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Antioxidant potential of crude extract, flavonoid-rich fractions, and a new compound from the seeds of *Cordia dichotoma*

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The current study assessed the antioxidant activity of methanolic extract and different fractions of the seeds of *Cordia dichotoma* by 2,2-diphenyl-2-picrylhydrazyl hydrate method. Phytochemical screening of *C. dichotoma* seed extract was done using thin-layer chromatography technique and phytochemical methods. The percentage yield of secondary metabolites like alkaloids and saponins was also determined. The methanolic extract was subjected to isolation by Column Chromatography. Phytochemical screening revealed the presence of significant amounts of phenols and flavonoids in the extract. TLC analysis confirmed the presence of phytoconstituents with the application of derivatizing agents like aluminium chloride and anisaldehyde. Total phenolic and flavonoid contents obtained were 37.7 and 32.16% w/w, respectively. The crude seed extract of *C. dichotoma* showed inhibition at all concentrations in a dose-dependent manner. Maximum scavenging activity was exhibited by the methanolic extract with a low IC_{50} value. A new compound named Cordioside was also isolated from the same extract. The phytochemical screening of the seed extract showed the presence of rich amounts of phenolic compounds and flavonoids, which may be acting as the key factors responsible for the antioxidant activity. The results revealed that methanolic extract and the aqueous fraction of *C. dichotoma* seed possess a significant antioxidant activity.

Keywords: Antioxidant activity, *Cordia dichotoma*, DPPH, Flavonoids, Phytochemical, Thin-layer chromatography.

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Introduction

Antioxidants are important not only for health reasons but also play a crucial role in the food, cosmetics, and chemical industries. Free radicals produced in our body under various circumstances are responsible for degeneration and disorders in the body[1]. Hence, these antioxidants are an essential part of the medicinal system that acts adjunctly with other therapeutic agents in the management of various ailments. Antioxidants are available in synthetic and natural forms and are in use for many decades. Sati *et. al.*, have reported data of traditionally used medicinal plants possessing significant antioxidant activity[2]. Although synthetic ones are cheap, readily available, and more competent, their safety is disputed. Research findings revealing the toxicity and carcinogenic potential of synthetic antioxidants in animals have led to an increased interest in natural antioxidants[3]. Natural antioxidants are beneficial for our health that act by scavenging the free radicals after intake without having unfriendly effects on the body. Hence, they can be a safe replacement for toxic and harmful synthetic antioxidants. It would be valuable to create awareness among the consumers about natural sources of antioxidants and their capability to recognize synthetic antioxidants in packaged foods[4]. Plants produce secondary metabolites in response to some circumstances that have proven health benefits for humans. These secondary metabolites belong to different classes of phytochemicals like flavonoids, phenols, condensed and hydrolyzable tannins, and carotenoids, all having various pharmacological actions along with potent antioxidant activity[5]. *Cordia dichotoma* (Boraginaceae) also known as Indian cherry or Manjack is commonly used in Ayurvedic medicine, the Unani system, and folk remedies. It grows in

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forests and gardens bearing fragrant flowers and small edible fruits[6,7]. The seeds of this plant possess a wide range of phytochemicals, like α-amyrins, betulin, lupeol-3-rhamnoside, β-sitosterol, taxifolin-3, 5-dirhamnoside, and hesperitin-7-rhamnoside and are reported to have anti-inflammatory activity[8]. Therefore, the present investigation was undertaken to determine the amount of total phenolic and flavonoid content and evaluate the *in vitro* antioxidant activity of the crude methanol extract of *C. dichotoma* and its different fractions through free radical scavenging assay.

Materials and Methods

Plant sample collection and identification

The dried seeds of *C. dichotoma* were collected from a local retailer in Aminabad, Lucknow, Uttar Pradesh, India. For identification and taxonomic authentication, a sample was given to the National Botanical Research Institute (NBRI), Lucknow, India, which confirmed the authenticity of the plant specimen (voucher specimen no. NBRI/263/2011).

Extraction and fractionation

The dried seed powder was extracted with methanol in a Soxhlet apparatus for 24 h. The extract was concentrated under reduced pressure by a rotary vacuum evaporator to obtain a sticky residue. The crude methanolic extract, after removal of the solvent, was dissolved in 10% sulfuric acid. This extract was then fractionated by column chromatography into three flavonoid-rich fractions, namely chloroform, ethyl acetate, and residual water. The methanolic extract and its fractions were screened for antioxidant activity.

Qualitative phytochemical screening

Phytoconstituents were determined qualitatively using different chemical methods and thin-layer chromatography (TLC) analysis[9].

Phytochemical tests

The methanolic extract of *C. dichotoma* and chloroform, ethyl acetate, and aqueous fractions were qualitatively evaluated to detect the presence of different phytochemicals like alkaloids, flavonoids, saponins, and phenols by the standard procedure[10].

Thin-layer chromatography

TLC of various extracts of *C. dichotoma* seeds was performed using three different solvent systems, Chloroform: methanol (9:1), Benzene: acetone (2:1), and Chloroform: ethylamine benzene to detect flavone, anthocyanidin flavonols, and phenolic acids respectively. The plate was developed and observed under a UV lamp with a 366 nm filter. Several fluorescent bands were observed on the TLC on visualization under UV 366 nm. The plates were sprayed with 1% ethanolic solution of aluminium chloride to observe the spots and those sprayed with anisaldehyde reagent were heated at 110 °C until spots were visible. Every spot with a specific R_f on the TLC plate indicates the presence of a specific class of compound, as per the method described by Markham[11].

Quantitative determination of total phenolic and flavonoid contents

Total soluble phenolics in different extractives of seeds of *C. dichotoma* were determined spectrophotometrically by Folin-Ciocalteu reagent using gallic acid as a standard[12]. An aliquot (1 mL containing 10 mg of seed powder) of the extract in a 25 mL volumetric flask, was diluted with 9 mL of distilled water. About 1 mL Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 minutes, 3 mL of sodium carbonate was added and made up to the mark. A reagent blank was prepared using distilled water. The mixture was allowed to stand in dark for 1 h with intermittent shaking and absorbance was measured at 760 nm. The phenolic content was calculated from the calibration curve prepared by repeating the operation using 1 mL gallic acid solutions at different concentrations (25, 50, 100, 200, 300, and 500 µg/mL). The total flavonoid content was estimated by the aluminium chloride colourimetric method using rutin as a standard[13]. An aliquot (1 mL containing 10 mg/mL) of the extract was added in a 10 mL volumetric flask containing 4 mL distilled water and mixed with 0.3 mL of 5% sodium nitrite. After 5 minutes, 0.3 mL of 10% aluminium chloride was added. At the sixth minute, 2 mL of 1 M-sodium hydroxide (NaOH) was added, the volume was made up to 10 mL with distilled water, and the content of the flask was mixed thoroughly. Reagent blank using distilled water was prepared and the absorbance of the reaction mixture was measured against the blank mixture at 510 nm. The calibration curve was prepared by measuring the absorbance of the routine standard solutions at different concentrations (25, 50, 100, 200, 300, and 500 µg/mL) in distilled water. All determinations were performed in triplicate.

Percentage yield of phytoconstituents

Standard procedures were used to screen the phytochemicals (qualitatively and quantitatively). The

phytochemical tests as per Edeoga *et al.* were carried out using aqueous specimens[14].

Alkaloid was extracted from 5 g of the powdered and weighed sample. The powdered sample was dissolved in 100 mL of 10% acetic acid in ethanol in a 250 mL beaker. The mixture was covered and allowed to stand for 4 h. It was then filtered and the filtrate was concentrated on a water bath to one-fourth of its original volume. Thereafter, concentrated ammonium hydroxide was added dropwise until precipitation was completed. The solution was then allowed to settle, and this precipitate of alkaloid was collected, washed with dilute ammonium hydroxide, and filtered.

Phenols were quantified from 1 g of seed sample and defatted with 100 mL of diethyl ether using a Soxhlet apparatus for 2 h. The fat-free sample was boiled with 50 mL of ether for 15 minutes to extract the phenolic components. Further, 5 mL of the ether extract was transferred to a 50 mL volumetric flask, followed by the addition of 10 mL distilled water, 2 mL ammonium hydroxide, and 5 mL concentrated amyl alcohol. The sample volume was made up to the mark using distilled water and kept aside for 30 minutes. The colour produced was measured spectrophotometrically at 505 nm.

Flavonoids were determined by the methods developed by Boham and Kocipai-Abyazani[15]. About 10 g of the plant sample was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The hydro-alcoholic extract was subjected to filtration and concentrated on a water bath to remove all the solvent and to get the flavonoid sample.

Saponins were determined according to the method described by Obadoni and Ochuko[16]. According to this method, 10 g of the powdered sample was transferred into a conical flask, to which 50 mL of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 h while stirring continuously at 55 °C. Thereafter, the mixture was filtered and the residue was re-extracted with another 100 mL of 20% ethanol.

The combined extracts were concentrated to 40 mL over a water bath at about 90 °C, poured into a 250 mL separatory funnel, and partitioned with 10 mL diethyl ether. The aqueous layer was recovered while the ether layer was discarded. This process was repeated by adding 30 mL n-butanol. The combined n-butanol extract was washed twice with 5 mL of 5% aqueous sodium chloride. The remaining solution was then heated on a water bath. After evaporation, the samples were dried in a hot air oven at 70°C to a constant weight.

Isolation of phytoconstituents[17,18]

Apparatus, chemicals and instrument

Silica gel (Qualigens, Mumbai, India) was used for column chromatography. Silica gel G (Qualigens) was used for analytical TLC. Spots were visualized in presence of iodine vapours, UV Lamp 254 nm and by spraying with anisaldehyde sulphuric acid reagents. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., MO, USA. The solvents for isolation were obtained from Merck Mumbai, India.

UV spectra were scanned in methanol on Lambda Bio 20 Spectrophotometer, Shimadzu, Singapore. I.R spectra were recorded in KBr pellets on a Win IR FTS 135 instrument (Biorad, USA). 1H NMR (300 MHz) and 13C NMR (75 MHz) spectra were recorded on a Brucker Spectrometer (Brucker, USA) in CDCl3 with TMS as internal standard. The MS was measured in DART dried Helium was used for ionization mode with a JEOL-Accu TOF JMS-T100LC.

Preparation of extract

The seeds were coarsely powdered and extracted with methanol separately by using the Soxhlet apparatus. The methanolic extracts were then concentrated on the water bath and dried under reduced pressure to get a dark brown mass.

Preparation of slurry

The concentrated extract of the drug was taken in a China dish and heated continuously on a water bath by gradually adding methanol in small portions with stirring, till required consistency was obtained. A weighed quantity of silica gel for column chromatography was then added slowly with continuous mixing with a steel spatula until the whole methanolic solution of plant extracts was adsorbed on silica gel. It was dried and the larger aggregates were broken by rubbing between hands and finally passed through a sieve (No. 8) to get a uniform particle size.

Packing of column and Isolation of phytoconstituents

A column of 1.5 feet, height and 16 mm internal diameter was taken, cleaned properly and dried. The lower end of the column was plugged with non-absorbent cotton wool. The column was clamped and fitted in a vertical position on a stand. The column was then half-filled with petroleum ether (B.P. 60-80 ºC). Silica gel (for column, 60/120 mesh) was then poured in small portions and allowed to settle down and the dried plant extract slurry was loaded over the column and then eluted successively with different solvents, in their order of increasing polarity. The

developments and elution of the column were carried out with successive series of different solvents in various combinations such as pet. ether (100), pet. ether: chloroform (75:25, 50:50, 25:75), chloroform (100), chloroform: methanol (75:25, 50:50, 25:75) and Ethyl acetate: Methanol (99:1, 98:2, 97:3, 95:5, 90:10, 80:20) to isolate the possible compounds[19].

Homogeneity of the fractions

The fractions collected were subjected to TLC to check the homogeneity of various fractions. Fractions were subjected to TLC using TLC plates cut to about 6 cm in length and 20 cm in width. Fractions were loaded on TLC plates in a sequence as fine spots using TLC spotters on a line drawn 2.5 cm above the bottom. The mobile phase was adjusted and TLC plates were developed under UV light at 254 nm. The identical fractions having the same R_f values were combined and concentrated. They were then recrystallized with solvent system[20-25].

Isolation of phytoconstituents

The methanolic extract was subjected to column chromatography on silica gel using solvents of increasing polarities from petroleum ether, chloroform, ethyl acetate and methanol in different ratios to yield several sub-fractions. The fractions were eluted using chloroform and ethyl acetate in different ratios.

Fractions of 10 mL each were collected. A new compound was isolated from fractions 72-80 of chloroform- ethyl acetate (6:4) which were pooled due to their similar TLC pattern and showed an Rf value of 0.51. The fractions were eluted using the chloroform and ethyl acetate in different ratios. Fractions of 10 mL each were collected. This was then allowed to stand overnight. Pale colour crystals were obtained. This was further purified and recrystallized by dissolving the compound in hot water. The crystals were then filtered, collected and dried to yield 105 mg of the new compound (Cordioside).

DPPH antioxidant assay

The radical scavenging activity was determined by the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) method[26]. The DPPH molecule is a stable, free radical due to the delocalization of the spare electron over the molecule; this delocalization produces a deep violet colour, characterized by an absorption band in ethanol or methanol solution centred at about 517 nm.

Statistical analysis

Data of cell inhibition was expressed as mean±SD from three independent experiments. One-way ANOVA and Dunnett's multiple comparison tests were performed using GraphPad Prism (Version 5.01) software. Data were considered significant if the *P*-value was ≤ 0.05.

Results

Phytochemical screening

Phytochemical analysis of extracts and other fractions was done using TLC and qualitative tests were conducted for different phytoconstituents. Table 1 summarizes TLC analysis data of methanol, chloroform, and petroleum ether extracts using different solvent systems and derivatizing agents that indicate the presence of flavones, anthocyanin, and phenolic compounds. Depending upon the R_f value and colour change after derivatization with aluminium chloride and anisaldehyde reagent, the presence of flavones, anthocyanin, and phenolic compounds was inferred. Table 2 shows the observations of qualitative phytochemical analysis of a methanolic extract of *C. dichotoma* seeds and organic solvent fractions, such as chloroform, ethyl acetate, and aqueous fraction. The seed extract and different fractions show

Table 1 — TLC analysis of *C. dichotoma* extract

Extract	Solvent system	UV (366 nm) (R_f)	Aluminium chloride (R_f)	Anisaldehyde/H_2SO_4 (R_f)	Possible presence of Phytochemicals
Methanol	Chloroform: methanol (9:1)	0.90 (red), 0.72 (blue) 0.37 (red)	0.90, 0.84, 0.72 (yellow)	0.54, 0.31, 0.87, 0.96	Flavones
Chloroform	Benzene: acetone (2:1)	0.63 (yellow) 0.84 (blue) 0.33 (yellow)	0.63, 0.26 (yellow)	0.76,0.27, 0.42,0.57, 0.65,0.94	Anthocyanidin Flavonols
Petroleum ether	Chloroform: diethylamine benzene (2:2:6)	0.70 (blue) 0.91 (blue) 0.84 (blue)	0.70,0.24 (yellow)	0.44, 0.68, 0.77, 0.88	Phenolic acids

R_f- Retention factor

the presence of alkaloids, flavonoids, saponins, and tannins. Besides, a compound named Cordioside was also isolated (Fig. 1 and 2).

Quantitative determination of TPC and TFC

The total flavonoid content of *C. dichotoma* seeds was found to be 32.16±0.208%, and the total phenolic content was 37.7±0.057% (Table 3). This experiment was done in triplicate and the data were presented as mean±standard deviation.

The percentage yield of phytoconstituents

The extraction process was followed by an evaluation of the percentage yield of phyto-constituents like alkaloids, flavonoids, saponins, and phenols. The methanolic extract of seed powder exhibited a high quantity of flavonoids and phenolic compounds. The seed extract was found to have flavonoid content of 0.15 g/g and phenolic content of 0.17 g/g (Table 4).

Antioxidant activity of methanolic extract and fractions of *C. dichotoma* by DPPH assay

The crude seed extract of *C. dichotoma* showed inhibition at all concentrations in a dose-dependent manner with a nominal amount of 66.1% inhibition at a concentration of 10 µg/mL and a marked inhibition of 93.1% at a concentration of 100 µg/mL. A marked decrease in percentage was found at higher concentrations. The chloroform fraction of *C. dichotoma* showed inhibition at all concentrations in a dose-dependent manner with a nominal amount of 42.1% inhibition at a concentration of 10 µg/mL and marked percentage inhibition of 77.5% at a concentration of 100 µg/mL. A marked decrease in percentage inhibition was seen at higher concentrations (between 50-100 µg/mL). The ethyl acetate fraction of *C. dichotoma* showed inhibition at all concentrations in a dose-dependent manner with a nominal amount of 57.3% inhibition at a concentration of 25 µg/mL and marked percentage inhibition of 73% at a concentration of 100 µg/mL. A marked decrease in percentage inhibition was seen at higher concentrations (between 50-100 µg/mL). The aqueous fraction of *C. dichotoma* showed inhibition at all concentrations in a dose-dependent manner with a nominal amount of 61.2% percentage inhibition at a concentration of 10 µg/mL and marked percentage inhibition of 78% at a concentration of 100 µg/mL. A marked decrease in percentage inhibition was seen at higher concentrations (between 50-100 µg/mL) (Fig. 3 and 4).

DPPH assay in terms of IC_{50} (µg/mL) value of methanolic extract and different fractions of

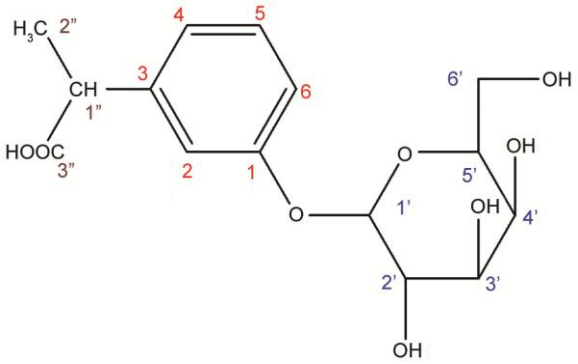

Fig. 1 — Cordioside: 3-Isopropanoic acid phenyl –α-D-glucopyranoside.

Table 2 — Qualitative phytochemical analysis of the methanolic extract and solvent fractions of *C. dichotoma*

Phytoconstituent	Crude methanol extract	Solvent fractions		
		Chloroform	Ethyl acetate	Aqueous
Alkaloid	++	+	+	++
Flavonoid	+++	++	++	++
Saponins	++	+	+	+
Tannins	++	+	+	+

+++= highly present; ++= moderately present; += low; -= absence of constituents.

Table 3 — Phenolic and flavonoid content of dried seeds of *C. dichotoma*

Phytochemical	Amount of drug taken (g)	Amount of product obtained (g)	Total content (% w/w)
TPC	1	0.377	37.7±0.057
TFC	10	0.321	32.2±0.208

TFC, total flavonoid content; TPC, total phenolic content. Total content is presented as mean±SD.

Table 4 — Percentage yield of phytoconstituents in *C. dichotoma* seeds

Plant	Alkaloid	Flavonoid	Saponins	Phenols
Cordia dichotoma (g/g)	0.045±0.01	0.15±0.01	0.15±0.01	0.17±0.01

Data presented as mean±SD.

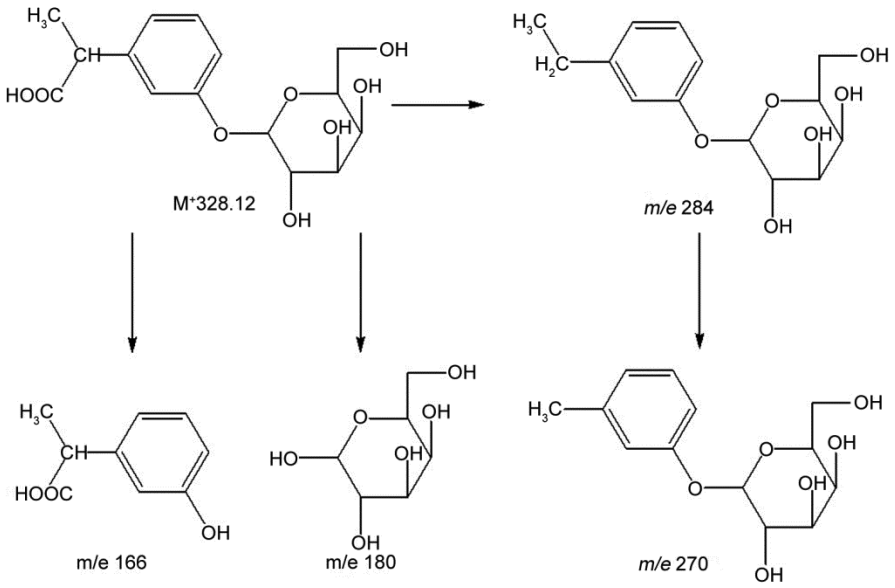

Fig. 2 — Mass fragmentation pattern of the isolated compound- Cordioside.

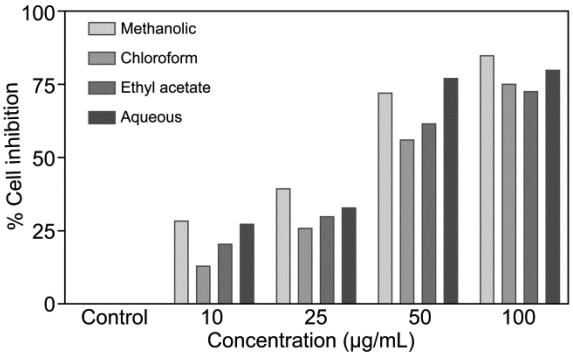

Fig. 3 — DPPH assay of *Cordia dichotoma* methanolic extract and its fractions.

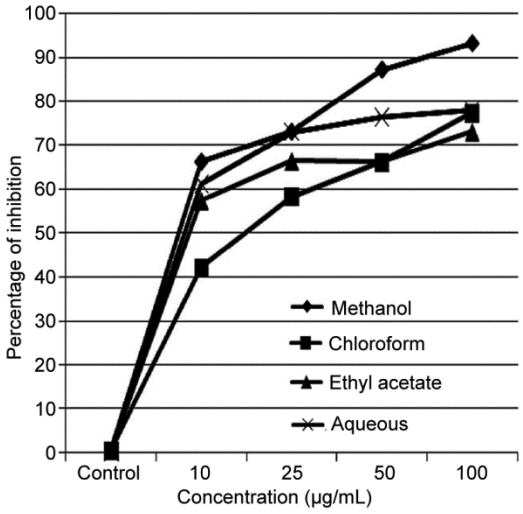

Fig. 4 — Percentage of inhibition of extracts of *Cordia dichotoma* by DPPH assay.

C. dichotoma seeds in comparison to ascorbic acid is represented in Fig. 1. Maximum scavenging activity was exhibited by methanolic extract with IC_{50} (5.5 µg/mL). Aqueous and ethyl acetate fractions showed lower IC_{50} values (6.31 and 7.24 µg/mL) than ascorbic acid (12.40 µg/mL), thus demonstrating higher scavenging activity.

Discussion

The present study was undertaken to evaluate the antioxidant potential of crude methanolic extract and flavonoid rich fractions of the *C. dichotoma* seeds. The presence of different flavonoids and phenolic acids in *C. dichotoma* plant exhibiting antioxidants properties have been reported in the literature. Thus, *C. dichotoma* seed extract and aqueous and ethyl acetate fractions exhibit virtuous free radical scavenging activity with a low IC_{50} value that signifies better antioxidant activity.

Primary phytochemical screening of *C. dichotoma* seed powder was done using the TLC technique and chemical methods. The TLC of different solvent extracts of *C. dichotoma* seeds reveals the presence of flavones, flavonols, and phenolic acids. The presence of significant content of flavonoids and phenolic compounds along with other secondary metabolites such as alkaloids and saponins was confirmed by phytochemical methods and found to be in accordance with earlier reported data[27]. In literature, the rich content of phenols and flavonoid compounds in leaves, bark, fruits, and seeds have been reported that

are responsible for various pharmacological actions including antioxidant activity[28-30]. In the present study, flavonoids were found to be present in higher amounts in the methanolic extract and moderately in chloroform, ethyl acetate, and aqueous fractions. Phenolic compounds were present in good amounts in the methanolic extract, but in lower amounts in other fractions indicating methanol to be a very good solvent for extraction of a majority of the secondary metabolites. The seeds were found to be rich in flavonoids and phenolic compounds as indicated by TPC (37.7%) and TFC (32.2%).

Plants and botanicals are very good sources of natural antioxidants and are being investigated by many researchers for the presence of antioxidant activities. Singh *et al.* have assessed the antioxidant activity of methanolic extract of *C. dichotoma* leaves and seeds and found it to be a good source of natural antioxidants[31]. In the present study, similar results were obtained with methanolic seed extract and in addition, the chloroform, ethyl acetate, and aqueous fractions were evaluated for antioxidant activity.

There are various *in vitro* assays to assess antioxidant activities of different extracts and isolated phytoconstituents. DPPH antioxidant assay is a very common and convenient method developed to determine the antioxidant potential in terms of IC_{50} values of the drugs to be studied. In the current study, the IC_{50} value of methanolic extract (5.50 µg/mL**)**, aqueous (6.31 µg/mL), and ethyl acetate fractions (7.24 µg/mL**)** were less than that of ascorbic acid (12.40 µg/mL**)**. The IC_{50} values of the above-mentioned samples were less than 10 µg/mL thus implying higher radical scavenging activity than ascorbic acid.

Compound Cordioside, named 3-Isopropanoic acid phenyl-α-D-glucopyranoside, was obtained as a yellow crystalline mass, from Chloroform: Ethyl acetate eluants, Rf (0.51) (6:4). It gave effervescence with sodium bicarbonate solution indicating the presence of the carboxylic group in the molecule. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3408 cm^{-1}) and carboxylic function (3271, 1690 cm-1). Based on ESI mass spectra, the molecular weight of A was established at m/z 313, which corresponded to the molecular formula of a phenolic glycoside, $C_{15}H_{20}O_8$. The 1H NMR spectrum of the compound exhibited a one-proton doublet at 6.76 (J = 8.0 Hz) and 7.44 (J = 6.8 Hz) assigned to aromatic H-4 and H-6 proton respectively. One-proton multiplet at 6.29 and one-proton broad singlet as was ascribed to aromatic H-5 and aromatic H-2 respectively. The one-proton doublet appeared at δ 5.26 (J = 4.8 Hz) assigned to the sugar H_1' proton. The two-proton double doublet appeared at δ 3.01 (J = 4.8 Hz) assigned to H-6' methylene proton. The one-proton multiplet appeared at δ 3.30, 3.20, 3.18, and 3.63 assigned to sugar H-2', H-3', H-4' and H-5' proton respectively. A one-proton multiplet at δ 2.41, a three-proton doublet at 0.90 (J = 5.6 Hz), and a one-proton broad singlet at δ 12.50 were accounted to CH proton, methyl proton, and COOH of isopropanoic acid respectively. The 13C NMR spectrum of cordioside showed the presence of fifteen carbon signals and the important signals appeared for carboxylic carbon at δ 177.70 (C-3″), methyl carbons at δ 17.35 (C-2″), methylene carbon at δ 37.17 (C-1″), anomeric carbons at δ 101.13 (C-1′), other sugar carbons from δ 71.66 to 76.38, aromatic carbons in the range of δ 115.18-156.37, and hydroxymethylene carbons at δ 70.34 (C-6'). Based on spectral data analysis and chemical reactions, the structure of Cordioside has been determined as 3-Isopropanoic acid phenyl-α-D-glucopyranoside.

Based on the previous data, it can be predicted that the powerful antioxidant activity of polar extracts is due to the presence of compounds with free hydroxyl groups. In this context, flavonoids and phenolic acids possess an ideal structure for the scavenging of free radicals, since they present several hydroxyl groups acting as hydrogen donors, which makes them important antioxidant agents[31]. The present study indicates that methanolic extract, aqueous as well as ethyl acetate fractions obtained from dried seeds of *C. dichotoma*, exhibited potent DPPH scavenging activity due to the ability to donate hydrogen by flavonoids and phenolic acids.

Conclusion

In the current study, the methanolic extract exhibited a significant free radical scavenging action and the aqueous and ethyl acetate fraction showed strong antioxidant activity. This bioactivity was mainly attributed to the presence of flavonoids and phenolic compounds, which was confirmed by the IC_{50} data correlation. Further, this action can be of therapeutic importance to combat oxidative stress-associated diseases. The different parts of the plant *C. dichotoma* can be used to extract, isolate, and

characterize phytoconstituents that possess an ability to scavenge free radicals, albeit *in vivo* studies will help to gain a better understanding of this activity.

Conflict of interest

No conflict of interest

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