

ROLE OF *CORDIA DICHOTOMA* SEEDS AND LEAVES EXTRACT IN DEGENERATIVE DISORDERS

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ABSTRACT:

A common theme which underlies etiology of several degenerative disorders is free radical induced stress. Free radicals prime the immunomodulatory response, recruit inflammatory cells and are innately bactericidal. In the body, excess production of free radicals affects lipid cell membranes to produce lipid peroxides and reactive oxygen species (ROS) which leads to decline in membrane fluidity and many biological changes, such as DNA damage, ageing, heart disease and cancer etc. Antioxidants serve as free radical scavengers, neutralizing and defending the body from a number of diseases which are born because of generation of free radicals. They offer defense against radical toxicity by antagonizing the damages caused by free radicals. The current study is therefore carried out to investigate the free radical scavenging potential of methanolic extract of seeds and leaves of *Cordia dichotoma* using *in-vitro* models viz. DPPH and hydrogen peroxide model. These models demonstrate positive antioxidant activity in a concentration dependent manner and demonstrate that highest concentration exhibits highest (100µg/ml) antioxidant activity. This activity was more pronounced in leaves as compared to seeds.

Keywords: Reactive Oxygen Species, *Cordia dichotoma*, Anti-oxidant, DPPH, Reducing Power

INTRODUCTION

The potentially imprudent derivatives of oxygen, endorsed as ROS such as O_2^- , H_2O_2 and OH radical are incessantly generated within the human body as a consequence of revelation to a superfluity of exogenous chemicals in our ambient milieu and/or a number of endogenous metabolic processes linking redox enzymes and bioenergetic electron transmit. Under normal state of affairs, the ROS generated are detoxified by the antioxidants nearby in the body and there is symmetry between the ROS generated and the antioxidants present. However due to ROS overproduction and/or derisory antioxidant argument, this equilibrium is hindered favoring the ROS gain that culminates in oxidative hassle. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (1). This oxidative damage is a decisive etiological factor concerned in quite a lot of chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing course (2). Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the expediency of antioxidants in protection against these diseases is defensible. Epidemiological studies have brought into being that the intake of antioxidants such as Vitamin-C (ascorbic acid) reduces the risk of coronary heart disease and cancer (3). The antioxidants may reconcile their upshot by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions (4). Several synthetic antioxidants, e.g., BHA and BHT are commercially accessible but are quite perilous and their toxicity is a problem of disquiet (5). In recent years one of the areas, which attracted a great treaty of attention, is antioxidant in the control of degenerative diseases in which oxidative dent has been implicated. Several plant

extracts and different lessons of phytochemicals have been shown to have antioxidant activity. Plant extracts and plant products such as flavonoids and other polyphenolic constituents have been reported to be effective racial scavengers and inhibitors of lipid peroxidation (6-10).

Cordia dichotoma L. (Boraginaceae) is tree of tropical and subtropical regions, commonly known as Lasaura/Lasura. It is a medium sized tree with short crooked trunk, leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with round and cordate base, flower white, fruit drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part (11). It grows in sub-Himalayan tract and outer ranges, ascending up to about 1500 m elevation (12). It is used as immunomodulator, antidiabetic, anthelmintic, diuretic and hepatoprotective in folklore medicine. *Cordia dichotoma* seeds has disclosed the presence of α -amyrins, betulin, octacosanol, lupeol-3-rhamnoside, β -sitosterol, β -sitosterol-3-glucoside, hentricontanol, hentricontane, taxifolin-3, 5-dirhmnoside and hesperitin-7-rhamnoside (13). The seed contain α -amyrin and toxifolin 3, 5, dirhamnoside, which shows significant anti-inflammatory activity by an oral dose of 1gm/kg in albino rats (11). The seeds of this plant reported to contain fatty acids and flavonoids (13).

The goal of the present study was to investigate the antioxidant profile of methanolic extract of *Cordia dichotoma* seeds and leaves by different antioxidant models including hydrogen peroxide scavenging, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method & to correlate it with diseases known to be associated with generation of free radicals.

MATERIAL AND METHODS

Collection of Plant Material:

The seeds, leaves and fruits of *Cordia dichotoma* were collected by self in rainy season of 2009 from district Lucknow of Uttar Pradesh and authenticated by the division of Taxonomy, National Botanical Research Institute, Lucknow. A voucher specimen no. NBRI/CIF/128/2010 was deposited for future reference. The seeds and leaves of plant material was washed with water, air dried and placed in a drying cabinet at 55-60°C. The dried material was pulverized into fine powder and stored in a covered jar at room temperature.

Preparation of Plant Extracts:

The extracts was prepared by extracting 50g of powdered drug (both seeds and leaves separately) with 500ml of methanol in a Soxhlet apparatus. The extract was subjected to vacuum evaporation on a Buchi rota evaporator. 100 mg of extracts was taken, dissolved in methanol and phosphate buffer respectively and final volume of flask was made up to 100 ml with same solvents. The final concentrations of solutions were 1000µg/ml which was used as stock solutions for further study. The different concentrations (20, 40, 60, 80, 100

µg/ml) from stock solutions was prepared by diluting with methanol and phosphate buffer respectively.

Preparation of DPPH Solution:

Solution of DPPH (0.1mM) in methanol was prepared by dissolving 1.9 mg of DPPH in methanol and volume was made up to 100ml with methanol. The solution was kept in darkness for 30 minutes to complete the reaction.

Preparation of Hydrogen Peroxide Solution:

0.2M Potassium dihydrogen phosphate and 0.2M sodium hydroxide solutions were prepared as per the Indian Pharmacopoeia 1996 standards. 50 ml of Potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask and 39.1 ml of 0.2M sodium hydroxide solution was added in this and finally volume was made up to 200 ml with distilled water to prepare phosphate buffer (pH- 7.4). 50 ml of phosphate buffer solution was taken and an equal amount of hydrogen peroxide was added in this to generate the free radicals and solution was kept a side to complete the reaction.

Table 1: Table showing % inhibition by different concentrations of drug extracts

Concentration in µg/ml	% Inhibition			
	Leaves		Seeds	
	DPPH Method	H ₂ O ₂ Method	DPPH Method	H ₂ O ₂ Method
20 µg/ml	23.76	29.44	19.46	28.68
40 µg/ml	38.42	44.65	34.78	39.86
60 µg/ml	59.68	67.86	51.66	60.48
80 µg/ml	75.42	77.22	74.34	82.56
100 µg/ml	85.46	88.62	81.24	90.28
IC ₅₀ Value µg/ml	51.83	44.73	57.22	48.28

Figure 1: Showing percent inhibition of free radicals by leaves in both models

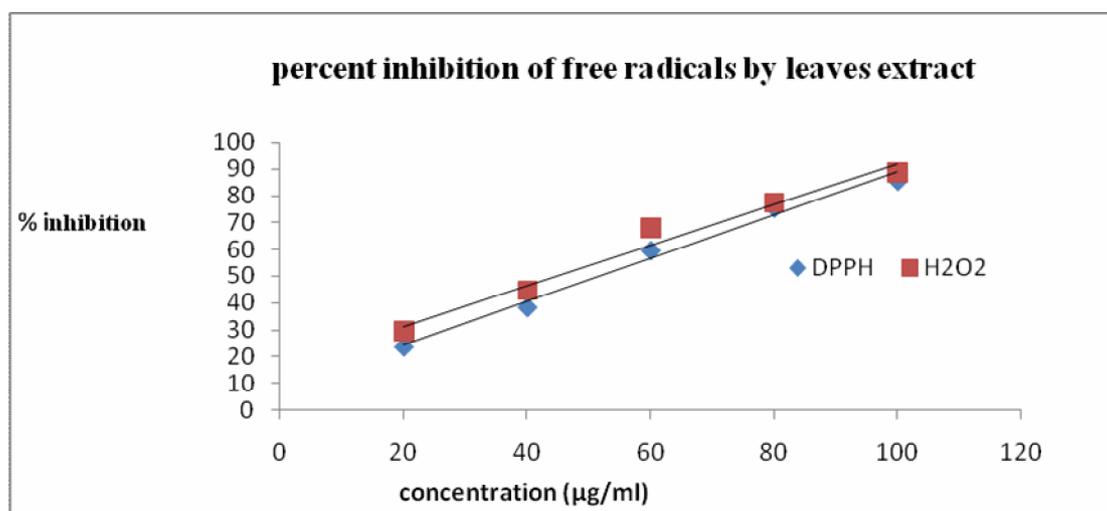
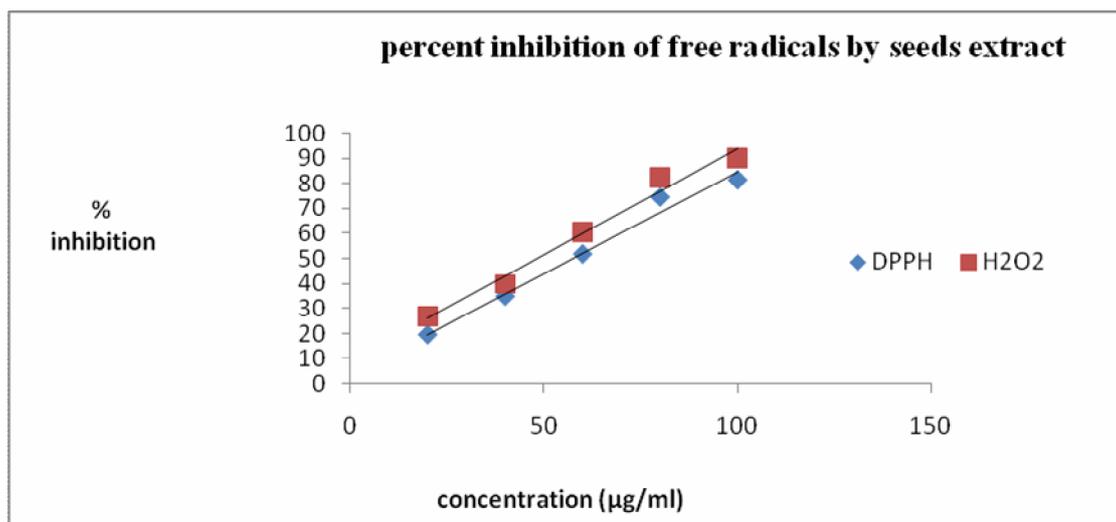


Figure 2: Showing percent inhibition of free radicals by Seeds in both models

Determination of Anti-Oxidant Activity:

1ml of DPPH solution was added to 1ml of different extracts and allowed to stand at room temperature for 30 min, and then absorbance was measured at 517 nm in a spectrophotometer. Similarly 1ml Extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer.

The percentage inhibition was measured by following formula (14).

$$\% \text{ inhibition} = (A_c - A_t) \times 100 / A_c$$

Where A_c is the absorbance of control

A_t is the absorbance of test sample

RESULTS

Table 1: Table showing % inhibition by different concentrations of drug extracts

Figure 1: Showing % inhibition of free radicals by leaves in both models

Figure 2: Showing % inhibition of free radicals by Seeds in both models

DISCUSSION

The phytochemical screening of plant extract showed the positive reaction for flavonoids, steroids, etc. DPPH and H₂O₂ methods exhibited inhibition of free radicals by *Cordia dichotoma* seeds and leaves extracts. Methanolic extracts of seeds and leaves which were taken up for the research work showed significant activity. A 100 µg/ml of methanol extract of seeds exhibited 81.24 & 88.57 percent inhibition of DPPH & H₂O₂ free radicals respectively whereas A 100 µg/ml of methanol extract of leaves exhibited 85.46 & 88.62 percent inhibition of DPPH & H₂O₂ free radicals respectively. In both the models the methanolic extract of the leaves exhibited low IC₅₀ values as compared to the seeds' extracts. The IC₅₀ value for leaves was found 51.83µg/ml and

55.73µg/ml for DPPH and H₂O₂ models respectively. The IC₅₀ values for seeds were found 57.22µg/ml and 48.28µg/ml for DPPH and H₂O₂ models respectively. The obtained result of absorbance & percent inhibition showed decrease in the concentration of DPPH & H₂O₂ radicals due to scavenging potential of extract. The crude methanolic extract exhibited low IC₅₀ values in these two methods justify the traditional usage of plant in various free radical induced diseases/disorders.

CONCLUSION

The present study suggests that methanolic extract of *Cordia dichotoma* seeds and leaves possess powerful antioxidant activity, the leaves are more potent than seeds. Therefore it is suggested that *Cordia dichotoma* could be a potential source of natural antioxidants that could have great importance as therapeutic agent in preventing or slowing down the progress of ageing and age associated oxidative stress related degenerative diseases. Further research is recommended for better characterization of important active constituents, responsible for antioxidant activity. The revealed antioxidant property of extracts may provide potential therapeutic intervention against oxidative threats and degenerative disorders.

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