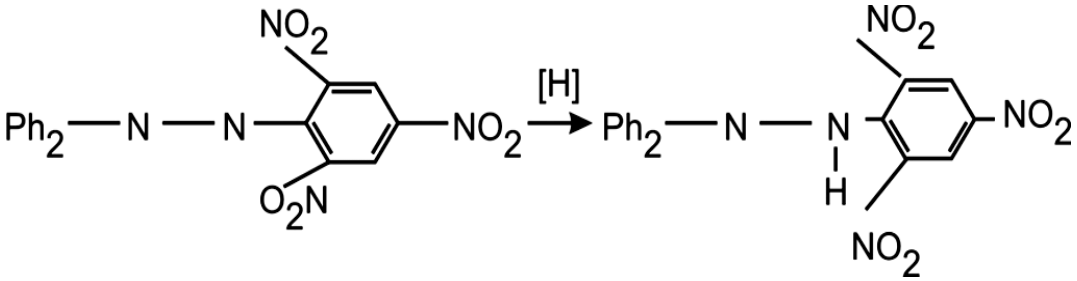
Fractionation was continued by using chloroform: methanol (40: 60) and fractions similarly obtained and treated as above.

Lastly 50 ml methanol was used as eluent and fractions were collected.

Screening For Antioxidant Activity [8]:

Reagents used for Antioxidant activity: Butylated hydroxyl toluene, DPPH, Phosphate Buffer saline (pH 7.4), Stock TBA-TCA-HCl reagent (Trichloroacetic acid (15 % w/v), thiobarbituric acid (0.375% w/v), hydrochloric acid (0.25 N). The solution was heated to assist in the dissolution of thiobarbituric acid)

DPPH Method: DPPH is stable nitrogen centered free radical and has been extensively used to characterize an antioxidant. The reduction of DPPH radical serves as a quick and simple method to detect the antioxidant potential of compounds especially those with phenol group. It is known that DPPH react rapidly with compound containing weak N–H or O–H bonds. Electron transport is also an important mechanism for its reduction. It is reversible, reduced and due to its unpaired electron, densely colored. This property makes it suitable for spectrophotometric studies.



REDUCTION OF 1, 1 DIPHENYL - 2 - PICRYL HYDRAZYL

Procedure: The free radical scavenging effect of fractions was assessed by the decoloration of a methanolic solution of DPPH with minor modifications. Samples were dissolved in 0.1 ml DMSO and added to 0.1 ml of 0.1 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand for 10 min at room temperature in the dark. The absorbance at 565 nm by DPPH was measured by a spectrophotometer. BHT was used as a positive control.

Statistical Analysis: One way analysis of variance (ANOVA) followed by Newmann Keul's, was carried out & $p<0.001$ was considered as significant. Groups were compared with control group.

RESULT:

Column Fractionation and Chromatographic Profile of the Methanolic Extract [9]: The methanolic extract on column chromatography gave the following fractions with the following characters.

Solvent system for TLC:

1) Chloroform: Methanol (9: 1)
2) Forestal
3) Phenol

Spraying agent: Anisaldehyde reagent 0.5%

TABLE 1:

S. No.	Fraction No.	Eluent	No. of Spots	R_f value	UV Values
1.	C1	$CHCl_3$	2	0.3, 0.95	--------
2.	C2	$CHCl_3$	2	0.29, 0.95	--------
3.	C3	$CHCl_3$	1	0.961	--------
4.	C4	$CHCl_3$	1	0.961	---------
5.	C5	$CHCl_3$	No spot	--------	---------
6.	CM1	$CHCl_3$:MeOH (7:3)	1	0.86	288.8
7.	CM2	$CHCl_3$:MeOH (7:3)	1	0.863	288.8
8.	CM3	$CHCl_3$:MeOH (7:3)	No spot	--------	--------
9.	CM4	$CHCl_3$:MeOH (7:3)	1	0.83	--------
10.	CM5	$CHCl_3$:MeOH (7:3)	1	0.83	---------
11.	CMM1	$CHCl_3$:MeOH (4:6)	1	0.86	264.8
12.	CMM2	$CHCl_3$:MeOH (4:6)	1	0.83	264.8
13.	CMM3	$CHCl_3$:MeOH (4:6)	1	0.83	268.4
14.	CMM4	$CHCl_3$:MeOH (4:6)	1	0.81	---------
15.	CMM5	$CHCl_3$:MeOH (4:6)	2	0.81, 0.17	---------
16.	M1	MeOH	1	0.12	305.6
17.	M2	MeOH	1	0.12	305.6
18.	M3	MeOH	No spot	---------	---------
19.	M4	MeOH	1	0.124	303.2
20.	M5	MeOH	1	0.12	302.0

The fractions C_3 and C_4 (chloroform fractions) from methanolic extract of *Cordia dichotoma* seeds gave single component spot on TLC with R_f value 0.941with mobile phase Chloroform: Methanol:: 9:1 which gave typical positive reactions for steroidal glycosides and matched comparably with sitosterol. The melting point of the substance obtained was 84-85^o which was in confirmation to earlier reported data for hentricontanol, a substance earlier reported for the seeds [10]. This fraction was designated as hentricontanol fraction (HTF).

The fractions CM_1 and CM_2 (Chloroform: Methanol:: 7:3) from methanolic extract of *Cordia dichotoma* seeds showed single component spot on TLC with R_f value 0.86 with mobile phase Chloroform : Methanol :: 9:1 which gave strong positive reactions for flavonoid glycosides. The fractions were combined, concentrated and precipitated to yield yellow component with melting point 148^o which on spectroscopic analysis showed UV (CH_3OH) λ_{max} (288 nm) and IR (KBr) peaks (3400, 2900, 2935, 1683, 1536, 1558, 1455, 1417, 1280, 1271, 1124, 867 and 822 cm^{-1} which matched comparably to earlier reported data for Taxifolin which is reported as a constituent [11] and to standard data for flavonoids.

This fraction was designated as Taxifolin fraction (TF). The fractions CMM_2 and CMM_3 (Chloroform: Methanol:: 4:6) from methanolic extract of *Cordia dichotoma* seeds showed single component spot on TLC with R_f value 0.83 with mobile phase Chloroform : Methanol :: 9:1 which gave strong positive reactions for flavanoid glycosides. This could not be further identified and was designated as flavanoid fraction (FF).

The fractions M_1 and M_2 (Methanol) from methanolic extract of *Cordia dichotoma* seeds showed single component spot on TLC with R_f value 0.12 with mobile phase Chloroform: Methanol:: 9:1 and BAW and matched comparably with hesperitin and rutin standards. Acid hydrolysis of the precipitate obtained after combining the fractions and precipitation with Ethyl acetate: Petroleum ether, gave a compound with melting point 227-228^o which on spectroscopic analysis showed IR (KBr) peaks (3400, 3128, 2939, 2345, 1647, 1508, 1458, 1294, 1062 and 808 cm^{-1}) which matched comparably to earlier reported data for Hesperitin [12]. Thus, the fractions must be Hesperitin glycoside (HF).

Effect of Extracts and Fractions on Dpph Free Radical Scavenging Activity:

TABLE 2: SHOWING THE RESULT OF ANTIOXIDANT ACTIVITY OF THE VARIOUS EXTRACTS AND FRACTIONS FROM OF THE SEEDS OF *C. DICHOTOMA*.

S. no.	Sample	Concentration (µg/ml)	DPPH Radical Scavenging Activity	
			ABSORBANCE	%
1.	Control Blank	-------	0.892	----
2.	Ref (BHT)	1000	0.175	84
3.	I	100	0.542	39.2
4.	II	100	0.788	11.6
		10	0.732	17.9
5.	III	100	0.213***	76.12***
		10	0.365**	59.0*
6.	IV	100	0.436*	51.1*
		10	0.498*	44.17*
7.	V	100	0.285***	68.0**
		10	0.376**	57.5*
8.	VI	100	0.611	31.5
		10	0.591	33.7
9.	VII	100	0.198***	77.8***
		10	0.229**	66.5**

Sample I- Petroleum ether extract; Sample II- Chloroform extract; Sample III- Methanol extract; Sample IV- Hentricontanol (HTF) Fraction; Sample V- Taxifolin (TF) fraction ; Sample VI- Flavanoidal (FF) fraction; Sample VII- Hespitin (HF) fraction. Values are expressed as Mean ±SEM (n=6). P*<0.05, P**<0.01, P***<0.001

DISCUSSION AND CONCLUSION:

Effect of Extracts and Fractions on DPPH Free Radical Scavenging Activity: In the study of antioxidant by scavenging free DPPH radicals, the various extracts and fractions showed following responses. The methanolic extract ($P<0.001$) of seeds of *Cordia dichotoma* Linn. showed promising DPPH free radical scavenging activity at a concentration of 100 µg/ml whereas the petroleum ether extract and chloroform extract and the FF was non-significant at 100µg/ml and 10 µg/ml concentration. The fraction HTF showed ($P<0.05$) significance whereas HF and TF showed ($P<0.001$) significance at the 100 µg/ml concentration. At 10 µg/ml concentration the methanolic extract, TF and HF showed significance ($p<0.01$) (**figure 1**).

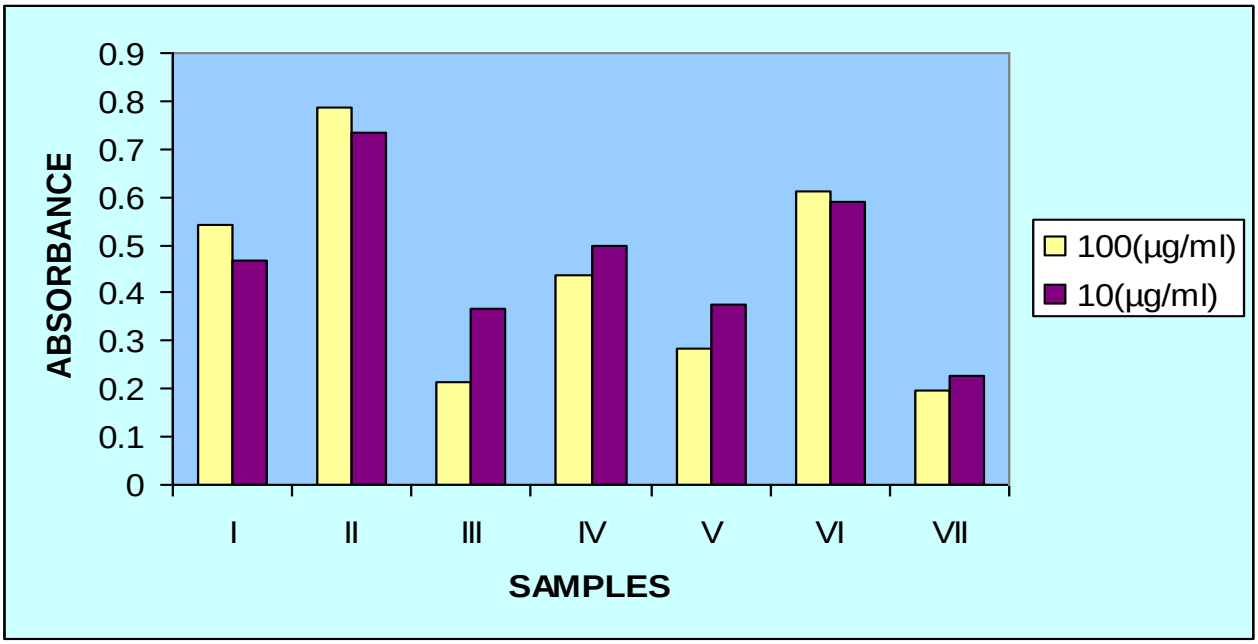


FIG. 1: GRAPH SHOWING THE EFFECT OF THE VARIOUS EXTRACTS AND FRACTION ON THE DPPH FREE RADICAL SCAVENGING ACTIVITY. Sample I- Petroleum ether extract; Sample II- Chloroform extract; Sample III- Methanol extract; Sample IV- Hentricontanol (HTF) Fraction; Sample V- Taxifolin (TF) fraction ; Sample VI- Flavanoidal (FF) fraction; Sample VII- Hesperitin (HF) fraction.

REFERENCES:

1 Aqil F, Ahmed I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J Biol 2006; 30: 177-183.

2 Devasagayam TPA, Tilak JC, Boloor KK, Review: Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI 2004; 53:794-804.

3 Kirtikar, K.R and Basu, B.D., "Indian medicinal plants", edition 11th, Vol. III, Oriental enterprises, 1935; 1675

4 The Wealth of India, Raw material, "A dictionary of Indian raw material and industrial products", Council of Scientific and Industrial Research , New Delhi, 1950; 2: 232

5 Agnihotri, V.K. and Srivastava, S.K. "Constituents from the seeds of *Cordia dichotoma* as potential anti-inflammatory agent", Indian journal of pharmaceutical sciences, March-April 1987; 66-68.

6 Kuppasta I.J. and Nayak P.V. "Antihelminthic activity of fruits of *Cordia dichotoma*", Indian Journal of Natural Products 2003; 19(3): 27-29.

7 Kuppasta I.J. and Nayak P.V. "Wound healing activity of *Cordia dichotoma* Forst. fruits", Natural Product Radiance, 2006; 5: 103-107.

8 Vogel G. and Vogel H., 1997, "Drug discovery and evaluation", Pharmacological assay, II edition, Vol. 3, pp. 950-951.

9 Wagner H., Bladet S. and Zgainski E.M. "Plant Drug Analysis: A TLC atlas", edition 2nd, Verlag Berlin Heidelberg, Germany, 1996; 163-200.

10 Agnihotri, V.K. and Srivastava, S.K, "Constituents from the seeds of *Cordia dichotoma* as potential anti-inflammatory agent", Indian journal of pharmaceutical sciences, March-April 1987; 66-68

11 Srivastava, Santosh K. and Srivastava, Savitri D. "Taxifolin 3, 5-dirhamnoside from the seeds of Cordia dichotoma", Phytochemistry, 1979; 18: 2058-205

12 Chauhan, J. S. and Srivastava, S. K. "5, 7-Dimethoxytaxifolin-3-O-α-L-rhamnopynoside from the methanolic extract of the roots of Cordia dichotoma", Indian J. Chem, Vol 15B Aug, 1977; 760-761.

How to cite this article:
Roy M, Kumar B, Kumar N, Patel A and Kumar B: Antioxidant activity of taxifolin obtained from methanolic extracts of *cordia dichotoma linn*. Seeds. *Int J Pharm Sci Res* 2014; 5(7): 2896-2901.doi: 10.13040/IJPSR.0975-8232.5 (7).2896-2901.