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IN VITRO ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENTS AND PHYTOCHEMICAL EVALUATION FROM CRUDE EXTRACTS OF AN ENDANGERED PLANT *CORDIA MACLEODII* HOOK. F. THOMSON

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ABSTRACT:

Cordia macleodii, an important medicinal plant traditionally used for the treatment of various disorders, including wound healing, jaundice, gastric ulcer, hepatoprotective activity and antivenom potential, thus the plant showed the high antioxidant properties. Successive extraction of leaf and barks powder with methanol and water was tested for quantitative determination of total phenolics, total flavonoids and various *in vitro* antioxidant activities. Phytochemical screening of crude plant extracts revealed the presence of sterols, alkaloid, flavonoid, phenolic, Reducing sugar, glycosides, tannins and saponins. All extracts showed the significant total phenolic and flavonoid contents and as well as radical scavenging and iron-chelating activities. Among all, methanol leaf extract was observed to be higher antioxidant activity than that of other extract. The methanol extracts showed greater antioxidant activity by DPPH scavenging the free radical with IC_{50} values of 7.63 ± 0.38 µg gm^{-1} along with scavenged free radical in a concentration depended manner, which is compare to the standard ascorbic acid. Our results revealed that the presence of grater antioxidant activity of *C. macleodii* extract may be due to the high level of flavonoid and phenolic content in the plant. These results clearly indicated that *C. macleodii* is an effective natural antioxidant.

Key words: *Cordia macleodii,* antioxidant, radical scavenging, phenolics, flavonoid, phytochemical.

1. Introduction

Antioxidants are vital substances which have the ability to protect the body from damages caused by free radical induced oxidative stress which could affect and damage biological molecules. Reactive oxygen species (ROS) are highly diversified and reactive molecules such as, hydroxyl radical ($^{\cdot}OH$), peroxide ($ROO^{\cdot}$) and superoxide radicals ($O_2^{\cdot}$), can damage to cells and tissues during various diseases. Natural antioxidants, e.g., polyphenols are present in most of medicinal plants, which prevents oxidative damages **[1,2,3]**. Previous studied have shown that plants contains variety of substances called "Phytochemicals" are naturally occurring components and these chemical compounds exhibiting antioxidant properties **[4]**. In recent years, several researches have succeeded in finding the phytochemicals and identifying natural antioxidants and that may occur in all plant parts *viz* leaves, stem, bark, roots, flowers and fruits **[5,6].** However, synthetic antioxidants such as butylated hydroxylanisole (BHA), propyl gallate (PG), butylated hydroxyl toluene (BHT) have been used to prevent oxidation but their use is currently challenging because of its carcinogenic effects. Hence, the natural antioxidants particularly polyphenols have been act as suitable antioxidants to replace synthetic ones and to inhibit the circulation of free radical reaction, to protect the human body from the diseases **[7,8,9]**.

The genus *Cordia macleodii*, an endangered medicinal plant belongs to family Boraginaceae, traditionally known as Dahipalas or Dahiman and it is distributed in moist-dry deciduas forest of central India. It is well known that the leaf and bark extracts of *C. macleodii* have various pharmaceutical activity such as hepatoprotective, acute toxicity, inflammatory, antioxidant, antibacterial, antifungal, wound healing, antivenom potential activity **[10,11].** In addition, screening of preliminary phytochemical analysis of leaf and bark has indicated the presence of several phytochemicals *viz* tannins, phenols, flavonoids, saponins, alkaloids and glycosides **[12,13]**. In present time, complete analysis of these plants provides a variety of bioactive molecules for development of newer pharmaceutical products. Recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine especially *Cordia macleodii* due to their healing properties. Till date no data available related to phytochemical study of adopted plant especially in Bilaspur region Chhattisgarh so far. Hence this study was carried out to develop standards methods of antioxidants activity of its bark as well as leaf, which will be lead to utilize by researchers for exploring the possibility for herbal medicine and drug discovery.

2. Materials and methods

2.1 *Collection of plants materials*

Plant part of *Cordia macleodii* for the proposed work was collected from Korba district, Chhattisgarh in the month of May, 2017. The plants were identified using the Flora of Madhya Pradesh; district Bilaspur **[14]**. The botanical identification was authenticated by Dr. A. K. Dixit. The herbarium specimen (GGV/BOT/T/BOR/DKS/129) was submitted at institute for future reference.

2.1.1 *Preparation of plant extracts*

Fresh and healthy plant materials were washed separately with tap water followed by distilled water to remove the dust on the surface and dried at room temperature to constant weight and pulverized into fine powder. Then the powdered of leaf and bark were separately extracted with methanol and distilled water in soxhlet apparatus. After that the extracts were filtered through Whattman no. 1 filter paper and at last the filtrate was concentrate under reduced pressure using a rotary evaporator then the dried extracts were kept at 4^0C for further study.

2.2 *Chemicals*

Folin Ciocalteu's phenol reagent, Gallic acid, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, quercetin, 2,2-diphenyl-1- picrylhydrazyl, ferrous sulphate, ferrozine, ferric chloride, phenanthroline, hydozen peroxide, potassium ferricyanide, trichloroacetic acid and ascorbic acid. All the chemicals used were of analytical grade.

2.3 *Phytochemical screening*

The crude methanolic and aqueous extracts of leaves and bark of *C. macleodii* were tested for the presence of its phytoconstituents such as sterols, alkaloid, flavonoid, phenolic, Reducing sugar, glycosides, tannins and saponins by using standard protocol of world health organization (WHO) guidelines **[15]**.

2.4 *In vitro antioxidant activity*

2.4.1 *DPPH radical scavenging activity*

Antioxidant activity of the extracts on the basis of the scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, was determined by the method of Braca et al. **[16]**. For this method, 0.1 mM solution of DPPH in methanol was prepared and it must be protected from light influence by maintaining the dark condition and 3 ml of this solution was added to 1ml various concentration of extracts or standard solution. Absorbance was taken after 30 minutes at 517 nm and percentage inhibition activity was calculated as:

$$Inhibition\ (\%) = [(A_0 - A_1) / A_0]_x\ 100$$

Where A_0 = Absorbance of the control, A_1 = Absorbance of extract or standard.

2.4.2 *Ferrous ion chelating activity*

The chelating of ferrous ions by leaf and bark extracts of *C. macleodii* was estimated by Dinis et al. **[17]**. The different concentrations of extracts were mixed with 100 µl of 2 mM ferrous sulphate solution and 300 µl of 5 mM ferrozine. Then the mixture was incubated at room temperature for 10 minutes. The absorbance of the solution was

measured at 562 nm and percentage inhibition was calculated by using this formula:

Inhibition (%) = [(A_0 - A_1) / A_0] x 100

Where, A_0 = Absorbance of the control, A_1 = Absorbance of extract or standard.

2.4.3 *Hydroxyl radical scavenging activity*

Scavenging activity of the extract on hydroxyl radical was measured by the method of Chung et al. **[18]**. In 1 ml of each diluted different plant extract 60 µl of $FeCl_3$ (1 mM), 90 µl of 1,10-phenanthroline (1 mM), 2.4 ml of 0.2 M phosphate buffer, pH 7.8 and 150 µL of H_2O_2 (0.17 mM) were added. After that mixture was incubated at room temperature for 5 minutes and absorbance was at 560 nm against blank. The percentage inhibition was calculated from the equation below:

Inhibition (%) = [(A_0 - A_1) / A_0] x 100

Where, A_0 = Absorbance of the control, A_1 = Absorbance of extract or standard.

2.4.4 *Determination of reducing power activity*

The reducing potential of extracts was determined by the method of Oyaizy **[19]**. Ascorbic acid and different concentrations of the extract were mixed in 1 ml distilled water with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 % w/v). After that the mixture was incubated at 50^0C for 20 minutes and a portion of 2.5 ml of trichloroacetic acid (10 %) was added to the mixture which was then centrifuged for 10 minutes at 4000 rpm. The upper layer of 2.5 ml of solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1 %) and absorbance was measured at 700 nm in a spectrophotometer. The percentage inhibition was calculated from the equation below:

Inhibition (%) = [(A_0 - A_1) / A_0] x 100

Where A_0 = Absorbance of the control, A_1 = Absorbance of extract or standard.

2.5 *Determination of total phenolic contents (TPC)*

Total phenolic content was estimated by Folin Ciocalteu's method of Miliauskas et al. **[20]**. The reaction mixture contained 1 ml of leaf and bark extract, 0.5 ml of Folin Ciocalteu's reagent, after 5 minutes, 1.5 ml of 20 % sodium carbonate and 10 ml of distilled water was mixed and the mixture was incubate for 2 hours at room temperature. After incubation intense blue colour was developed and absorbance was measured at 750 nm. Gallic acid is used as a standard to calculate the total phenolics contents and expressed as gallic acid equivalents (GAE) in mg gm^{-1}dry sample.

C = (c × V) / m

Where, C = Total content of phenolic compounds (GAE mg gm^{-1} of plant extract), c = concentration of gallic acid established from the calibration curve (mg ml^{-1}), V = The volume of extract in ml and m = The weight of crude plant extract in gm.

2.6 *Determination of total flavonoid content (TFC)*

Total flavonoid content was measured by the aluminium chloride colorimetric assay of Chang et al. **[21]**. The reaction mixture contains 1 ml of leaf and bark extract, 0.3 ml of sodium nitrite solution, 0.3 ml of 10 % aluminum chloride and 2 ml of 1 M solution hydroxide was added after 2 minutes. Finally, volume was making up to 10 ml with distilled water then shake well. Orange yellowish color was developed. The absorbance was measured at 510 nm and the calibration curve was plotted using standard quercetin. The data of total flavonoids were expressed as mg of quercetin equivalents, in mg gm^{-1} of dry mass.

C = (c × V) / m

Where, C = Total content of flavonoids (QUE mg gm^{-1} of plant extract), c = concentration of quercetin established from the calibration curve (mg ml^{-1}), V = The volume of extract in ml and m = The weight of crude plant extract in gm.

2.7 *Statistical analysis*

The statistical analyses were carried out using the statistical package for the social science, version 22 (SPSS 22, IBM, Chicago, USA). The data were subjected to one way analysis of variance (ANOVA) and the significance of different between means was determined by Posthoc Tukey test ($P < 0.05$) and the results are shown in the form of graphs and different alphabets demonstrate significant variance. Values expressed were means of three replicates determined ± standard deviation.

3. Results and Discussion

3.1 *General*

Several methods have been used to determine antioxidant activity of plants. For this purpose, qualitative phytochemical screening were performed and antioxidant activity was assessed using different tests *i.e.* DPPH radical scavenging activity, hydroxyl radical scavenging activity, Ion chelating activity respectively and also determined the Total phenolic content and total flavonoid contents of plants. The results obtained from each analysis are considered below.

3.2 *Phytochemical screening*

The phytochemical screening of crude methanolic and aqueous extracts of leaf and bark of *C. macleodii* revealed the presence of its phytoconstituents such as sterols, alkaloid, flavonoid, phenolic, Reducing sugar, glycosides, tannins and saponins **(Table 1).** The phytochemical isolated from the plant extract were known to have some medicinal properties. For example, saponins derived from the plants have been reported as antibacterial, wound healing **[22,23]**. Similarly, phenolic compound have been reported as a good antioxidants and alkaloid derived from *C. macleodii* has been also reported as antibacterial, cytotoxicity activity, acute toxicity and hepatoprotective activity **[24,25]**. The presence of flavonoids in plants might be responsible for the protective effect against snake venom poisoning though it's anti-inflammatory and antihistaminic activity **[26]**. In other study sterols derived from plants possess antibacterial properties **[27,28]**. These phytoconstituents present in leaf and bark extracts of *C. macleodii* may be responsible for the biological activities and also the reason of their use as a traditional medicine. Three flavonoids were isolated from the ethanolic extract of *C. macleodii* bark i.e. quercetin, kaempferol and apigenin using preparative thin layer chromatography **[29]**. Similarly, three phytosterols i.e. Stigmasterol, Cholest-5-EN -3OL (3-beta) -carbonyl chlorinated and Camphesterol were isolated from the petroleum ether extract of *C. macleodii* bark using chromatographic and spectrometric method **[30]**.

3.3 *DPPH radical scavenging activity*

A number of methods are used to determine the radical scavenging activity effects of antioxidants. The DPPH method is most preferred method because it is stable, easy, fast, reliable method and does not require any special reaction or devices. The free radical scavenging activities of the extracts depend on the capability of antioxidant compounds to lose hydrogen **[31,32]**. The DPPH can easily receive an electron and hydrogen from antioxidant molecule to become a stable diamagnetic molecule at 517nm wavelength. DPPH is considered to have a radical scavenging property due to its ability to bind with hydrogen **[33,34,35,36]**. A solution of DPPH prepared in methanol to convert it into DPPH-H (diphenylhydrazine) molecules in presence of an antioxidant agent. It is noticeable that the reduction capacity of DPPH was determined by the color decreasing effect which induced by antioxidants and absorbance also decreased with the colorless solution simultaneously. Hence, DPPH is used as a substance to evaluate the antioxidant activity. In general, the values of all tasted samples through DPPH radical scavenging activity were ranging from 63.7 - 87.5 % at a concentration of 1mg ml^{-1} in reaction mixture and all the activity is arranged in the following descending order ASC>ML>MB>AL>AB ($p < 0.05$). In this study, DPPH radical scavenging activity is highly related to the amount of phenolic compound that present in the extracts. Among all tested extracts methanol leaf extract exhibited the strongest, whilst aqueous bark extract showed the least DPPH scavenging radical activity (Fig. 1).

3.4 *Hydroxyl radical scavenging activity*

Hydroxyl radical is highly reactive biomolecules among all the oxygen radical. This type of activity was made by the donation of hydrogen by the hydroxyl substitution of phenolic compounds of plant extracts. Since phenolic compounds present in the extract are good electron donors because they may go faster the conversion of H_2O_2 into H_2O **[37,38]**. Different parts of plant extract showed good hydroxyl radical scavenging activities and ranging from 47.9 - 65.5 % at a concentration of 1mg ml^{-1} in reaction mixture. Each extracts showing hydroxyl radical-scavenging activity which was increased with increasing concentration of the extract sample. Methanol leaf extract had higher activity than the other extract but lower than ascorbic acid and the whole activity is arranged in following descending order ASC>ML>AL>MB>AB ($p < 0.05$) (Fig. 2).

3.5 *Ferrous ion chelating activity*

An antioxidant's ability to chelate Fe II and other element like copper is an important antioxidant to measure antioxidant activity. Ferrous ion chelating activity of an antioxidant is measured by the absorbance of iron II-ferrozine complex after the treatment of an iron II with the sample. Ferrozine, a ferroin compound is react with free Fe^{2+} and forms a red color iron II-ferrozine complex. The formation of complex is interrupt in the presence of chelating agent and the color of the complex is also decreased that indicates the presence of antioxidant chelators **[39,40]**. The values of all tasted samples threw ferrous ion chelating activity were ranging from 51.7 - 74.8 % at a concentration of 1mg ml^{-1} in reaction mixture and all the activity is arranged in the following descending order ASC>ML>MB>AL>AB (p < 0.05). Among all tested extracts methanol leaf extract exhibited the strongest, whilst aqueous bark extract showed the least ion chelating activity (Fig.3).

3.6 *Determination of reducing power activity*

The ability of a compound to donate an electron can be used to measure an antioxidant activity, is referred as its reducing capacity. The reducing capacity of crude extract can be measured by the direct reduction of $Fe^{3+}(CN)^6$ to $Fe^{2+(}CN)^6$. Addition of free Fe^{3+} to the reaction mixture leads to the formation of the intense blue color complex. An increase in absorbance of the reaction mixture would indicate an increase in reducing capacity due to an increase in the formation of the complex **[41,42]**. Reducing power activity of different plant extract had reducing power but not at same level and the activity is ranging from 42.24 - 62.65 % respectively at a concentration of 1mg ml^{-1} in reaction mixture. Results clearly indicate that methanol leaf extract had highest reducing power and the lowest was obtained in aqueous bark extract. From this point of view it was confirmed that the methanol leaf extract possessed the potent antioxidant compounds. The reducing power activity is arranged in the following descending order ASC>ML>AL>MB>AB (p < 0.05) respectively (Fig. 4).

3.7 *Total phenolic and flavonoid content*

Table 2 represent the total phenolic and flavonoid content of the methanol and aqueous extracts of leaf and bark of *Cordia macleodii*. The total phenolic content of different extracts was determined by a linear gallic acid standard curve (R^2 = 0.978). The highest content of TPC was determined in methanol bark extract 477.32 ± 0.24 µg GAE gm^{-1}; whereas the lowest content of TPC was measured in aqueous leaf extract 155.99 ± 3.32 µg GAE gm^{-1}. TPC of different extract is arranged in the following descending order MBE>MLE>ABE>ALE respectively (p < 0.05). Numerous studies have revealed that the phenolic contents are associated with their antioxidant activities, most likely due to their redox properties, which allow them to act as singlet oxygen quenchers, hydrogen donors and reducing agents **[43,44,45]**. Plants have diverse groups of phenolic compounds such as simple phenolics, phenolic acids, anthocyanins and flavonoids. All these phenolic classes have gained extensive attention because of their physiological functions including free radical scavenging, anti-inflammatory effects, anti-mutagenic and anti-carcinogenic **[46,47,48]**.

Similarly, total flavonoid content (TFC) of different extracts of leaf and bark showed that highest TFC was observed in methanol leaf extract *i.e.* 293.73 ± 1.57 µg QCE gm^{-1} of dry sample, whereas the lowest was observed in aqueous bark extract *i.e*. 41.51 ± 0.47 µg QCE gm^{-1}, of dry sample. TFC of extracts is arranged in following sequences MBE>MLE>ALE>ABE respectively (p < 0.05). Our results also support the previous work and showed the high phenolics content as well as flavonoid content. Flavonoids are the widely distributed plant phenolic compound, which are usually very important natural antioxidant **[49,50]**.

Statistical correlations have been studied between total phenolic content and antioxidant activity determined by various assays which are shown in **Table 3**. Total phenolic content was highest association with reducing power assay in the present study (R^2 = 0.988). A strong positive correlation was observed between TPC and reducing power activity which was supported by **[51]**. Similar results were also observed for the ion chelating activity (R^2 = 0.942) and DPPH (R^2 = 0.948). In previous work a significant correlation between total phenolic content and DPPH scavenging activity was found **[52,53]** and also a close relationship between TPC and hydroxyl radical scavenging assay was found **[54]**. However, in our study there was no significant correlation

found between TPC and hydroxyl radical scavenging assay.

When the total phenolic and flavonoid contents of *Cordia macleodii* is compared with available data of the same species and other member of the same family, it is clearly shown that *C. macleodii* plant extracts have phenolic and flavonoid contents which are shown in **Table 4.** Polyphenols of plants are major group of compound acting as free radical scavenger or antioxidants; therefore it is valid to determine phenolic content in different extracts of plant.

4. IC50 value of different antioxidant

Table 5 depicted that the IC50 of the different antioxidant activity and result revealed that the IC_{50} values of all four extracts were also determined and the effective IC_{50} value for DPPH radical-scavenging activity (7.63 µg ml^{-1}), hydroxyl radical-scavenging activity (7.81 µg ml^{-1}), Ion chelating activity (8.13 µg ml^{-1}) and reducing power activity (6.74 µg ml^{-1}) were observed. Our results also showed significant correlation along with ascorbic acid as a standard. On the basis of our findings leaf was showed effective antioxidant activity as compared to the bark of *Cordia macleodii*.

Table 1: Phytochemical screening of leaf and bark extracts of *Cordia macleodii.*

S.No.	Tested for	Leaf		Bark	
		MLE	ALE	MBE	ABE
1.	Phenolics	+	+	+	+
2.	Saponins	-	-	-	+
3.	Flavonoids	+	+	+	+
4.	Tannins	-	-	-	+
5.	Reducing sugar	+	-	+	+
6.	Glycosides	+	-	+	-
7.	Alkaloids	+	+	-	+
8.	Phytosterols	-	+	+	+

MLE = Methanol leaf extract, ALE = Aqueous leaf extract, MBE = Methanol bark extract, and ABE = Aqueous bark extract.

Table 2: Total phenolic content (TPC) and Total flavonoid content (TFC) of extracts

Extracts	Total phenolic content in µg GAE/g extract	Total phenolic content in µg QUE/g extract
MLE	324.99 ± 7.65^{a}	293.73 ± 1.57^{a}
ALE	155.99 ± 3.32^{b}	69.30 ± 3.34^{b}
MBE	477.32 ± 0.24^{c}	180.52 ± 4.26^{c}
ABE	238.56 ± 1.58^{d}	41.51 ± 0.47^{d}

Where MLE = methanol leaf extract, ALE = Aqueous leaf extract, MBE = Methanol bark extract, ABE = Aqueous bark extract respectively (n = 3). Values with different superscript letters within the same column are statistically different ($p < 0.05$).

Table 3: Correlation between total phenol content (TPC) and antioxidant activity determined by different assays.

	TPC	DPPH	Hydroxyl	Ion chelating	Reducing
TPC	1				
DPPH	0.256	1			
Hydroxyl	0.184	0.933	1		
Ion chelating	0.493	0.876	0.942*	1	
Reducing	0.157	0.948*	0.988**	0.926	1

* Significantly correlated at $p < 0.05$, $n = 4$; ** significantly correlated at $p < 0.01$, $n = 4$.

Table 4: Comparison of total phenolic and flavonoid contents with other published data of some Species of *Cordia* plant.

S.N.	Plants	Plant part	Extracting solvent	TPC (mg/g)	TFC (mg/g)	References
1.	*Cordia macleodii* Hook F. Thomson	Bark, Leaf	MtOH	1.65 ± 0.12	0.98 ± 0.09	**[28]**
			MtOH	0.65 ± 0.05	1.98 ± 0.11	
2.	*Cordia retusa* Vahl. masamune	Aerial part	MtOH	1.86±0.65	2.71±0.75	**[37]**
3.	*Cordia monoica* Roxb.	Leaf	EtOH	71.33±5.01	1.65±0.11	**[55]**
			EtAc	22.07±1.39	1.11±0.05	
4.	*Cordia multispicata* Cham.	Leaf	Acetone	2.31±0.07	ND	**[56]**
5.	*Cordia verbanaceae* L.	Leaf	EtAc	72 ± 2	ND	**[57]**
			EtOH	97 ± 3	ND	
			Acetone	82 ± 2	ND	
			Aqueous	111 ± 3	ND	
			Dichloro-methane	82 ± 3	ND	
			Hexane	42 ± 2	ND	
				In µg/g		
6.	*Cordia macleodii* Hook F. Thomson	Bark	MtOH	324.99 ± 7.65	293.73 ± 1.57	Present study
			Aqueous	155.99 ± 3.32	69.30 ± 3.34	
		Leaf	MtOH	477.32 ± 0.24	180.52 ± 4.26	
			Aqueous	238.56 ± 1.58	41.51 ± 0.47	

TPC = Total phenolic content, TFC = Total flavonoid content, EtOH = Ethanol, MtOH = Methanol, EtAC = Ethyl acetate and ND = Not detected.

Table 5: IC50 values of different antioxidant activity (µg ml^{-1}).

Plant extract	DPPH	HYDROXYL	ION	REDUCING
MLE	7.63 ± 0.38	16.9 ± 0.85	8.99 ± 0.45	8.8 ± 2.24
ALE	16.48 ± 0.82	20.46 ± 1.02	37.5 ± 2.88	6.74 ± 0.33
MBE	9.81 ± 0.49	28.35 ± 2.42	28.65 ± 1.93	14.42 ± 0.72
ABE	12.73 ± 0.64	7.81 ± 0.39	8.13 ± 0.41	18.3 ± 1.61
STD	7.58 ± 0.37	7.15 ± 0.35	6.76 ± 0.33	5.12 ± 0.25

Where MLE = methanol leaf extract, ALE = Aqueous leaf extract, MBE = Methanol bark extract, ABE = Aqueous bark extract and STD = Standard respectively.

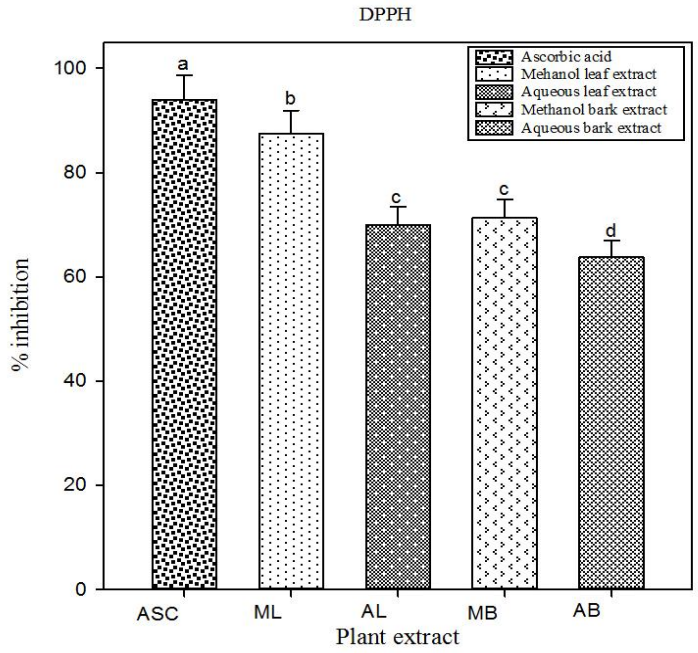

Fig. 1: DPPH free radical scavenging activity of methanol and aqueous extract of *Cordia macleodii* leaf and bark. Values are means ± standard deviation (n = 3). For each column values with different superscript letters are statistically different ($p < 0.05$).

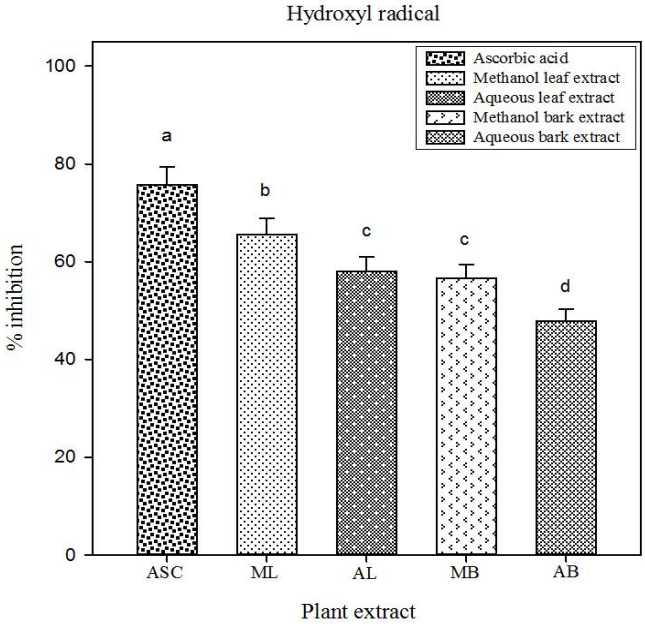

Fig. 2: Hydroxyl radical scavenging activity of methanol and aqueous extract of *Cordia macleodii* leaf and bark. Values are means ± standard deviation (n = 3). For each column values with different superscript letters are statistically different ($p < 0.05$).

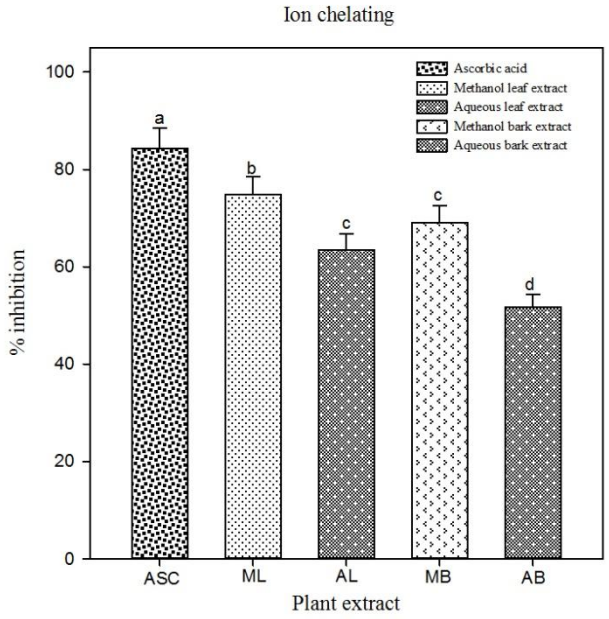

Fig. 3: Ion chelating activity of methanol and aqueous extract of *Cordia macleodii* leaf and bark. Values are means ± standard deviation (n = 3). For each column values with different superscript letters are statistically different ($p < 0.05$).

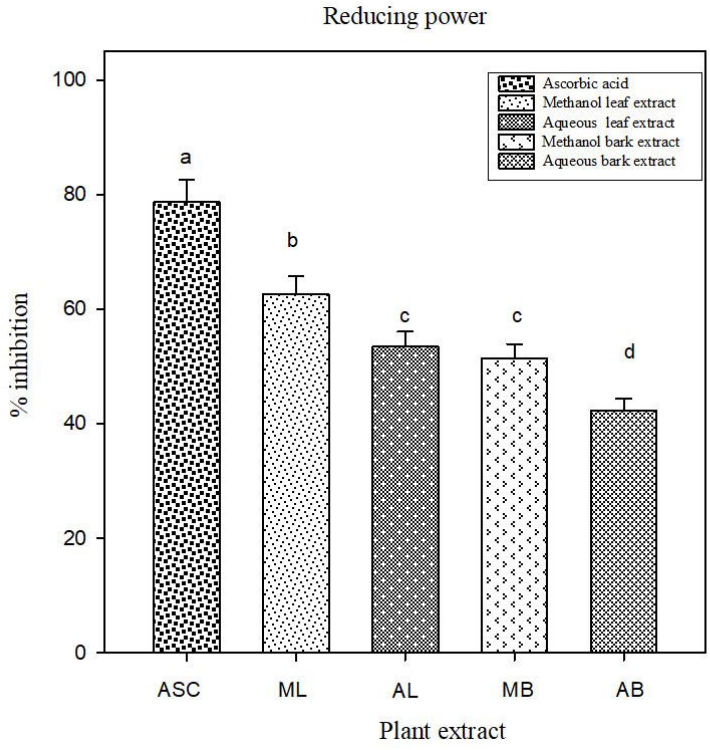

Fig. 4: Reducing power activity of methanol and aqueous extract of *Cordia macleodii* leaf and bark. Values are means ± standard deviation (n = 3). For each column values with different superscript letters are statistically different ($p < 0.05$).

5. Conclusion

In conclusion, result obtained in present study has shown that the medicinal properties of *C. macleodii* might be due to the different phytoconstituents and some phenolic compounds. The results also confined that the phenolic content existing in *C. macleodii* leaf and bark play an important role in the antioxidant activity through the free radical scavenging activity with mutual action of ion chelating activity and reducing activity mechanism. This suggested that the plant is a good source of natural antioxidants and contributes scientifically to recognizing the importance of *C. macleodii* as a medicinal healer and explore the new findings of pharmacology investigation related to endangered *Cordia macleodii* plants.

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