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Antioxidant, Antibacterial and Cytotoxic activities of Ethanol extract and its different fractions of *Sterculia cordata* leaves

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ABSTRACT

Background: *Sterculia cordata* is a flowering plant and belongs to the family Malvaceae. The goal of our investigation was to determine the significant medicinal properties of the leaf of this plant.

Methods: Leaves of *Sterculia cordata* was extracted with pure ethanol, then fractioned with n-hexane, Ethyl acetate, chloroform and methanol. The extract and fractions were tested for secondary metabolites and the determination of total phenol, total flavonoid content and antioxidant activity. The antibacterial screening was done by disk diffusion technique. The cytotoxic bioassay was carried out with the method as described by brine shrimp bioassay.

Results: Alkaloid, flavonoid, terpenoid, and tannins are present in all extracts and fractions of *Sterculia cordata* leaves. Among all extract and fractions, MFEESC exhibited strong antioxidant activity in both reducing powers. Phenol content was at a range of 3.06 ± 0.34 mg to 45.61 ± 0.43mggallic acid/g, flavonoid content was 25.87 ± 0.20 mg to 36.57 ± 0.34 mg quercetin/g. In antibacterial screening, the moderate zone of inhibition (8-12 mm in diameter) was observed against gram-positive Bacillus subtilis, and more promising zone of inhibition (7.8-14 mm in diameter) against gram-negative *pseudomonas aeruginosa, Escherichia coli. Bacillus cereus, Staphylococcus aureus, Salmonella paratyphi* did not show any sensitivity. In brine shrimp lethality bioassay, chloroform fraction revealed the highest cytotoxic activity having LC50 value 95.23 mg/ml.

Conclusions: The overall results of the study indicated significant Antioxidant, antibacterial and cytotoxic activities of *Sterculia cordata* leaves. So this plant deserves further investigation to isolate the active constituents responsible for these activities and to establish the mechanism of action.

Keywords: *Sterculia cordata*, Malvaceae, antioxidant, antibacterial, cytotoxic.

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BACKGROUND

The investigation of medicinal properties of various plants is increasing day by day because of their potent pharmacological activities, convenience to users, economic viability and low toxicity.[1] In a biological system, an antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Recently, increasing attention has been focused on the use of natural antioxidants, such as ascorbic acid, tocopherols, phenolic compounds including flavonoids, phenolic acids, and volatile compounds for preventing oxidation of biomolecules which can lead to cell injury and death.[2] The medicinal properties of some plants have been investigated throughout the world, due to their potent antioxidant activities. Reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide ion (O_2^-), hydroxyl ion (OH), and hydrogen peroxide (H_2O_2) are highly reactive and toxic molecules generated in cells under normal metabolic activities. ROS can cause oxidative damage to proteins, lipids, enzymes, and DNA molecules.[3] The natural antioxidant has a wide range of biochemical activities including inhibition of reactive oxygen species generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential.[4]

Plant-derived preparations and isolated phytochemicals or their model derivatives may be potentially useful to treat infectious diseases, especially in the light of the emergence of drug-resistant microorganisms and the need to produce more efficacious and cost-effective antimicrobial agents. The use of antibiotics has revolutionized the treatment of various bacterial infections. However, their

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indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms. This necessitates the need for the development of novel antimicrobials. One way of preventing antibiotic resistance of pathogenic species is the development of new compounds that are not based on existing synthetic antimicrobial agents. Plant-derived traditional medicines can be used to treat different diseases as they contain a variety of secondary metabolites to which the bacterial species may not be resistant.[5] Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties.[6]

Plants produce a wide range of bioactive principle and constitute a rich source of medicines. In many developing countries, traditional medicine is one of the primary healthcare systems.[7] Large-scale evaluation of the local flora exploited in traditional medicine for various biological activities is, therefore, necessary. Isolation and characterization of the bioactive principles ultimately leading to new drug development. In view of this, our attention has been focused particularly *Sterculia cordata* Blume, Bijdr. belongs to the family "Malvaceae". Sterculia cordata is a deciduous tree growing up to 46 metres tall. The bole can be 76cm in diameter. The large seeds of many species in this genus are used for food. Usually cooked, they are rich in oil and have a flavour described by some as like peanuts. This plant distributed at Bangladesh, Thailand, Peninsular Malaysia, Sumatra, Java, Borneo, Philippines.[8,9] The wood is harvested from the wild for commercial usage. There is no previous systemic pharmacological investigation done on this plant.

The present study was conducted on various fractions of ethanolic extract (chloroform n-hexane, ethyl acetate, and methanol fraction) of *Sterculia cordata* leaves in order to evaluate the plant as a source of natural antioxidant, antibacterial and cytotoxic.

METHODS

Collection of plant materials

Fresh leaves of *S. cordata* were collected from Bandarban (A district in South-Eastern Bangladesh which is one of the three districts that make up the Chittagong Hill Tracts) in March 2015. The plants were identified by Dr. Sheikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Bangladesh with a voucher specimen (Accession No. 5625CTGUH). And the voucher specimen of the plant has been preserved in the botanical herbarium of the University of Chittagong for future reference.

Extract preparation and fractionation

The fine powder of fresh leaves of *S. cordata* (600 g) was placed in a clean, flat-bottomed glass container and soaked in ethanol. The container with its contents was sealed and kept at room temperature for 7 days accompanied by occasional shaking and stirring. The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness at 50°C under reduced pressure using a rotary evaporator to obtain the ethanol crude extract (approximately 11 g). And the crude ethanol extract was successively partitioned by three solvents such as n-hexane, Chloroform, and methanol. The resultant partitionates i.e., n-hexane (NHFEESC), chloroform (CHFEESC), ethyl acetate (EAFEESC), methanol (MFEESC) and crude ethanol extract (EESC) of *S. cordata* leaves were used for the analyses outlined below.

Preliminary phytochemical screening

Qualitative phytochemical analysis of the extracts was carried out to determine the presence of alkaloids, glycosides, tannins, reducing sugar, flavonoids, steroids, terpenoids, and anthraquinone respectively as described.[10,11]

Determination of total phenolic content

Total phenolic content of all the extracts and fractions were evaluated with Folin-Ciocalteu method.[12] Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent thereby producing a blue colored complex. The phenolic concentration of extracts was evaluated from a Gallic acid calibration curve. To prepare a calibration curve, 0.5mL aliquots of 12.5, 25, 50, 100, 200, and 400 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantitative phenolic estimation was performed at 765 nm against reagent blank by the Shimadzu Biospec 1601 UV-visible spectrophotometer. The total content of phenolic compounds was calculated in Gallic acid equivalents (GAE) using the formula: A= (CXV)/m; where A is the total content of phenolic compounds, mg/g plant extract in GAE; C is the concentration of Gallic acid established from the calibration curve, mg/ml; V is the volume of extract in ml and m is the weight of plant extracting.

Determination of total flavonoid content

Total flavonoid content of the extracts and fractions was evaluated with the method of Jiao.[13] One ml of extracts, fractions or standard of different concentrations was taken in a test tube, and 3 mL of methanol was added. Then 200 µL of 10% aluminum chloride solution was added to the same test tube followed by the addition of 200 µL of 1M potassium acetate. Finally, 5.6 mL of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 min at room temperature to complete the reaction. Then the absorbance of the solution was measured at 415 nm using a spectra-photometer against blank. Methanol served as blank. The Total content of flavonoid compounds in the extracts and fractions were expressed in mg/g quercetin equivalent (QE).

Determination of antioxidant activity

Ferric reducing power assay

The reducing power of the extracts and fractions were evaluated using the method of Oyaizu.[14] Different concentrations of leave extracts and fractions of *S. cordata* (125, 250, 500, and 1000 µg/mL) in 1 mL of distilled water were mixed 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). The mixture was incubated at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) solution was added to each tube, and the mixture was centrifuged at 3000 rpm for 10 min. Subsequently, 5 mL of the upper layer solution was mixed with 5 mL of distilled water and 1 mL of ferric chloride solution (0.1% w/v), and the absorbance was measured at 700 nm. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as a reference standard. Phosphate buffer (pH 6.6) was used as a blank solution.

Assessment of antibacterial activity

Antibacterial screening by disk diffusion technique

All the extract and fractions were screened at three concentrations (500, 800, and 1000 µg/disc) against three Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) and four gram-negative bacteria (*Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*, *Pseudomonas aeruginosa*) using the disc diffusion method.[15] Solutions of known concentration (33.3 mg/mL) of the test samples were prepared. Dried and sterilized filter paper discs (about 5 mm diameter) were then impregnated with known amounts (30µl for 1000 µg/disc, 24µl for 800 µg/disc and 15µl for 500 µg/disc) of the test substances using a micropipette. Discs containing the test material were placed on nutrient agar medium (Merck, India) uniformly seeded with the pathogenic test microorganisms. The prepared inoculums size was approximately106 cfu/mL. Standard antibiotic discs (kanamycin, 30 µg/disc) and blank discs (impregnated with solvents) were used as positive and negative controls, respectively. These plates were then, kept at 4°C for a 1-h diffusion of the test material. There was a gradual change in concentration surrounding the discs. The plates were then, incubated at 37°C for 24 h to allow organism growth. The test materials having antibacterial activity inhibited microorganism growth, and a clear, distinct zone of inhibition surrounding the discs was visualized. The antibacterial activity of the test agents was determined by measuring the diameter of the zone of inhibition expressed in millimeters (mm).

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula.[16,17]

Relative percentage inhibition of the test extract:

$$\frac{100 \times (x - y)}{(z - y)}$$

Where,

x = total area of inhibition of the test extract

y = total area of inhibition of the solvent

z = total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of the zone of inhibition.

Assessment of cytotoxic activity

Brine shrimp bioassay was carried out with the method as described by Meyer et al.[18] to investigate the cytotoxicity of the extracts. The dried extract preparations were re-dissolved in DMSO to obtain a solution of 10 mg/ml which was subjected to serial dilution to get the concentrations between 12.5 µg/ml- 800 µg/ml. Standard drug Vincristine Sulphate (VS) was used as positive control at concentrations of 5µg/ml - 0.312 µg/ml. A 5.0 ml of artificial seawater was added to all the test tubes. Simple zoological organism (*Artemia salina*) was used as a convenient monitor for cytotoxic screening. The eggs of the brine shrimps were collected from local aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (Prepared by using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 24 h under the light. The hatched shrimps were allowed to grow for 48 h to get shrimp larvae called nauplii. After 48 h,

active nauplii were attracted to one side in a glass petri dish by using a micropipette. The nauplii were then separated from the eggs by aliquoting them in another glass petri dish containing artificial seawater and used for the assay. A suspension containing 10 nauplii was added into each test tube and was incubated at room temperature (25 ± 1°C) for 12 h under the light. The tubes were then examined after 24 h and the number of surviving larvae in each tube was counted with the aid of a 3X magnifying glass. Experiments were conducted along with VS in a set of three tubes per dose. The concentration that would kill 50% of the nauplii (LC_{50}) was determined from a linear regression equation using the software "Microsoft excels 2007".

Statistical analysis

The statistical analysis was carried out by using SPSS, version 16.0 (SPSS for Windows, IBM Corporation, New York, USA). One-way ANOVA was used for analysis of data and results were expressed as mean ± SEM and mean ± SD. The values were considered significant at $P < 0.05$.

RESULTS

Phytochemical screening

Preliminary qualitative analysis revealed that the leaf part of *S. cordata* is rich in steroids, terpenoids, alkaloid, carbohydrate, glycosides and flavonoids (Table 1).

Total phenols and flavonoid content

Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the different extracts and fractions of *S. cordata* was solvent dependent and expressed as milligrams of gallic acid equivalents (GAE) equivalent. Table 2 summarizes that total phenolic compounds in extracts varied widely, ranging from 3.06 ± 0.34 to 45.62 ± 0.43 mg/g expressed as gallic acid equivalents (GAE). MFEESC exhibited the highest total phenolic content. The content of flavonoid expressed as quercetin equivalents, varied from 25.87 ± 0.20 to 36.57 ± 0.34 mg quercetin equivalent/g extract (Table 2). The MFEESC showed the highest amount of phenol and flavonoid contents.

ANTIOXIDANT ASSAY

Ferric reducing power

Figure 1 shows the dose response bars for the reducing powers of all extracts and fractions (125 -1000 µg/mL) from *S. cordata* leaves. It was found that the reducing power increased with the concentration of each sample. The ranking order for reducing power was NHFEESC>MFEESC >EESC > CHFEESC > EAFEESC.

ANTIBACTERIAL ACTIVITY

Determination of zone of inhibition

The antibacterial activity of EESC, NHFEESC, CHFEESC, EAFEESC and MFEESC were

Table 1 **Result of chemical group's test of the extract and fractions of leaves of *S. cordata***

Sample	Steroids	Terpenoids	Alkaloids	Carbohydrates	Glycosides	Tannins	Flavonoids	Anthraquinones
EESC	-	++	+	-	++	+	++	-
NHFEESC	-	+	++	-	++	+	++	-
CHFEESC	-	++	++	-	++	++	++	-
EAFEESC	-	++	++	-	++	++	++	-
MFEESC	-	+	++	-	++	+	++	-

(+): Present; (-): Absent
EESC = Ethanolic extract; NHFEESC = n-hexane fraction of ethanolic extract; CHFEESC = Chloroform fraction of ethanolic extract; EAFEESC: Ethyl acetate fraction of ethanolic extract; MFEESC: Methanolic fraction of ethanolic extract of *S. cordata* leaves

Table 2 **Total phenol and flavonoid content of the extract and fractions of *S. cordata* leaves**

Sample	Total Phenol (mg gallic acid /g)	Total Flavonoid (mg quercetin/g)
EESC	30.78 ± 0.15	36.57 ± 0.34
NHFEESC	32.50 ± 35	27.35 ± 0.25
CHFEESC	3.06 ± 0.34	25.87 ± 0.20
EAFEESC	13.61 ± 0.34	27.25 ± 0.30
MFEESC	45.61 ± 0.43	36.00 ± 0.31

Each value represents a mean ± SEM (n = 3). EESC = Ethanolic extract; NHFEESC = n-hexane fraction of ethanolic extract; CHFEESC = Chloroform fraction of ethanolic extract; EAFEESC: Ethyl acetate fraction of ethanolic extract; MFEESC: Methanolic fraction of ethanolic extract of *S. cordata* leaves

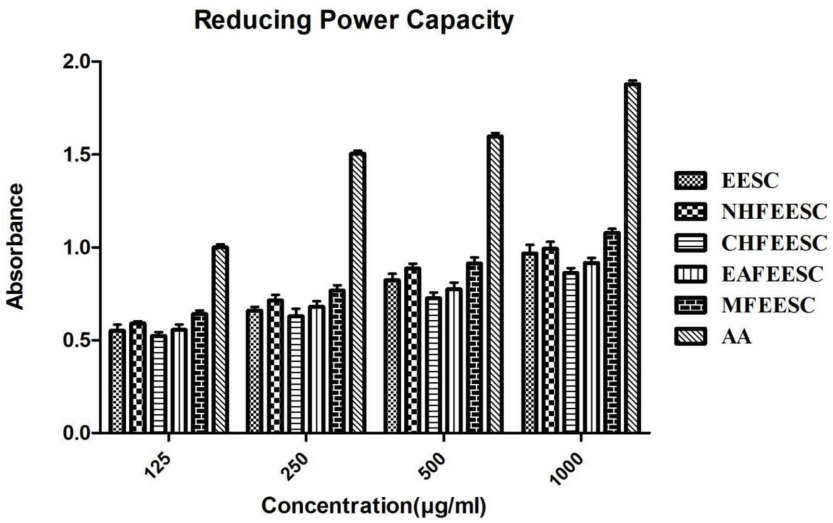

Figure 1 Reducing power capacity of Ascorbic acid (AA) and different extracts and fractions of ***S. cordata*** at different concentrations. Each value represents a mean ± SEM (n = 3). EESC = Ethanolic extract; NHFEESC = n-hexane fraction of ethanolic extract; CHFEESC = Chloroform fraction of ethanolic extract; EAFEESC: Ethyl acetate fraction of ethanolic extract; MFEESC: Methanolic fraction of ethanolic extract of *S. cordata* leaves

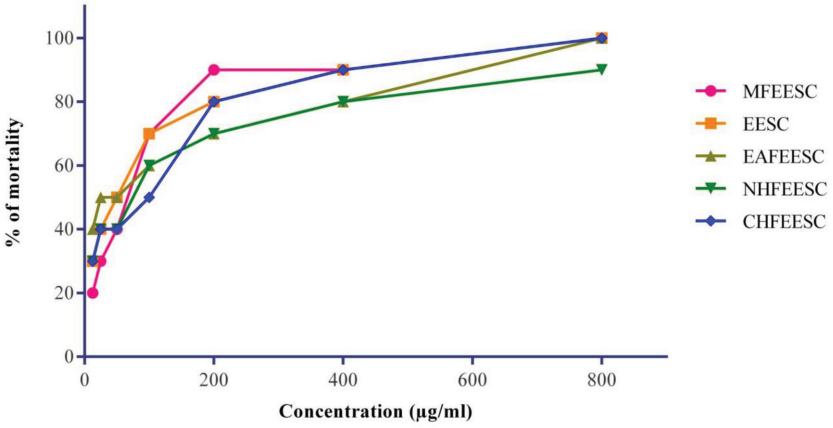

Figure 2 Cytotoxic effect of the extracts and fractions of ***S. cordata*** leaves. Each value represents a mean ± SEM (n = 3). EESC = Ethanolic extract; NHFEESC = n-hexane fraction of ethanolic extract; CHFEESC = Chloroform fraction of ethanolic extract; EAFEESC: Ethyl acetate fraction of ethanolic extract; MFEESC: Methanolic fraction of ethanolic extract of *S. cordata* leaves

tested against 6 pathogenic bacteria and EESC, NHFEESC and MFEESC exhibited a significant antibacterial activity against both gram positive and gram negative bacteria at the concentration of 800 and 1000 µg/disc which is shown in Table 3. The inhibitory activities showed the test samples were compared with standard broad spectrum antibiotic Kanamycin (30µg/disc). The zone of inhibition produced by EESC against gram-positive bacteria (*Bacillus subtilis*) were found to be 10.5 ± 0.5mm and against gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) were found to be 11.5.0 ± 0.87 mm to 13.5 ± 1 mm at different concentration. But *Staphylococcus aureus, Bacillus cereus* and *Salmonella paratyphi* showed resistant for EESC. NHFESC produced 8 ± 0.58 mm to 10.5 ± 0.58 mm zone of inhibition against gram-positive bacteria *Bacillus subtilis* and 9.00 ± 0.50 mm to 12.0 ± 1 mm zone of inhibition against gram-negative bacteria (Escherichia coli, *Pseudomonas aeruginosa*) at 700 and 1000 µg/disk. But NHFESC didn't show zone of inhibition for *Staphylococcus aureus, Bacillus cereus* and *Salmonella paratyphi*. MFEESC produced zone of inhibition against gram-positive bacteria in the range of 9.0 ± 0.50 mm to 12.0 ± 0.50 mm and against gram-negative bacteria in range of 12.0 ± 0.87 mm to 14 ± 1 mm at different concentration. *Staphylococcus aureus, Bacillus cereus and Salmonella paratyphi* showed resistant for MFEESC. On the other hand, Kanamycin showed a zone of inhibition against gram-positive bacteria in the range of 18.2 ± 0.29 mm to 25 ± 0.50 mm and against gram-negative bacteria in the range of 20.3 ± 0.29 mm to 25.5 ± 0.50 mm.

Determination of relative percentage inhibition

The results of antimicrobial activity of plant extract were compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition. The four organic extracts exhibit maximum relative percentage inhibition against the tested bacteria are presented in Table 4.

Cytotoxic activity

The regression analysis for brine shrimp bioassay was presented in Table 5. Comparative mortality of brine shrimps and LC50 values for different extracts was shown in Figures 2, respectively. EAFEESC had exhibited highest cytotoxic effect and showed the LC_{50} value of 20.87 ± 2.17 µg/ml and all the fractions also showed LC_{50} below 250 µg/ml as like as vincristine sulfate (0.76 ± 0.04 µg/ml), positive control indicating that the extract and fractions have a cytotoxic effect (Figure 2).

DISCUSSION

Free radicals play a definite role in a wide variety of pathological manifestations. Antioxidants fight

Table 3 **Results of antibacterial activity testing of the extracts and fractions of *S. cordata* leaves**

Name of the bacteria	EESC		NHFEESC		CHFEESC		EAFEESC		MFEESC		Kanamycin
	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	30µg/disc
Gram Positive											
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	22.2 ± 0.76
Bacillus subtilis	10.5 ± 0.50[b]	8.5 ± 0.50[b]	10.5 ± 0.58[b]	8.0 ± 0.58[b]	9.5 ± 0.50[b]	8.2 ± 0.76[b]	10.3 ± 1.04[a]	8.5 ± 0.50[b]	12.0 ± 0.50[a]	9.0 ± 0.50[a]	18.2 ± 0.29
Bacillus cereus	-	-	-	-	-	-	-	-	-	-	25 ± 0.50
Gram Negative											
Salmonella paratyphi	-	-	-	-	-	-	-	-	-	-	20.3 ± 0.29
Escherichia coli	13.0 ± 1.00[a]	11.5 ± 0.87[a]	12.0 ± 1.00[a]	11.0 ± 0.87[a]	10.0 ± 1.04[a]	8.5 ± 0.50[b]	10.9 ± 1.04[a]	9.0 ± 0.50[b]	13.5 ± 1.00[a]	12.0 ± 0.87[a]	23.5 ± 0.50
Pseudomonas aeruginosa	13.5 ± 1.00[a]	12.0 ± 0.87[a]	10.5 ± 1.04[a]	9.0 ± 0.50[b]	10.0 ± 0.50[b]	8.5 ± 0.76[b]	9.5 ± 0.50[b]	7.8 ± 0.76[b]	14.0 ± 1.00[a]	12.5 ± 0.87[a]	25.5 ± 0.50

Values are mean inhibition zone (mm) ± S.D of three replicates. Bold text indicates the highest antibacterial activity of extracts on each test bacteria. The different superscripted (a, b) values have significantly different (aP< 0.01 and bP< 0.001) as compared with standard (Kanamycin) in same row in Dunnett's test by SPSS. - - - = no zone of inhibition.

Table 4 **Relative percentage inhibition of different extracts with their doses compare to standard antibiotics**

Name of the bacteria	Relative percentage inhibition (%)									
	EESC		NHFEESC		CHFEESC		EAFEESC		MFEESC	
	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc	1000µg/disc	700µg/disc
Gram Positive										
S. aureus	0	0	0	0	0	0	0	0	0	0
B. subtilis	33.28	21.81	33.28	19.32	27.24	20.29	32.02	21.81	43.47	24.45
B. cereus	0	0	0	0	0	0	0	0	0	0
Gram Negative										
S. paratyphi	0	0	0	0	0	0	0	0	0	0
E. coli	30.6	23.9	26.07	21.9	18.1	13.08	21.5	14.6	33	26.07
P. aeruginosa	28.02	22.1	16.9	12.4	15.3	11.1	13.8	9.35	30.1	24.02

Values calculated from their mean values

Table 5 **Calculation of LC50 values, confidence limits and regression equations for the extracts and fractions of *S. cordata* leaves with reference to vincristine sulfate**

Sample	LC_{50} (µg/ml)	Regression equation
Vincristine sulfate (VS)	0.76 ± 0.04	Y= 2.98X +3.16
EESC	25.89 ± 2.32	Y= 0.078x + 47.98
NHFEESC	105.07 ± 3.38	Y = 0.069x + 42.75
CHFEESC	96.28 ± 3.24	Y= 0.086x + 41.72
EAFEESC	20.87 ± 2.17	Y= 0.069x + 48.56
MFEESC	84.05 ± 3.18	Y= 0.089x + 42.52

against free radicals and protect us from various diseases. They either scavenge the reactive oxygen species or protect the antioxidant defense mechanisms.[19] The electron donation ability of natural products can be measured by 2,2 0-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching.[20] The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test.[21] In the present study among all the fractions tested, ethanol, methanol, n-hexane, chloroform and ethyl acetate showed significantly higher inhibition percentage which is correlated with the total phenolic and flavonoid content. Results of this study suggest that the plant extract contains phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

Infectious diseases result from the infection, presence, and growth of pathogenic biological agents in an individual host organism. Antimicrobials prevent or slow down the transmission of infectious diseases either by killing pathogens or inhibiting the growth of the pathogen. In our study, the antimicrobial activity of various extracts of *S. cordata* is evaluated by disc diffusion method. Two different doses of extracts were used in the test and compared with the positive control. All extracts showed dose-dependent activity. The inhibition zone produced by the commercially available positive control was larger than those produced by the extracts. The presence of minor concentrations of bioactive compounds in the plant extract may contribute to the poor antimicrobial activity.[1] However, the plant extracts were found to be effective against both bacteria (Gram-positive and Gram-negative). It gives an indication of the presence of antimicrobial compounds with a broad spectrum or simply general metabolic toxins.[22] Therefore, extracts of *S. cordata* should be subjected to further investigation for finding antimicrobial compounds.

Since any compound or extract can exert antimicrobial and antioxidant activity as a result of its toxic effects on the cells, determination of the toxic effect of antimicrobial and antioxidant agents on host cell is mandatory.[22] Forth is the purpose, brine shrimp lethality test was performed. The results observed in 24 h were found to be dose dependent for all extracts. Any extract or pure compound is considered as a potential cytotoxic and toxic substance only if LC50 is less than 1000 µg/mL.[23,24] In this study, no extracts were found to be significantly toxic compared to positive control.

CONCLUSION

The overall results of the study indicated significant antioxidant and antimicrobial activity of different extracts and fractions of leaves of *S. cordata*. Therefore, considering the potential bioactivity, the plant materials can further be studied extensively to find out their unexplored efficacy to identify the phytoconstituents responsible for these bioactivities and to establish the mechanism of action of such activities.

LIST OF ABBREVIATIONS

EESC: Ethanolic extract of *S. cordata*; NHFEESC: n-hexane fraction of ethanolic extract; CHFEESC: Chloroform fraction of ethanolic extract; EAFEESC: Ethyl acetate fraction of ethanolic extract; MFEESC: Methanolic fraction of ethanolic extract of *S. cordata* leaves; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TCA: Trichloroacetic acid; DMSO: Dimethyl sulfoxide; SPSS: Statistical Package for the Social Sciences; LC_{50}: Lethal concentration 50; SEM: Standard error mean; SD: Standard deviation.

DECLARATIONS

Acknowledgments

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Availability of data and materials

All data and materials are presented in the manuscript.

Consent for publication

Not applicable.

Authors' contributions

Authors SMAK and MH were collected, dried, powdered, extracted the plant sample. SMAK, MH, SA, KN, FK, MSHK, MNUC and MNHJ did the literature study, participated in experimental works, interpreted the data, performed statistical analysis and prepared the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

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