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Antioxidant Activity of Methanolic Extract of *Cordia dichotoma* Collected from Different Geographical Region

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Abstract: ***Herbals are still used widely by World population ,because of better compatibility with the human body and lesser side effects(Rawls R. 1996) .The antioxidative active of a total 11 genotypes of leaves and fruit materials of Cordia dichotoma by using different reagent.. The methanolic extract of leaves and fruit were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant .Free radical scavenging activity was evaluated using 1,1 diphenyl picryl hydrazine (DPPH) free radical. The methanolic extracts of leaves and fruits were screened for phytochemical constituents as per standard method. The extracts shows positive test for phenols,flavonoids,tannins and alkaloids among few genotype. The extracts that showed the greatest DPPH antioxidant activity of leaves were of NNd4 (54.23%),NNd8 (54.33%),NNd7(53.09%),NNd9(50.45%)and fruit extracts of NNd2 (58.09%), NNd4(54.23%), NNd9(52.12%), NNd7(51.06%).The Leaves extracts that showed the greatest reducing power antioxidant activity was of NNd7(48.23%), NNd4(43.66%) , NNd9(42.89%) and fruit extracts of NNd7(45.12%), NNd9(41.94%), NNd2(44.76%), NNd(40.65%) . The leaves and fruit extracts showed weak nitric oxide scavenging activity***.

Keywords: *Cordia dichotoma* , phytochemical, antioxidant activity ,scavenging activity, DPPH

1. Introduction

In recent years, there is an increasing interest in antioxidants. The main reason for this interest is the protection of cells, their organelles and metabolic pathways against oxygen free radicals and their reactive derivatives (ROS). (Tiwari A,2001) Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton N. and Brown D.1999). Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc., are some of the antioxidants produced by the plant for their sustenance.(Mccall M. and Frei B. 1999). Several studies have demonstrated the antibacterial and/or antioxidant properties of these plants, mainly using in vitro assays. Moreover, some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the plants and their above mentioned functional properties (Dean S.and Svoboda K. 1989; Farag R.*et al.,* 1989). The objectives of the present study were to determine the antioxidant activity.Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and there by improve the quality and nutritional value of food. (Lo-liger J. 1991) . Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks , roots, spices , herbs, and crude plant drugs (Ramarathnam N.*et al* 1995) Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks (Larson R 1988). Free radicals are highly reactive species produced in the body during normal metabolic function or introduced from the environment. These are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Reactive oxygen species (ROS) react with free radicals to become radicals themselves. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function (Gulcin I, 2005) Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cells. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases (Hsu W. and Cheng K. 2003) Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton N. and Brown D.1999).

2. Materials and Methods

Plant material was collected from surrounding area of Nanded (MS) district. Leaves and Fruit of elven genotypes of *Cordia dichotoma* was selected for the study ,from different geographical region on the basis of morphological variation.The eleven genotype was named as NNd1,NNd2, NNd3, NNd4, NNd5, NNd6, NNd7, NNd8, NNd9, NNd10 and NNd11 respectively . The leaves and fruit of plant was 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.1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and potassium ferricyanide was obtained from Sigma Aldrich Co. Ascorbic acid was obtained from (Merck, Germany) chem. Naphthyl ethylene diamine dihydrochloride was obtained from Sigma Chemical Co, India. Sodium nitro prusside was obtained from Loba

chemie, India. All other chemicals used were of analytical grade.

Preparation of Plant Extracts

The extracts was prepared by extracting 50g of powdered drug (leaves and fruit separately) with 500ml of methanol in a Soxhlet apparatus. The extract was subjected to evaporation 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.

Preliminary Phytochemical Screening

The methanolic extracts of leaves and fruit of *C.dichotoma* was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures **(**Sofowara A.1993, Trease G. *et al* 1989, Harborne J. 1973)

Test for Alkaloids: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloid

Test for Phenols The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compounds .

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Tannins: 1ml of the sample was taken in a test tube and then 1ml of 0.008 M Potassium ferricyanide was added. 1ml of 0.02 M Ferric chloride containing 0.1NHCl was added and observed for blue-black colouration.

Test for Saponins: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

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.

DPPH radical scavenging activity of selected genotypes and calculation of IC_{50} value.
The stable 1-1 biphenyl 2 picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extracts (Koleva *et al* 2002).Different extracts were added at an equal volume to methanolic solution of DPPH (100 micromolar) .After 15min at room temperature ,the absorbance was recorded at 517nm. The experiment was repeated for two times .IC50 values denote the concentration of sample, which required scavenging 50% of DPPH free radical (Pourmorad F. and Hosseinimehr S.2006).The %DPPH radical scavenging activity was calculated as follows. Methanol (1.0 ml) plus the plant extract solution (2.5ml) was used as the blank, while the DPPH solution plus methanol was used as the control.

$$\% \text{ DPPH scavenging activity} = \frac{1\text{-O.D. of Test} * 100}{\text{O.D.of control}}$$

Determination of Reducing power and calculation of IC_{50} value

The reducing power of selected extracts was tested by the virtue of conversion of ferric to ferrous ions .In this case 0.5 ml of 0.1mM test extract was added to 3 ml of 1 mM potassium ferricyanide solution .The mixture was shaken thoroughly and incubated for 10 min at room temperature. Finally the reducing capacity of the compound was tested spectrophtometrically at 420nm using an appropriate blank of 3.5 ml potassium ferricyanide solution after every 10 min interval (Irene crespoV. 2008).This is reducing power activity was calculated as follows .

$$\% \text{ Reducing Power activity} = \frac{1\text{- O.D.of Test} * 100}{\text{O.D.of control}}$$

Determination of NO radical scavenging activity and IC50 value

Nitric oxide scavenging activity was determined according to the method reported by (Garrat D. 1964) Sodium nitroprusside in aqueous solution at physiological pH,spontaneously generates nitric oxide ,which can be determined by the use of the Griess Illosvoy reaction .2 ml of 10 mM sodium nitroprussside in 0.5 ml phosphate buffer (pH 7.4) was mixed with 0.5 ml of extract and the mixture incubated at 25°c for 150 min .From the incubated mixture 0.5 ml was taken out added into 1 ml of sulfanilic acid reagent(33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally 1 ml of naphthylene diamine dihydrochloride (0.1%W/V) was mixed and incubated at room temperature for 30 min .The absorbance at 540 was measured with a spectrophotometer (Garrat D. 1964) .The % nitric oxide scavenging activity was calculated as follows.

$$\% \text{ Nitric Oxide scavenging activity} = \frac{1\text{- O.D.of Test} * 100}{\text{O.D.of control}}$$

3. Result and Discussion

Morphological diversity

Wide variation was observed among the eleven accessions of *cordia dichotoma* germplasm on various morphological parameters of leaf, flowers, fruits and stone (Table 2). Morphological variation was observed among the eleven accessions of *C. dichotoma* germplasm on various morphological parameters of leaf, flowers, fruits and stone The leaf shape of most of the accessions is round or obovate as they have length and width almost equal except NNd9 and NNd10. Most of the fruits are oval or round shape. Leaf

length and width, fruit length, width and weight and pulp:stone ratio were found highly heritable.The table 2 indicates the average value of each calculated from 100 nos.

Results obtained in the present study revealed that the level of t hese phenolic compounds in the methanol extracts of the leaves and fruit of *Cordia. dichotoma* were considerable (Table 3) Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extract is due to these compounds (Okudu T. *etal* 1994, Tepe B. and *etal* 2006) This activity is believed to be mainly due to their redox properties, which play an important , role in adsorbing and neutralizing free radicals quenching singlet and triplet oxygen, or decomposing peroxides (Zheng W. and Wang S.2001). In fact, many medicinal plants contain large amount of antioxidantssuch as polyphenols. Many of these phytochemicals , possess significant anti-oxidant capacities thatare associated with lower occurrence and lower mortality rates of several human diseases(Anderson K.and *et al* 2001, Djeridane A.and *et al* 2006). Plant extract was tested for chemical constituents such as alkaloids, glycosides,carbohydrates, protein, tannins,flavinoids, saponins. Out of all test ,leaves and fruit extract was positive for phenol, flavinoids saponin and tannins. Behaviour of powder of *Cordia dichotoma* leaves and fruit with different chemical reagents like concentrated HNO_3, H_2SO_4 and HCl, as well as picric acid, acetic acid, ferric chloride and iodine solution were also tested, which showed orange colour, dark brown, blackish green, yellowish green, light brown, black colour and light green colour respectively (Table .3) If the extract of *C.dichotoma* plant show positive results for many number of tests, indicates that methanol is used as a best solvent for extraction of phytochemicals. Many chemical components can not show +ve results for other solvents like Chloroform,Acetone and Hexane. The phytochemical screening of plant extract showed the positive reaction for flavonoids, phenols, tannins , etc

Oxygen free radicals and their reactive derivatives are generated endogenously.The exogenous sources of free radicals are eg. smoking, air pollution, UV radiation and metabolism of xenobiotics. In excess they can cause multiple damages by attacking biomolecules like proteins, lipids, DNA etc. (Marnett L. and *et al* 2003, Valko M. and *et al* 2004). Naturally, there is a dynamic balance between the amount of ROS generated and degraded in cells. They are degraded to non-reactive forms by enzymatic and non-enzymatic antioxidant defenses produced in cells or by others supplied with the diet. If there is a depletion of these compounds, The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee S.*et al.*, 2003). Phenols and polyphenolic compounds, such as flavonoids, are widely found in plant sources, and they have been shown to possess significant antioxidant activities (van Acker S.*et al.*, 1996). The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables (Klimczak I.*et al* 2007, Jayaprakasha G.*et al* 2008).

It is known that only flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity (Mensor L.*et al* 2001). Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities (Mensor L.*et al* 2001, Hou W. *et al* 2003). DPPH ,FRAP and NO methods exhibited inhibition of free radicals of *Cordia dichotoma* leaves and fruit extracts. Methanolic extracts of leaves and fruit which were taken up for the research work showed significant activity. A 100 µg/ml of methanol extract of leaves exhibited 56.23, 54.09, 53.33 percent inhibition of DPPH free radicals of NNd4, NNd7, NNd8 respectively . where as a 100 µg/ml of methanol extract of fruit exhibited 58.09, 54.23, 52.12 percent inhibition DPPH free radicals of NNd2, NNd4, NNd9 respectively. The DPPH scavenging assay of reference compound ascorbic acid given in table .4.

In this assay the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The ferric reducing/antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary polyphenols (Luximon-Ramma A.2005) The reducing capacity of the extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe3+ to Fe2+ by donating an electron.In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe+3) in ferric chloride to ferrous (Fe+2). The results of the reducing power assay are given in (Table .4) The reducing ability of the extracts was in the range of 23.73- 48.23mg/ml . The relative reducing power of the different methanol extract of leaves of *Cordia dichotoma* from the results are NNd7 > NNd4>NNd8 > NNd9 and reducing power of different methanol extract of fruit of *Cordia dichotoma* from the result are NNd7>NNd2>NNd9>NNd>NNd4. The FRAP values for the methanol extracts of the leaves and fruit of *C. dichotoma* were significantly lower than that of ascorbic acid. For the reducing power assay , reference compound ascorbic acid given in table no.4.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes (Lata H.*et al.* 2003). Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as N02, N204, N304, N03_. and N02 are very reactive. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans *et aI.*, 1997). The nitric oxide scavenging activity of flavonoids and phenolic compounds are known (Kim H.*et aI* 1999) Our findings suggest that all of the extract have the property to counteract the effect of NO formation due to the presence of tannins and f1avonoids and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in vivo. The NO scavenging ability of the extracts was in the range of 16.14-42.43 mg/ml (Table .4). The results of NO scavenging activity of the selected plant extracts are shown as percent of NO scavenging in Table .4. The NO scavenging activity of methanol extract of the leaves and fruit of *C.dichotoma* were significantly lower than that of quercetin

4. Conclusion

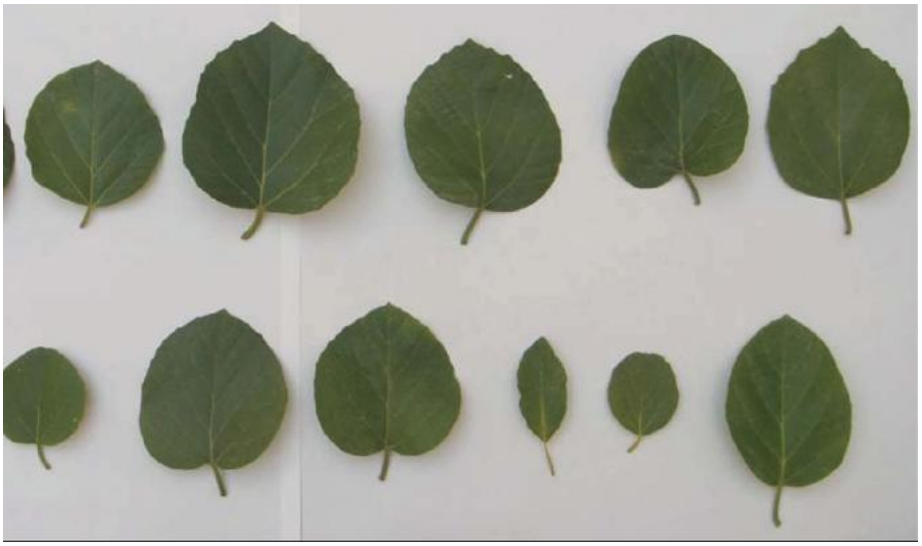
There is no doubt that plants have continued to offer a large range of natural compounds belonging to different molecular families which have various properties to humans as earlier reported by (Zabri H.*et al 2008)*. co*rdia dichotoma* leaves appears
to be rich in bioactive compounds which are widely used for various activities including traditional medicine. The leaves extract of *C.dichotoma* shows higher potential as antioxidant activity, comapare to fruit extract.

Table 1: Details of genotypes and collection sites

Sr.No.	*Accession No.*	*Village/district*	*Collection site*
1	NNd1	Ardhapur/Nanded	Farmers' field
2	NNd2	Mudkhed/Nanded	Farmers' field
3	NNd3	Barad/Nanded	Farmers' field
4	NNd4	Tamsa/Nanded	Forest area
5	NNd5	Vishnupuri/Nanded	Farmers' field
6	NNd6	Bhokar/Nanded	Farmers' field
7	NNd7	Pawadiwadi/Nanded	Farmers' field
8	NNd8	Limbgaon/Nanded	Farmers' field
9	NNd9	Patnoor/Nanded	Farmers' field
10	NNd10	Vishnupuri /Nanded	Farmers' field
11	NNd11	Asarjan/Nanded	Farmer's field

Table 2: Morphological diversity of *Cordia dichotoma* germplasm used in this study

Sr.No.	*Accessions No.*	*Leaf length (cm)*	*Leaf width (cm)*	*Fruit length (cm)*	*Fruit width (cm)*	*Fruit weight At pickle stage (g)*	*Fruit weight at ripe stage (g)*	*No. of fruits per cyme*	*Leaf shape*
1	NNd1	6.9	5.5	1.07	1.02	1.35	2.01	6±3	oval
2	NNd2	7.4	5.9	1.12	0.93	1.98	2.45	8±4	oblong
3	NNd3	10.1	7.8	1.26	0.96	1.19	3.02	4±3	oval
4	NNd4	8.1	6.5	1.06	0.80	.0.98	2.45	8±2	oval
5	NNd5	6.3	5.4	1.83	1.04	2.34	3.67	6±4	oval
6	NNd6	8.6	7.4	1.21	0.63	1.56	3.98	9±2	obtuse
7	NNd7	10.4	9.4	1.95	1.58	3.67	4.97	6±6	oval
8	NNd8	6.5	5.6	1.34	1.23	3.12	4.65	6±2	oblong
9	NNd9	4.6	3.5	1.03	0.92	1,47	3.43	10±3	oblong
10	NNd10	3.2	2.9	1.84	1.75	2.78	4.02	6±2	Obtuse
11	NNd11	9.6	5.4	1.67	1.56	2.11	4.12	5±4	oval



Morphological diversity of leaves and fruits among eleven accessions of *Cordia dichotoma* germplasm

Table 3: Phytochemical Screening of selected 11 genotypes

Sr.No.	*Accession No.*	*Methanol extracts*	*Alkaloid*	*Phenol*	*Flavonoids*	*Tannins*	*Saponin*
1	NNd1	Leaves	-	+	+	+	-
		Fruit	-	+	++	-	-
2	NNd2	Leaves	+	+	+	+	-
		Fruit	-	++	++	-	+
3	NNd3	Leaves	+	-	-	+	+
		Fruit	++	+	++	-	-
4	NNd4	**Leaves**	+	++	++	++	+
		Fruit	+	++	++	+	-
5	NNd5	Leaves	-	+	+	+	+
		Fruit	+	+	+	-	-
6	NNd6	Leaves	+	-	+	++	+
		Fruit	+	+	+	-	++
7	NNd7	**Leaves**	-	++	++	-	+
		Fruit	+	++	++	+	-
8	NNd8	**Leaves**	-	++	++	-	+
		Fruit	-	+	+	+	+
9	NNd9	**Leaves**	-	++	++	-	+
		Fruit	+	++	++	+	-
10	NNd10	Leaves	-	+	+	+	+
		Fruit	++	+	++	-	-
11	NNd11	Leaves	-	+	+	++	+
		Fruit	+	+	-	+	-

++ strong colouration, +weak colouration , – absent

Table 4: Antioxidant activity of selected 11 genotypes

Sr.No.	*Accession No.*	*Methanol Extract*	*% DPPH scavenging activity (mg/ml)*		*% Reducing power activity (mg/ml)*		*% No radical scavenging activity (mg/ml)*	
			Plant Extract	*Ascorbic acid(Std.)*	*Plant Extract*	*Ascorbic acid(Std.)*	*Plant Extract*	*Quercetin (Std.)*
1	NNd1	Leaves	32.12	72.34	23.98	67.54	19.87	59.97
		Fruit	34.67	72.34	34.89	67.54	26.89	59.97
2	NNd2	Leaves	38.55	72.34	22..56	67.54	21.45	59.97
		Fruit	**58.09**	72.34	**44.76**	67.54	**39.67**	59.97
3	NNd3	Leaves	33.78	72.34	37.87	67.54	**30.45**	59.97
		Fruit	44.12	72.34	31.77	67.54	23.67	59.97
4	NNd4	**Leaves**	**56.23**	72.34	**43.66**	67.54	**40.32**	59.97
		Fruit	**54.23**	72.34	**40.65**	67.54	**39.46**	59.97
5	NNd5	Leaves	39.78	72.34	31.00	67.54	28.67	59.97
		Fruit	41.88	72.34	38.04	67.54	18.87	59.97
6	NNd6	Leaves	22.10	72.34	23.73	67.54	28.56	59.97
		Fruit	35.77	72.34	27.45	67.54	19.67	59.97
7	NNd7	**Leaves**	**54.09**	72.34	**48.23**	67.54	**41.98**	59.97
		Fruit	**50.06**	72.34	**45.12**	67.54	**42.43**	59.97
8	NNd8	**Leaves**	**53.33**	72.34	**43.54**	67.54	**36.45**	59.97
		Fruit	34.76	72.34	25.46	67.54	29.21	59.97

9	NNd9	**Leaves**	**53.33**	72.34	**42.89**	67.54	**38.87**	59.97
		Fruit	**52.12**	72.34	**41.94**	67.54	**40.67**	59.97
10	NNd10	Leaves	36.87	72.34	37.33	67.54	27.12	59.97
		Fruit	42.98	72.34	27.86	67.54	16.14	59.97
11	NNd11	Leaves	32.43	72.34	30.76	67.54	22.44	59.97
		Fruit	39.87	72.34	35.25	67.54	21.37	59.97

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