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Research Article

COMPARATIVE ANALYSIS OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF LEAF, BARK, FLOWER AND FRUIT PULP OF *CASSIA FISTULA*

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ABSTRACT: The present study investigated total phenolic contents (TPC), antioxidant activity, and antibacterial activities of aqueous extracts of leaf, bark, flower, and fruit pulp of *C. fistula*. The TPC of the extracts was determined by the Folin-Ciocalteu method. The 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and superoxide radical scavenging assays were used to evaluate antioxidant activity. Antibacterial activities were assessed using the well diffusion assay, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The bacteria tested were *Staphylococcus aureus* (MTCC 7443) and *Escherichia coli* (MTCC 2991). It was found that bark extract contained the highest TPC (22.56 ± 0.91 mg of gallic acid equivalent /g of extract). The bark extract also showed the strongest ABTS scavenging activity (IC50=180.13µg/ml), DPPH scavenging activity (IC50=318.77µg /ml), and Superoxide radical scavenging activity (IC50=478.31µg/ml). Zones of Inhibition were observed in all the aqueous extracts against *Staphylococcus aureus* (MTCC 7443) and *Escherichia coli* (MTCC 2991). The antibacterial potential of the extracts was found to be dose-dependent. At 100 mg/ml, the highest average Zone of Inhibition of 14.33±0.33 mm and 16.0±0.58 mm was recorded in aqueous bark extract against *S. aureus* (MTCC 7443), and *E. coli* (MTCC 2991) respectively. The bark extract of *C. fistula* was most potent against *E. coli* (MTCC 2991) and *S. aureus* (MTCC 7443), with MIC values of 3.125 mg/mL and 6.25 mg/mL, respectively.

Keywords: Total phenolic contents, Antioxidant, Antibacterial, *C. fistula.*

INTRODUCTION

Since ancient times, people have used plants, plant parts, and isolated phytochemicals to prevent and treat a wide range of illnesses. Worldwide, there are roughly 500,000 different species of plants [1]. It is estimated that 121 active chemicals originated from plants and are used in medicine, accounting for around 25% of prescription medications worldwide. Eleven percent of the 252 medications on the WHO's essential medicine list are solely derived from plants. Approximately 80% of people living in rural India practice traditional medicine or use medicinal plants [2]. Although the Indian subcontinent is known to employ 2000 plant species for therapeutic reasons, 500 of those species are frequently used in the various indigenous medical systems that are prevalent there. [3]. It is well known that synthetic medications are expensive, poisonous, and potentially quite dangerous. Herbal remedies, on the other hand, are more affordable, environmentally friendly, and less hazardous. Additionally, people have been using them for generations. [4]. A wide range of chemicals are abundant in plants. Numerous are

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secondary metabolites that contain aromatic compounds, the majority of which are either phenols or their derivatives that have had their oxygen replaced, such as tannins. Numerous of these substances possess antioxidant qualities. [5].

The treatment of infectious diseases is facing significant concerns due to the increasing growth of microbial resistance to traditional antibiotics [6]. The current antibacterial medications are less effective or perhaps useless due to the emergence of resistance [7]. To tackle antibiotic resistance, several strategies have been proposed recently. One approach to achieve this goal has been proposed: combining ineffective medicines with other compounds seems to restore the appropriate antibacterial activity. These compounds might possess antibacterial qualities without being antibiotics, which could open up new therapeutic avenues [8, 9]. As far as this case is concerned, phytochemicals are effective in combating bacterial resistance, while many researchers have used natural products to do so [10, 11].

Common names for *C. fistula* include Amulthus, Raja Vriksha, Indian Laburnum, and Golden Shower. It originated in India, the Amazon, and Sri Lanka and spread to other nations such as China, Mexico, Mauritius, East Africa, South Africa, and the West Indies. The fruit pulp of *C. fistula* is used as a mild laxative for acid reflux and other gastrointestinal issues like constipation. Besides, the fruits of *C. fistula* are also used in fever, heart disease, and leprosy. When treating skin conditions, *C. fistula* bark is employed. Erysipelas, malaria, rheumatism, and ulcers are all treated using *C. fistula* leaves. Fever can be treated with *C. fistula* flowers, and skin conditions, constipation, leprosy, and fever can be treated with the buds. [12]. The leaves of *C. fistula* are known for their laxative, antiperiodic, ulcer-healing, and anti-rheumatic properties. In traditional medicine, the leaves and pods were widely used as potent laxatives and purgatives. Additionally, it was discovered that leaves were good against ringworm and cough. It is made up of many different kinds of ingredients, including potassium, sugar, rhein, and triterpenes [13]. Few researchers have conducted antioxidant and antibacterial research on one or the other parts of *C. fistula*. There is very little information on research that compares various plant sections. Therefore, the purpose of this study was to examine the antioxidant activity and antibacterial potential of *C. fistula*'s leaves, bark, flowers, and fruit pulp *in vitro*.

MATERIALS AND METHODS

Analytical grade solvents were utilized. Sigma-Aldrich provided the following materials: gallic acid, Folin-Ciocalteu reagent (FCR), 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), phenazinemethosulfate-nicotinamide adenine dinucleotide (PMS-NADH), and Nitro blue tetrazolium (NBT). Nutrient broth, absolute alcohol, Mueller Hinton agar (MHA), MacConkey agar (MLA), Mueller Hinton agar (MSA) (HIMEDIA), and Mueller Hinton broth (Merck Specialities Private Limited, Mumbai) were utilized.

Preparation of crude extract

The CSIR-Institute of Himalayan Bioresource Technology, High Altitude Biology Division, Palampur (H.P.) India (Voucher No. PLP 15392) identified the leaves, flowers, bark, and fruits of *C. fistula*, which were collected from multiple locations in and around District Ludhiana (Punjab), India, and shade dried at room temperature. A grinding machine was used to powder all of these parts after they had dried. The maceration method produced aqueous extracts. In this process, 100 grams of a whole or coarsely ground crude sample were soaked in one liter of distilled water [14]. The sample was then allowed to rest at room temperature, stirring often, for at least 48-72 hours, or until the soluble material dissolved. Following the maceration process, the extract was first filtered through a muslin cloth and then again through filter paper (Whatman filter paper No. 1, Cat. No. 1001125). At forty degrees Celsius, the filtrate was evaporated in an oven. The residue was collected and stored in airtight bottles at 4ºC until needed [15].

Total phenolic content (TPC)

An experiment based on the Folin-Ciocalteu reagent (FCR) was used to determine the total phenolic content in the leaves, flowers, bark, and fruit pulp extract of *C. fistula*. After adding and mixing 950 µl of distilled water with 50 µl of plant extract. Following mixing, 2.5 µl of the 20% sodium carbonate solution and 500 µl of FCR with distilled water (1:1) were added. After 40 minutes of room temperature storage, the mixture's absorbance at 725 nm was measured. A gallic acid standard curve was created [16] (Fig. 1). The absorbance was measured in comparison to a control solution that was made using distilled water rather than extract. The total phenolic contents (mg/g) in the extract of plant sections from *C. fistula* were expressed using the gallic acid equivalent (GAE).

Antioxidant activities

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) Scavenging assay:

The usual protocol for ABTS radical scavenging activity was followed [17].

% ABTS + inhibition = [1 - (A734nm test/A734nm control)] × 100

IC50 values were determined by a graph between the percent of inhibition and concentration.

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity:

Using a conventional technique, DPPH radical scavenging was carried out [18]. Similar to ABTS, the DPPH radical scavenging activity and IC50 were determined at 517 nm.

Superoxide anion radical scavenging activity

A systematic procedure was used to determine this activity [19]. The IC50 and percentage of radical scavenging activity were computed in a manner consistent with that of ABTS scavenging activity.

Antibacterial activity

Microbial strains and preparation of medium

Aqueous extracts of *C. fistula*'s leaves, bark, flowers, and fruit pulp were tested for their antibacterial efficacy against gram-negative *Escherichia coli* (MTCC-2991) and gram-positive *Staphylococcus aureus* (MTCC-7443) bacteria. These antimicrobial properties were compared to those of ampicillin, a standard drug. The Department of Livestock Products and Technology, GADVASU, Ludhiana, provided the test organisms. Each test organism was grown for eighteen hours at 37ºC in nutrient broth to prepare the inocula. After comparing the overnight broth culture to the Mac Farland turbidity standard, an approximate value of 10^8 cfu/ml was obtained. Approximately 10^8cfu/ml was obtained by seeding a molten Mueller Hinton agar (MHA) medium with 100 µl of this broth and allowing it to cool to 45°C [20].

Well diffusion assay

For an antibacterial investigation, various doses of the plant extracts (12.5, 25, 50, and 100 mg/ml) were diluted in 100% dimethyl sulfoxide (DMSO). Various plant extracts were tested for their antibacterial activities using the agar-well diffusion method. At a depth of roughly 4 mm (15 ml), the sterilized medium (MHA) was added to triplicate pre-sterilized petri plates and left to harden. Using a sterile cork borer, the wells were cut after the medium of each plate was surface infected with a suspension of the corresponding bacteria (10^8 cfu/ml). The wells were filled with a 50 µl solution of several plant extracts at concentrations of 12.5, 25, 50, and 100 mg/ml in the appropriate solvent. The solvents, ampicillin (100 µg/ml) and DMSO (50 µl) were kept as positive and negative controls, respectively. During 24 hours, the plates were incubated at 37°C. After incubation, the zones of inhibition's diameter were measured [21].

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

Using the microdilution method in accordance with CLSI guidelines (2008), the aqueous extracts of *C. fistula* leaves, bark, flowers, and fruit pulp were tested for pharmacodynamic characteristics such as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Escherichia coli* (MTCC-2991) and *Staphylococcus aureus* (MTCC-7443) in Mueller Hinton broth (MHB). Standardization of the bacterial count is necessary for the computation of MIC and MBC. Two to four medium-sized *E. coli* (MTCC-2991) colonies cultured on MacConkey Lactose Agar (MLA) (HIMEDIA) were used for this. After 9 ml of MHB was added to the plates, they were incubated for 2.5 hours at 37°C. After incubation, the turbid broth was diluted 1:100 times using fresh MHB. The diluted inoculum was mixed thoroughly, and then 10µl of broth from each dilution of 10^2, 10^4, 10^6 and 10^8 was taken and plated on MLA plates. After incubation at 37°C for 18h, growth was observed, and a bacterial count was done. The inoculum size was 1-5 x 10^8 cfu/ml before adding it to microwell plates for MIC determination [22]. The standardization of *Staphylococcus aureus* (MTCC-7443) was done similarly, taking 4-5 colonies grown on mannitol salt agar (MSA), inoculating them in 9 ml of MHB, and then incubating them at 37°C for 2.5 h. After incubation, the turbid broth was diluted and plated similarly as in the case of *E. coli* (MTCC-2991), using MSA as the selective medium.

Each well was initially filled with 50 µl of broth. Next, 50 µl of plant extract (100 mg/ml) was added to the microwell plate's first column, which was then serially diluted with the maximum dilution up to 1:2048. To each of these wells, 50µl of 1:100 times diluted *E. coli* (MTCC-2991) culture or *S. aureus* (MTCC-7443) culture was added so that the final inoculum of 1-5 x 10^8 cfu/ml was obtained. For

eighteen hours, the mixture including broth, medication, and bacterial culture was incubated at 37°C in its final volume of 100 µl [22].

The minimum inhibitory concentration, or MIC, is the lowest drug concentration that, in relation to the control, totally prevents the observable growth of bacteria. After adding 40 µl (0.2 mg/ml) of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and incubation for 30 minutes at 37°C, the minimum inhibitory concentration (MIC) was ascertained. MTT was digested by bacteria, giving it a dark blue tint, which was then utilized as an indication of the proliferation of bacteria. The wells with no color change following the administration of MTT were considered to have no microbial growth and were assigned MIC values. The MIC was carried out in three overlapping sets to improve accuracy. Following the MIC determination, MHB was spiked in a flat-bottom 96-well plate with varying extract concentrations. The concentrations spanning the most likely locations of MBC that were greater or equal to the MIC were chosen. Ten milliliters of broth containing bacterial cultures of *S. aureus* (MTCC-7443) and *E. coli* (MTCC-2991) with an inoculum size of 1-5 x 10^8 cfu/ml were added to these wells, stirred, and incubated for eighteen hours at 37°C. Controls for growth and sterility were conducted concurrently. [22]. Following incubation, quantities of *E. coli* (MTCC-2991) and *S. aureus* (MTCC-7443) at which neither visible growth nor turbidity was observed were chosen and plated on the MLA and MHA plates, respectively. Prior to counting, the plates were incubated for eighteen hours at 37°C. The lowest concentration, MBC, was shown to have a 99.9% reduction in bacterial counts.

Statistical analysis

A statistical analysis of the gathered data was performed. IBM SPSS version 20.0 statistical software was used to calculate the mean, standard error, one-way ANOVA, and other necessary statistical analyses for various parameters. We computed the IC50 value using linear regression analysis.

RESULTS AND DISCUSSION

The total phenolic content ranged from 1.19 to 22.56 mg of GAE/g of extract in different extracts from *C. fistula* leaves, bark, flowers, and fruit pulp (Table 1). The percent recovery of different *C. fistula* extracts ranged between 2.0 and 5.7 (Table 1). The variations in the percent recovery of *C. fistula* extracts might be attributed to the polarities of different compounds present in them. Among the extracts, the highest yield of 5.7% was recorded for *C. fistula* fruit pulp aqueous extract, and the lowest yield of 2% was recorded for *C. fistula* flower aqueous extract. A high percentage of soluble sugars in pulp extracts may be the cause of their high yield potential [23].

Out of all the *C. fistula* components, the aqueous extract from the bark had the highest total phenolic content, measuring 22.56 ± 0.91 mg of GAE/g of extract. With 1.19 ± 0.18 mg of GAE per gram of extract, the *C. fistula* fruit pulp aqueous extract had the lowest total phenolic content. Bark extracts had the highest total phenolic content among *C. fistula* aqueous extracts, followed by leaf, flower, and fruit pulp. Our findings are consistent with a prior study that found that *C. fistula* bark extracts had the highest concentration of total phenolics, followed by leaf, flower, and fruit extracts. Compared to stem, leaf, and root extracts, *C. fistula* bark extracts were shown to have higher total phenolic content, total tannin content, and antioxidant activity in one of the earlier investigations [23, 24]. The results of the present study showed that the aqueous extract of the bark of *C. fistula* contains a substantial amount of phenolics. The antioxidative activity of plants is directly correlated with their total phenolic content. Phenolic compounds primarily exhibit their antioxidant effects through redox characteristics such as quenching singlet oxygen, donating hydrogen, and scavenging free radicals. Free radicals are produced naturally by aerobic cell metabolism and can be utilized by the body to defend itself against them [25]. A high level of antioxidant activity is indicated by the total phenolic content [26]. Using a Folin-Ciocalteu reagent (FCR)- based analysis, the total phenolic content in the *C. fistula* extracts was ascertained [16]. The reducing capability, which is expressed as phenolic content, is obtained by the electron transfer-based Folin-Ciocalteu analysis.

The ABTS (2, 2'-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) technique was used to assess the total antioxidant activity of the *C. fistula* preparations. At a dosage of 1.0 mg/ml, the bark extract of *C. fistula* was found to have stronger activity than the leaf, flower, and fruit pulp extracts. Aqueous bark extract exhibited 82.03±0.39% scavenging activity. As illustrated in Fig. 2, the leaf, flower, and fruit pulp extracts were found to be 69.23±0.55%, 85.23±0.30%, and 67.70±0.92% activity, respectively. Based on a graph showing the percentage of scavenging action against the extract concentration, the IC50 values of the *C. fistula* extracts were determined. The IC50 values ranged from 180.13

Table 1. Total phenolic content (TPC) (Mean±SE) in leaves, bark, flowers and fruit pulp of *C. fistula* extracts [(n=3) measured as gallic acid equivalent (mg of GAE/ gram of extract) and percent recovery of extracts].

Extract	TPC (mg of GAE/ gm of extract)	% Recovery
Leaf (Aqueous)	4.94 ± 0.40	2.5
Bark (Aqueous)	22.56 ± 0.91	2.2
Flower (Aqueous)	2.42 ± 0.94	2.0
Fruit Pulp (Aqueous)	1.19± 0.18	5.7

Table 2. IC50 values (µg/ml) of ABTS, DPPH and superoxide radical scavenging assays of aqueous extracts of *Cassia fistula*.

Sample	ABTS	DPPH	Superoxide
Leaf	235.76µg /ml	388.77µg /ml	928.73 µg /ml
Bark	180.13 µg /ml	318.77µg /ml	478.31 µg /ml
Flower	265.42 µg /ml	1407.12µg /ml	935.28 µg /ml
Fruit Pulp	485.25 µg /ml	2004.23µg /ml	957.39µg /ml
Trolox	195.43 µg /ml	-	-
BHT	-	289.02 µg /ml	-
Ascorbic acid	-	-	90.37µg /ml

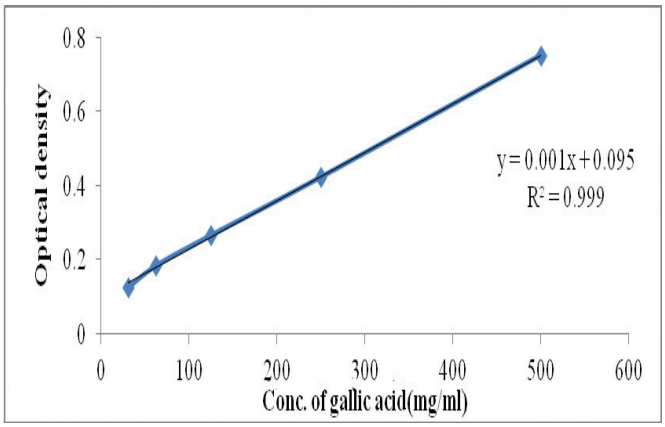

Fig. 1. Gallic acid standard curve for phenol content determination.

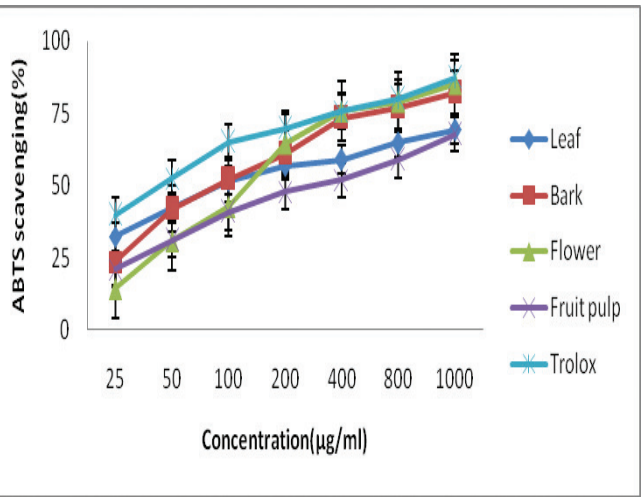

Fig. 2. ABTS radical scavenging activity (%) of leaf, bark, flower, fruit pulp aqueous extracts from *C. fistula* and control (Trolox). [Each value is expressed as mean±SE (n=3)].

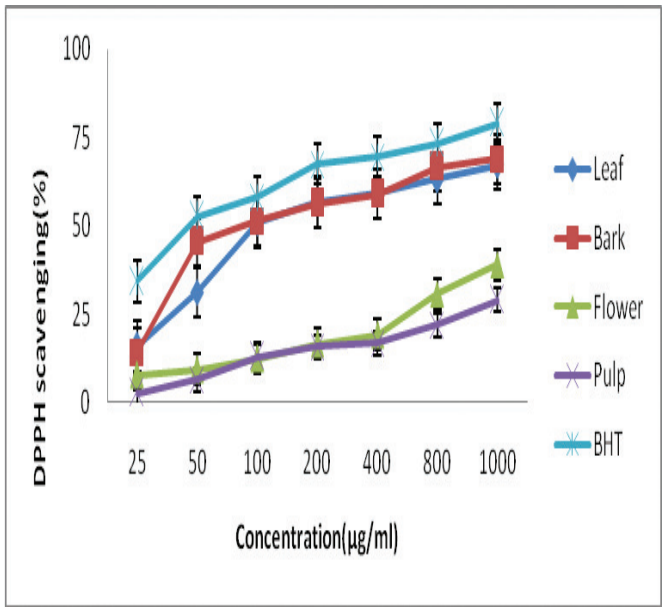

Fig. 3. DPPH radical scavenging activity (%) of leaf, bark, flower, fruit pulp aqueous extracts from *C. fistula* and control (BHT). [Each value is expressed as mean±SE (n=3)].

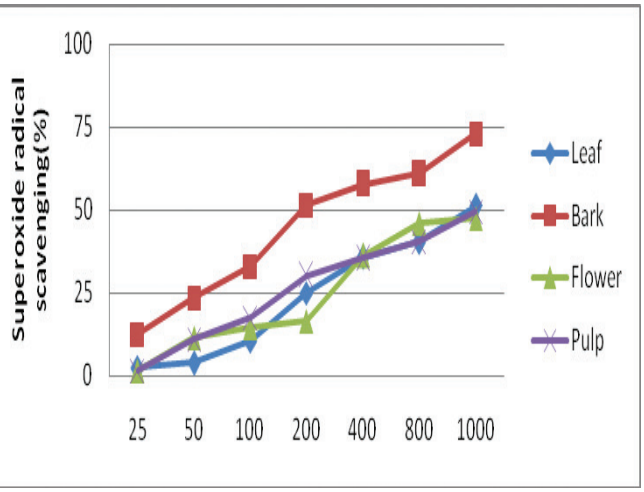

Fig. 4. Superoxide radical scavenging activity (%) of leaf, bark, flower and fruit pulp aqueous extracts from *C. fistula*. [Each value is expressed as mean±SE (n=3)].

µg/ml for bark extract to 485.25 µg/ml for fruit pulp extract (Table 2). The blue-green chromogen known as ABTS radical cation (ABTS+), which has a maximum absorption at 734 nm, is created when ABTS combines with potassium persulfate. One important determinant of the sample's antioxidant activity is the degree of decolorization. The ability of extracts to donate hydrogen is what causes the ABTS radical cation to be affected, as seen by the radical cation's color changing from ABTS+ to colorless ABTS. It was discovered that nearly every extract from various *C. fistula* sections was efficient at scavenging the ABTS radical [17]. The IC50 value served as the basis for determining the antioxidant activity. The concentration needed to inhibit free radicals by 50% is known as the IC50 value. The greater the scavenging potential, the lower the IC50 value. The percentage inhibition of ABTS radicals was concentration-dependent. When the IC50values of *C. fistula* leaf aqueous extracts, bark, flower, fruit pulp, and standard antioxidant Trolox are compared, the antioxidant activity is found in the following decreasing order: bark > Trolox > Leaf > Flower > Fruit pulp. This indicates that *C. fistula* bark aqueous extract has a good scavenging capacity for ABTS radicals. Bark extracts were discovered to have antioxidant activity that was even higher than that of Trolox, the typical antioxidant. High-molecular-weight phenolics, or tannins, have been shown in a prior study to be more effective at scavenging free radicals (ABTS+). Their efficacy is primarily dependent on their molecular weight, the number of aromatic rings, and the type of hydroxyl group substitution, rather than on the specific functional groups they replace [27]. The presence of high molecular weight phenolics such as gallic acid, ellagic acid, chebulinic acid, and quercetin may be the cause of the high free radical (ABTS+) scavenging activity of the ethanolic extract of *C. fistula* bark extract.

Using a DPPH-generated radical, the radical scavenging activity was examined with various sample extracts and contrasted with butylated hydroxytoluene (BHT) as the reference. The findings demonstrated that the percentage inhibition of the DPPH radical increased along with the sample concentration (Fig. 3). At a dosage of 1.0 mg/mL, it was found that the *C. fistula* bark extract exhibited greater efficacy than the leaf, flower, and fruit pulp extracts. Aqueous bark extract exhibited 68.9±0.83% scavenging activity, whereas extracts from leaves, flowers, and fruits demonstrated 67.19±0.40%, 39.04±0.18%, and 29.08±0.21% scavenging activity, respectively. The IC50 values for fruit pulp extract were 2004.23 µg/ml and for bark extract was 318.77 µg/ml (Table 2). A sensitive approach for assessing the antioxidant activity of plant extracts is the DPPH (2,2-diphenyl-1-picrylhydrazyl) stable free radical method [28]. When DPPH is combined with free electrons or hydrogen radicals, a stable free radical and stable diamagnetic molecule are produced [29]. The order of antioxidant activity is BHT>Bark>Leaf>Fruit pulp when comparing the IC50 values of aqueous extracts of *C. fistula* leaves, bark, flower, and fruit pulp with the standard antioxidant BHT. It was found that while bark extracts' radical-scavenging activity was higher than that of standard antioxidants, it was still lower than that of other extracts. [23]. A combination of several scavenging chemicals makes up extracts, and these compounds may work in concert to boost the antiradical action. Because of the extract's ability to donate hydrogen, which is observed as a shift in color from purple to yellow, it is thought to have an impact on DPPH radical scavenging [30]. In diverse ways, every plant extract examined suppressed the DPPH radical. This outcome demonstrated the extracts' ability to donate hydrogen or electrons, which might then react with the DPPH radical. The portion of the plant that was utilized for the study affected the difference in scavenging activity amongst the extracts. This discrepancy might be explained by the different portions of the plant having differing amounts of antioxidant chemicals, such as flavonoids and polyphenols. Increased DPPH radical scavenging activity was seen in the current study at higher plant extract concentrations. This finding may point to a greater capacity to donate hydrogen ions, resulting in a lighter solution that is proportionate to the amount of electrons obtained. [31, 32]. Therefore, due to their capacity to donate hydrogen ions, it is possible to hypothesize that the fruit pulp, leaves, bark, and flowers of *C. fistula* exhibit DPPH-scavenging activity by reducing the DPPH radical to equivalent hydrazine. An earlier study found that *C. fistula* bark extracts had higher total phenolic content and DPPH scavenging action than stem, leaf, and root extracts, which is consistent with the current findings [23]. Our findings, however, conflict with those of a previous study that found fruits to have stronger antioxidant activity than other sections [33].

Figs. 4 and Fig. 5 show the superoxide radical scavenging activity of *C. fistula* leaf, bark, flower, and fruit pulp extracts in comparison to the standard antioxidant ascorbic acid. At a concentration of 1.0 mg/ml, it was shown that the bark of *C. fistula* had

Table 3. Antibacterial activity of *Cassia fistula* aqueous extracts at various concentrations.

Extract	Dose (Concentration) (mg /ml)	Diameter of Zone of inhibition (mm)	
		E. coli	*S. aureus*
Leaf	12.5 (mg /ml)	8.00±0.00	8.0±0.58
	25 (mg /ml)	8.67±0.33	8.67±0.33
	50 (mg /ml)	9.00±0.58	8.67±0.33
	100 (mg /ml)	10.33±0.3	11.0±0.58
Bark	12.5 (mg /ml)	10.33±0.33	10.0±0.58
	25 (mg /ml)	12.0±0.58	11.33±0.33
	50 (mg /ml)	12.67±0.33	13.0±0.58
	100 (mg /ml)	16.0±0.58	14.33±0.33
Flower	12.5 (mg /ml)	7.33±0.33	7.67±0.33
	25 (mg /ml)	8.33±0.33	8.33±0.33
	50 (mg /ml)	10.0±0.58	11.33±0.33
	100 (mg /ml)	12.0±0.58	13.5±0.65
Fruit Pulp	12.5 (mg /ml)	8.33±0.33	7.67±0.33
	25 (mg /ml)	8.0±0.00	8.33±0.33
	50 (mg /ml)	9.33±0.33	11.33±0.33
	100 (mg /ml)	11.5±0.65	13.5±0.65
Ampicillin	100 (µg /ml)	25.00±0.58	23.0±0.58
Negative control	-------	------	-----

Negative (-ve) shows that the solvents used could not inhibit the growth of selected organisms.

Table 4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of aqueous extracts of *C. fistula*.

Extracts (Aqueous)	*E. coli*		*S. aureus*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Leaf	25	>100	25	>100
Bark	3.125	>100	6.25	50
Flower	25	>100	25	>100
Fruit Pulp	25	>100	25	>100

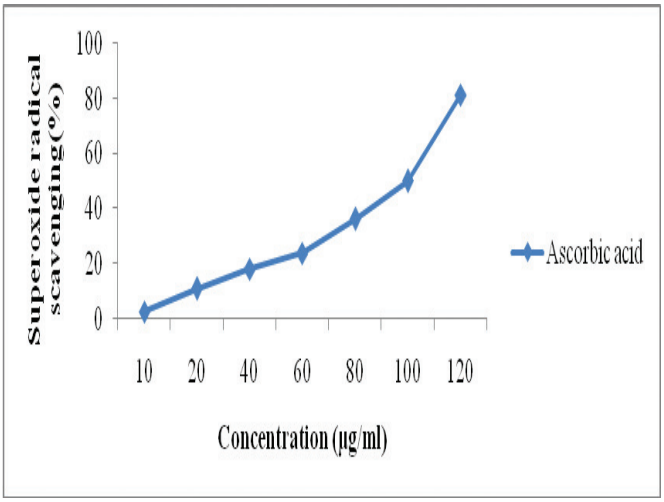

Fig. 5. Superoxide radical scavenging activity at different concentrations of ascorbic acid.

greater activity than extracts of the leaves, flowers, and fruit. Aqueous bark extract had a scavenging activity of 72.90±0.58%, whereas extracts from leaves, flowers, and fruits demonstrated scavenging activities of 51.22±0.40%, 47.45±0.63%, and 49.54±0.95%, respectively. For bark extract, the IC50 values were 478.31 µg/ml and for fruit pulp extract, they were 957.39 µg/ml. (Table 2). Superoxide anion radicals (O2-) are produced by active phagocytes, including neutrophils, eosinophils, monocytes, and macrophages [29]. The generation of superoxide anion is a crucial step in the phagocytes' ability to kill germs. Superoxide anion radicals were created in the PMS-NADH (Phenazine methosulfate-nicotinamide adenine dinucleotide) system by oxidizing NADH, and they were quantified by reducing NBT (Nitro blue tetrazolium). Reduced absorbance at 560 nm due to antioxidants indicates that superoxide anion consumption has taken place in the reaction mixture. [34]. Ascorbic acid is the standard antioxidant, while *C. fistula* preparations have a decreasing order of antioxidant activity: ascorbic acid > Bark > Leaf > Flower > Fruit pulp. Ascorbic acid's IC50 value was lower than that of *C. fistula* extracts, indicating that it is a more effective superoxide radical scavenger than *C. fistula*. The *C. fistula* bark extract exhibits the best scavenging activity of all the extracts. The greater polyphenolic content in the bark extract of *C. fistula* may be the cause of its improved superoxide radical scavenging action.

Antibacterial activity

Table 3 displays the findings for the antibacterial activity of various *C. fistula* extracts against gram-positive *S. aureus* (MTCC 7443) and gram-negative *E. coli* (MTCC 2991). Aqueous bark extract against *S. aureus* (MTCC 7443) showed the highest average inhibition zone (14.33±0.33 mm) at 100 mg/ml, whereas leaf extract showed the lowest inhibition zone (11.0±0.58 mm). Aqueous bark extract had the highest average inhibition zone (16.0±0.58) against *E. coli* (MTCC 2991) at the same concentration, while leaf

extract had the lowest inhibition zone (10.33±0.3). It was discovered that the extracts' antibacterial activity was dose-dependent. DMSO, the negative control, had no discernible effect on the growth of any of the tested bacteria. The inhibitory activity of each extract varied when it came to the tested bacterial strains. When compared to other extracts, bark extracts generally exhibited more antibacterial activity. Our results are consistent with earlier studies that used the agar disc diffusion method to find the significant antibacterial activity of the hydroalcoholic extract (8:2) of *C. fistula* leaves against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* [35]. Significant activity against *S. aureus* but not against *E. coli* was discovered in one of the tests. [36], whereas in our study, *C. fistula* stem bark showed activity against both *E. coli* and *S. aureus*. Antimicrobial activity of methanolic and ethanolic extracts of *C. fistula* flowers on *Bacillus cereus*, *Staphylococcus aureus*, *S. epidermidis, Salmonella* Typhi, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* was also seen in an earlier study [37]. Crude hydroalcoholic and chloroform extracts of *C. fistula* fruit pulp were reported to have moderate to strong activity against *S. aureus* and *E. coli* in one of the investigations. [38].

Table 4 displays the results of the MIC and MBC tests conducted on several *C. fistula* extracts against *E. coli* (MTCC 2991) and *S. aureus* (MTCC 7443). The MIC values of *C. fistula* against *E. coli* ranged from 3.125 mg/mL to 25 mg/mL for different sections of the plant. The MIC values of *C. fistula* against *S. aureus* ranged from 6.25 mg/mL to 25 mg/mL for different sections of the plant. The aqueous extracts of the leaves, bark, flowers, and fruit pulp of the *C. fistula* plant were examined using the broth dilution method to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in order to assess their bacteriostatic and bactericidal capabilities. The absence of bacterial growth in the tested strains streaked from the inhibitory zone corresponding to their lowest MIC served as confirmation of the MBC. The highest effective bark extract of *C. fistula* was 3.125 mg/mL against *E. coli* (MTCC 2991) and 6.25 mg/mL against *S. aureus* (MTCC 7443). Every extract had an MBC value of more than 100 mg/mL. The bark extract exhibited a 50 mg/mL MBC against *S. aureus*. Our results corroborate those of a previous study that determined the *C. fistula* extract was efficient against *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli*, and *K. pneumoniae*. It was shown that *K. pneumoniae* and *E. coli* were the most vulnerable bacteria to methanolic and ethanolic extracts. The bacteria that were least sensitive to ethanolic and methanolic extracts, respectively, were *B. cereus* and *S. aureus*. In contrast, the ethanolic extract's MBC and MIC values against *E. coli* were determined to be 5 mg/mL and 40 mg/mL, respectively, and 40 mg/mL against *S. aureus*. The variations in membrane composition and structure between the two groups may be the cause of the sensitivity discrepancies [39]. The MIC values of various *C. fistula* parts against *E. coli* ranged from 0.78 to 1.56 mg/mL in our previous research on ethanolic extracts of the plant, while the MIC values of various *C. fistula* parts against *S. aureus* were 6.25 mg/mL [40].

CONCLUSION

The investigations' findings demonstrated that *C. fistula* aqueous bark extracts had higher antioxidant activity, which is correlated with their total phenolic content. In comparison to the pulp of the leaves, flowers, and fruits of *C. fistula*, bark extracts exhibited the strongest antibacterial efficacy against both gram-positive and gram-negative pathogens. Because *C. fistula* bark has a high concentration of phenolic compounds, it can be utilized as a natural source of antioxidants in pharmaceutical preparations. To clarify the mechanisms underlying antioxidant and antibacterial properties, more research concentrating on the isolation and characterization of bioactive chemicals is required.

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