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RESEARCH ARTICLE

Phytochemical screening along with *in vitro* antioxidant, antibacterial and anticancer activity of *Senna auriculata* (L.) bark extracts

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ABSTRACT

This study aimed to evaluate the phytochemical content and biological properties of *Senna auriculata* (L.) Roxb. Four extracts—acetone, methanol, ethanol, and chloroform—were tested for antioxidant potential, enzymatic activity (peroxidase and polyphenol oxidase), antimicrobial, and anticancer effects. GC-MS analysis identified 34 bioactive compounds. The acetone extract exhibited the highest total alkaloid (5.8%), phenolic (752.78 ± 2.25 mg GAE/g), and flavonoid (285.78 ± 1.25 mg QE/g) content, along with the highest antioxidant (1489.42 ± 4.35 mg AAE/g) and enzyme activities. All extracts inhibited both Gram-positive and negative bacteria, with the acetone extract showing superior inhibition against *S. aureus* and *B. subtilis*. Additionally, the acetone and methanol extracts demonstrated anticancer effects on MDA-MB-231 breast cancer cells. These findings suggest that *Senna auriculata* has potential as a therapeutic agent for various diseases.

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KEYWORDS
Senna auriculata; phytochemical screening; GC-MS; antioxidant; antibacterial

Introduction

Plants are an important medication source because of their biological properties, which include antioxidant and antifungal properties. Nowadays, chemical-based and synthetic medications are typically used sparingly since they are frequently linked to unfavourable outcomes (George et al. 2018; Jahan et al. 2022). Conversely, traditional plant-based medicines are becoming more popular since they are natural, eco-friendly, and have fewer negative effects (Sahoo and Manchikanti 2013). Consequently, for the treatment of a variety of common illnesses, most people worldwide still prefer using herbal remedies over synthetic ones (Yuan et al. 2016; Ferrarini et al. 2022). Around the world, traditional medical systems have included a variety of plants with ethnomedical value as folklore remedies since ancient times (Pandey et al. 2013).

According to recent studies, more than 25,000 herbal formulations are utilized by medical practitioners worldwide to treat a wide range of illnesses, as well as in many traditional systems (Nayak et al. 2020). According to Yuan et al. (2016), Anand et al. (2019), Khan et al. (2020), and other studies, the bioactive chemical compounds found in medicinal plants have pharmacological activities including antidiabetic, antibacterial, antiviral, and thrombolytic actions in addition to antioxidant benefits. As a consequence, the potential for treating various pathological disorders is

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worthy to be investigated (Pandey et al. 2013, Yuan et al. 2016). Typically, plant crude extracts and their subsequent fractions are used to produce herbal medicines. Due to their distinct structural makeup, phytochemicals derived from plants may be able to treat a range of acute and long-term illnesses (Sahoo and Manchikanti 2013). Many medicinal plants have a multitude of bioactive constituents, but only a small number of these constituents have been thoroughly investigated and proved to possess biological activity (Keskes et al. 2017). The identification and extraction of medicinally active chemicals from various species of medicinal plants have made it possible to supply specific compounds endowed with high pharmacological potential (Konappa et al. 2020). The use of GC-MS for the identification of phytoconstituents has significantly expanded in recent years due to its ability to analyse different kinds of secondary metabolites, including steroids, organic acids, alcohols, hydrocarbons, amino acids, and esters Konappa et al. (2020).

In this context, *Senna auriculata* (L.) Roxb. syn. *Cassia auriculata* L. (Family: Fabaceae) is a traditional medicinal plant that is widely used for the treatment of various ailments in India's Ayurvedic and Siddha systems of medicine. This shrub is found throughout India, including the southern, western, and central dry zones, as well as in Sri Lanka (Gupta and Sharma 2007; Nille and Reddy 2017; Win and Min 2018). In addition to its antioxidant properties, such a species exhibits a vast variety of pharmacological activities such as anti-inflammatory, anticancer, anti-carcinogenic, antibacterial, antioxidant, antifungal, antiviral activities etc. and many of them have been recently reported. The main phytochemicals identified in *Senna auriculata* (L.) Roxb. are represented by alkaloids, anthraquinones, flavone glycosides, sugars, saponins, phenols, terpenoids, flavonoids, tannins, steroids, etc (Nille et al. 2021).

The present study aimed to investigate the bark extracts of *Senna auriculata* for their phyto-chemical content, free radical scavenging activity and antimicrobial/anticancer activities.

Experimental section

Plant materials and reagents

The bark of *S. articulata* was collected from India. All chemicals such as acetone, methanol, ethanol, chloroform, gallic acid, sodium carbonate, ammonium molybdate, sulfuric acid, sodium phosphate, ascorbic acid, potassium ferricynide, TCA (trichloroacetic acid), quercetin, 2,2-diphenyl-picrylhydrazyl (DPPH), ABTS reagent 2,2'-azinobis-(3-ethylbenzothiazillline-6-sulfonate), cate-chol, ferrozine, ferrous sulfate and guaiacol were purchased from Hi-Media, India. Hydrogen peroxide and Folin-Ciocalteu reagent were from Central Drug House (P) Ltd. India. All the other chemicals were of analytical grade purchased from Merck, Germany.

Preparation of plant extracts

The bark of *S. auriculata* were collected and washed in tap water. It was shade dried for 10 days and made into a fine powder of 40 mesh in size using the laboratory mill. 100 g of powder was filled in the thimble and was extracted successively with 70% of acetone, methanol, ethanol and chloroform. It was taken in a clean flat-bottomed glass container and soaked for 72 h. The container with its content was sealed and kept for a period of three days accompanying occasional shaking and stirring and the extracts were stored in sterile bottles at room temperature until use and it was filtered using Whatman no. 1 filter paper (Merck, Germany).

Bacterial cultures

The microbial strains were obtained from IMTECH, India: *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Bacillus megaterium* (MTCC 441), *Bacillus cereus* (MTCC 6840), *Bacillus aryabhattai* (MTCC 14,579), and Gram negative bacteria such as *Pseudomonas putida*

(MTCC 1194), *Escherichia coli* (MTCC 1302), *Klebsiella pneumoniae* (MTCC 4727), *Serratia marcescens* (MTCC 4822) and were grown in a nutrient broth at 37°C for 12 h under laboratory conditions.

GC-MS analysis for S. articulata leaf extracts

GC-MS analysis of *S. articulata* leaf extracts were performed on a Perkin Elmer Clarus 600 GC System (Markham, ON, Canada) equipped with a Rtx-5 MS capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness). Helium gas (99.999%) was used as carrier gas at a continuous stream of 1.73 mL/min and an injection volume of 0.5 µL was employed; the injector and ion-source temperature was 240°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 8°C/min, to 150°C, then 8°C/min to 250°C, ending with a 32 min isothermal at 280°C (Turbo mass over 5.2.0). Compound identification was carried out by using the database of National Institute Standard and Technology (NIST).

Phytochemical analysis

The preliminary phytochemical screening test was carried out according to Lawal et al. (2019).

Antioxidant activity, free radical scavenging capacity, and enzymatic activity of S. articulata bark extracts

Determination of total phenolic content (TPC)

The TPC was determined by using the Folin-Ciocalteu's method (Ahmed et al. 2022) Gallic acid was used as standard and different concentrations were prepared ranging from 0.05 mg/mL to 0.5 mg/mL. Absorbance was measured at 725 nm and results were expressed as mg GAE/g.

Determination of total flavonoid content (TFC)

The TFC was determined by using the $AlCl_3$ method (Ahmed et al. 2022). Quercetin was used as standard and different concentrations were prepared ranging from 20 mg/mL to 100 mg/mL. Absorbance was measured at 510 nm wavelength and results were expressed as mgQe/g.

Determination of total alkaloid content (TAC)

The TAC was evaluated according to the procedure by Archana et al. (2012).

Antioxidant Analysis

Total antioxidant activity

The TAA was evaluated following the procedure by Govindarajan et al. (2003) and Subhasree et al. (2009) with slight modifications. In brief, SAAE, SAME, ASEE and SACE stock concentrations were made at a concentration of 1 mg/mL. An aliquot of 0.2 mL of each extract was mixed with 1.8 mL of reagent (0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM $(NH_4)_6Mo_7O_{24}$). Then, the reaction mixture was incubated in water bath at 90°C for 90 min, followed by cooling at room temperature.

The absorbance of the samples was measured at 695 nm. TAA results were represented as mg of ascorbic acid equivalent per gram of plant extract.

DPPH radical scavenging activity assay

DPPH radical scavenging assay was carried out according to the protocols described by (Ahmed et al. 2022). The absorbance of the samples was measured at 517 nm.

Ferric Reducing/Antioxidant Power (FRAP) assay

FRAP assay was carried out in accordance with protocol described by (Ahmed et al. 2022). The absorbance of the samples was recorded at 593 nm.

ABTS scavenging assay

ABTS assay was performed according to the method described by Ahmed et al. (2022). The absorbance of the samples was measured at 734 nm.

Determination of iron-chelating ability

The approach developed by Khled et al. (2014) was utilized to calculate the extracts binding capacity to iron (II). The absorbance of the samples was measured at 562 nm.

Peroxidase (POD) assay

1 g of fresh bark was homogenized in 3 mL of 0.1 M Na_3PO_4 buffer (pH 7.0) with a mortar and a pestle. The whole mixture was centrifuged at 18,000 rpm at 5°C for 15 min. The supernatant was separated and used subsequently. Buffer solution (3.0 mL), guaiacol solution (0.05 mL), enzyme extract (0.1 mL), and H_2O_2 solution (0.03 mL) were mixed gently in the cuvette and the absorbance was measured at 470 nm.

Polyphenol oxidase (PPO) assay

The PPO assay was carried out in accordance with Sikora et al. (2019) procedure. The activity was shown as a variance in absorbance at 495 nm during a period from 30 s to 3 min. The enzyme activity was reported as U/mL/min.

Antimicrobial activity of S. auriculata crude extracts

The antibacterial activity of *Senna auriculata* extract was tested for their antibacterial efficacy against selected human pathogens by using the agar diffusion method. The pathogens, such as *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Bacillus megaterium* (MTCC 441), *Bacillus cereus* (MTCC 6840), *Bacillus aryabhattai* (MTCC 14,579), and Gram-negative bacteria such as *Pseudomonas putida* (MTCC 1194), *Escherichia coli* (MTCC 1302), *Klebsiella pneumoniae* (MTCC 4727), *Serratia marcescens* (MTCC 4822) were grown in a nutrient broth at 37°C for 12 h under laboratory conditions procured from the Microbial Type Culture Collection (MTCC), Chandigarh, Government of India. 100 mg of crude plant extracts was suspended separately in 1 mL of acetone, methanol, ethanol and chloroform. The fresh bacterial culture of 100 µL having 108 CFU/mL was inoculated into the agar medium and spread over the plates. Well of diameter 10 mm was punctured in the agar medium using sterile cork borer and filled 2 mg/mL added with 100 µL of each respective extracts and 100 µL of Amoxicillin, Kanamycine was added as positive control; 100 µL of acetone, methanol, ethanol and chloroform was added as a negative control. Amoxicillin was used as a reference compound for antibacterial activity. The culture plates were then incubated at 37°C for 24 h. The clear zone of inhibition around the plant extract was

measured in mm. The experiments were done in triplicate. Plates were then further kept in the laminar air flow for 30 mins for the diffusion of extracts and further incubated at 37°C for 24 h. The antimicrobial activity was assessed by visualizing the presence or absence of inhibition zone and measuring the zone of inhibition.

Time-kill assay

The Time-Kill assay was performed by the broth macro dilution technique (Aiyegoro et al. 2009). The extracts were incorporated into 50 µL of nutrient broth. Control consisted of nutrient broth included with the extract and the relevant alone at the test organism to a final inoculums' density of approximately 10–5 CFU/mL. Extracts of *Senna auriculata* bark were evaluated for the bactericidal activity effect against *Bacillus cereus*. The culture was harvested and added in the Luria broth for 3 mL in each test tube, 5% of previously prepared inoculums was added to each solution tube. Luria broth medium should be used in control, and it should be checked within 3 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h, and 24 h. The culture at different time duration was incubated and kept in the orbital shaker at 120 rpm 24 hr by measuring the optical density (OD) at 600 nm.

$$\begin{aligned} \textit{Bacteria cell viability} &= \textit{OD inoculums} - \textit{OD inoculums with extract} - \textit{OD blank} \\ &\times 100\% \textit{OD inoculums} \end{aligned}$$

In vitro anti-cancer activity

Cell lines and culture method

MDA-MB-231 and vero cells were purchased from ATCC, USA and were cultured in DMEM, MEM and RPMI 1640 medium correspondingly with 10% fetal bovine serum and 1% antibiotics (Penicillin/Streptomycin) and maintained in humidified cell incubator at 37°C and 5% CO_2.

Statistical evaluation

Data presented as mean ± SD of two duplicates of three independent experiments. Investigational data were evaluated by Student's t-test test and one or two way analysis of variance (ANOVA). Significant differentiation between every set of data were measured at the confidence level of $p < 0.05$ & $p < 0.001$.

RESULTS and DISCUSSION

Determination of phytochemical components

Qualitative phytochemical study demonstrated the presence of many bioactive compounds *e.g.* carbohydrates, alkaloids, flavonoids, glycosides, terpenoids, saponins, steroids, tannins, quinones, anthraquinones, phenols, coumarins, and anthocyanins which were heavily concentrated in the acetone extract, whereas they occurred only slightly in the methanol extract. The ethanolic extract contained all compounds except for glycosides and phenols, while the chloroform extract turned out to be the poorest one (Table 1). Alkaloids are secondary nitrogenous chemicals with demonstrated antioxidant activity (Quezada et al. 2006; Tiong et al. 2013). Terpenoids have been employed as a preventative measure against illnesses brought on by oxidative stress (González-Burgos and Gómez-Serranillos 2012. In addition to the antioxidant properties, saponins induce tumor cells to undergo apoptosis (Bi et al. 2012). The antioxidant qualities of tannins have been utilized as active ingredients in medications and drinks (Benzidia et al. 2019). Glycosides including flavonol diglycosides, and monoglycosides of quercetin have also been shown to be potent inhibitors of lipid peroxidation (Plumb et al. 1999). Quinones originating from plants exhibit superior antioxidant

Table 1. Qualitative phytochemicals analysis of *S. auriculata* bark extracts.

	Solvent extract			
Tests	Acetone	Methanol	Ethanol	Chloroform
Alkaloids	+++	++	++	+
Carbohydrates	+++	+++	+++	++
Flavonoids	+++	++	+	-
Glycosides	+	+	-	-
Terpenoids	+++	++	+	-
Saponins	+++	+++	++	+
Steroids	++	+++	++	-
Tannins	+++	+	++	-
Quinones	+++	++	+	-
Anthraquinones	-	+	++	+
Phenols	+++	++	-	++
Coumarins	+++	+++	++	+
Anthocyanins	+++	++	++	-

Heavily present,+++; Slightly present, ++ Present, +; Absent,-

activity when compared to synthetic antioxidants such as BHA (Prokopiev et al. 2024). Anthraquinones, coumarins, and anthocyanins are endowed with numerous biological activities, including antibacterial, antioxidant, anti-inflammatory, and anticancer properties (He et al. 2011; Al-Amiery et al. 2012; Choi et al. 2013).

Total alkaloids, phenolics and flavonoids

Total phenol, total flavonoid and total alkaloid contents, antioxidant activity, free radical scavenging capacity, and enzymatic activity of *S. auriculata* bark extracts are reported in Table 2. The highest total alkaloid content was 5.8% was for the SAAE followed by 4.3% for SAEE and 4.5% for SAME. Only the 3.2% was obtained for the SACE. A large amount of phenolics (752.78 ± 2.25 mg GAE/g) was attained for the SAAE, whereas values of 642.28 ± 2.35, 587.32 ± 3.2, 456.32 ± 2.31 mg GAE/g were found in the SAME, SAEE, and SACE, respectively. The flavonoid content of SAAE was found to be higher (285.78 ± 1.25 mgQe/g) compared to that of SAME, SAEE, and SACE with values of 201.23 ± 12, 182.25 ± 2.3, and 174.20 ± 3.21 mgQe/g. The highest content of phytochemicals in the SAAE may be associated to the biological properties (antihyperglycemic) of the bark, which include increased insulin sensitivity,

Table 2. Total phenol, total flavonoid and total alkaloid contents, antioxidant activity, free radical scavenging capacity, and enzymatic activity of *S. articulata* bark extracts.

	Phytochemical composition			
	Acetone	Methanol	Ethanol	Chloroform
Total phenol content (mg GAE/g)	752.78 ± 2.25	642.28 ± 2.35	587.32 ± 3.2	456.32 ± 2.31
Total flavonoid content (mgQe/g)	285.78 ± 1.25	201.23 ± 12	182.25 ± 2.3	174.20 ± 3.21
Total alkaloid content (%)	5.80 ± 0.64	4.50 ± 0.41	4.32 ± 3.2	3.25 ± 2.32
Antioxidant activity				
Total antioxidant activity (mg AAE/g)	1489.42 ± 4.35	1342.12 ± 3.21	125.12 ± 5.2	95.12 ± 3.12
Reducing power (Abs at 700 nm)	2.40 ± 0.09	1.12 ± 2.31	8.14 ± 12.2	5.15 ± 2.31
Iron chelating ability (%)	32.14 ± 5.38	25.02 ± 4.52	20.15 ± 2.30	15.16 ± 5.41
Free radical scavenging activity				
DPPH radical scavenging activity (% inhibition)	99.45 ±1.23	85.12 ± 3.21	80.32 ± 2.31	50.21 ± 3.22
ABTS radical scavenging activity (%)	88.45 ± 0.98	45.21 ± 6.23	40.15 ± 2.36	30.12 ± 6.52
Enzyme activity				
Peroxidase (U/mL/min)	70.00 ± 2.89	60.32 ± 5.32	52.12 ± 3.52	45.12 ± 5.26
Polyphenol oxidase (U/mL/min)	83.38 ± 1.48	70.14 ± 5.26	65.25 ± 2.15	30.56 ± 8.53

Data are expressed as mean ± SD of 3 replicates.

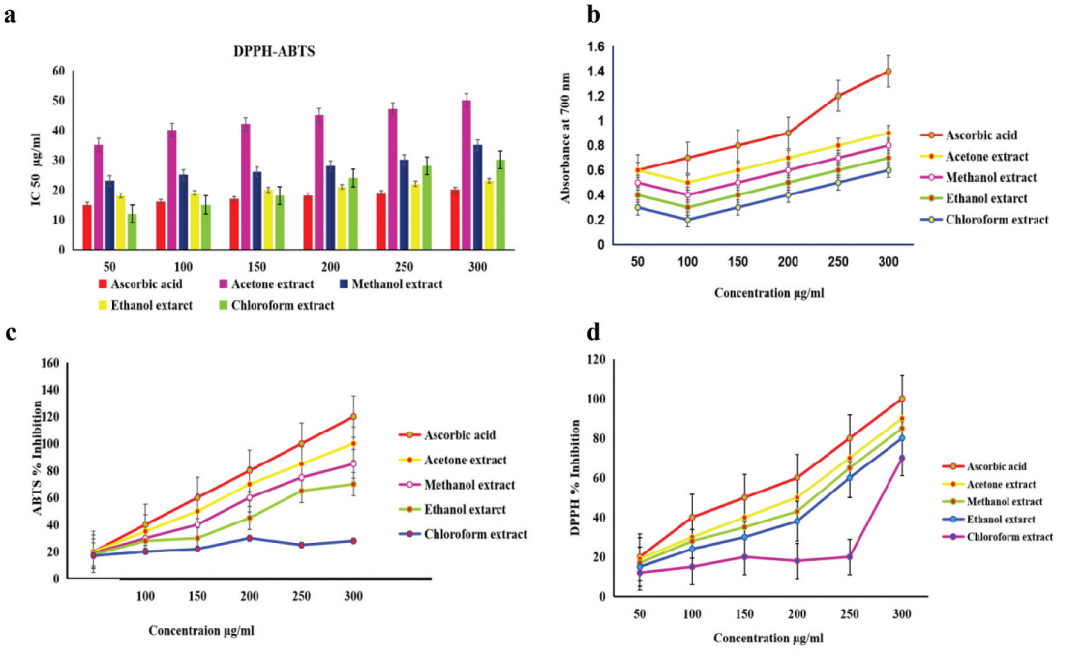

Figure 1. DPPH radical scavenging activity of *senna auriculata* bark extracts at various concentrations (µg/mL).

stimulation of pancreatic β-cell proliferation, improvement in insulin resistance through sensitization, and promotion of glycogen formation (Salehi et al. 2020). The higher antioxidant capacity of SAAE in comparison to SAME, SAEE, and SACE is consistent with the higher content of polyphenolic compounds playing the hydroxyl groups in the phenolic compounds a crucial role in their capacity to scavenge (Figure 1).

GC-MS analyses of acetone extract

GC-MS analysis of the SAAE's extract was performed according to Semwal and Painuli (2019) and a total of 34 compounds were positively identified based on retention times and match with WILEY8. LIB and NIST08.LIB enlisted compounds (Table 3). Most of the components identified and reported here do possess different biological activities e.g. antioxidants, anticancer, antifungal, antibacterial, antiviral, anti-inflammatory, etc. The major components were represented by tetradecanoic acid, 4-Aminodihydro-2(3 H)-furanone and 2-Pentanone, 4-hydroxy-4-methyl. Many of the components reported in this study have already been reported as responsible for many biological activities in other plants; additionally, some of them have been also employed in the fragrance and perfume industries (Swamy and Sinniah 2015; Semwal and Painuli 2019).

Total antioxidant activity

The phosphomolybdate method was used to assess the TAA of the extracts. The different levels of activity that both extracts showed are shown in Table 2. The findings demonstrated that SAAE has a much higher antioxidant capacity (1489.42 ± 4.35 mg AAE/g, followed by 1342.12 ± 3.21, 125.12 ± 5.2 and 95.12 ± 3.12 mg AAE/g for SAME, SAEE, and SACE, respectively. Overall antioxidant activity may be explained by the high content of polyphenols occurring especially in the SAAE and SAME.

Table 3. Volatile compounds identified in the SAAE by GC-MS analysis.

No.	RT	Area%	Compound name	Molecular formula	Molecular weight
1	4.73	0.23	2-Pentanone, 4-hydroxy	$C_5H_{10}O_2$	102.1
2	4.99	1.93	4-Amino-4,5-dihydro-2(3H)-furancarboxylic acid	$C_7H_{12}O$	128.2
3	5.15	7.42	2-Pentanone, 4-hydroxy-4-methyl	$C_6H_{12}O_2$	116.1
4	10.88	0.46	Pentadecanal	$C_{15}H_{30}$	226.4
5	10.91	4.93	Chromium, cycloheptatriene	$C_{17}H_{22}Cr$	278.4
6	11.22	0.51	Cyclotetradecane, 1,7,11-4--(1-methylethyl)	$C_{20}H_{40}$	280.5
7	12.17	4.92	6-(Adamantyl-1)naphthol-2	$C_{20}H_{22}$	278.4
8	12.33	0.56	2-Propenoic acid, 3-(hydroxy-3-methoxyphenyl)	$C_{11}H_{12}O_3$	192.2
9	12.77	0.81	1-Eicosene	$C_{20}H_{40}$	280.5
10	13.02	5.88	Trisiloxane, octamethyl	$C_8H_{24}O_2Si_3$	236.5
11	14.55	0.66	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	697.0
12	16.93	0.76	2-Ethylhexyl trans-4-methoxycinnamate	$C_{18}H_{26}O_3$	290.4
13	16.21	5.84	Benzo[b]triphenylene	$C\ 22\ H_{14}$	278.3
14	17.73	8.00	4-Aminodihydro-2(3H)-furanone	$C_7H_{12}O$	128.2
15	17.74	0.16	Cyclotetradecane	$C_{14}H_{28}$	196.4
16	17.81	0.80	Tricosyl pentafluoropropionate	$C_{26}H_{47}F_5O_2$	486.6
17	18.02	0.81	Tetrapentacontane, 1,54-dibromo	$C_{54}H_{108}Br_2$	917.2
18	18.45	0.83	Tetradecanal	$C_{14}H_{28}O$	212.4
19	19.01	0.18	Hexacosane	$C_{26}H_{54}$	366.7
20	19.13	8.63	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.4
21	19.43	0.49	1,19-Eicosadiene	$C_{20}H_{38}$	278.5
22	20.68	0.58	Tetrapentacontane, 1,54-dibromo	$C_{54}H_{10}Br_2$	917.2
23	20.89	1.32	4-Hydroxybenzyl alcohol, bis(tert-butyldimethylsilyl) ether	$C_{15}H_{24}O$	236.3
24	21.10	0.47	Z-11-Pentadecenol	$C_{15}H_{30}O$	226.4
25	29.28	1.32	Mercaptoacetic acid	$C_2H_4O_2S$	92.1
26	28.97	1.31	Oxalic acid, isobutyl tetradecyl	$C_2H_2O_4$	90.0
27	39.92	1.80	Isopropyl myristate	$C_{17}H_{34}O_2$	270.4
28	42.65	0.19	2-Pentanone, 4-hydroxy-	$C_5H_{10}O_2$	102.1
29	46.43	2.10	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116.1
30	51.31	2.32	Cyclohexadecane	$C\ _{16}H_{32}$	224.4
31	54.63	2.47	Palmitoleic acid	$C_{16}H_{30}O_2$	254.4
32	69.32	0.31	2,6-Diisopropylnaphthalene	$C_{16}H_{20}$	212.3
33	72.45	3.27	Piperidine, 1-(5-trifluoromethyl.	CHFN	153.1
34	81.99	3.70	Phenol, 4-fluoro	C_6H_5FO	112.1

Radical scavenging activity by DPPH

Radical scavenging is an essential procedure for mitigating the deleterious impacts of free radicals. A popular technique for examining the antioxidant capabilities of plant extracts is the DPPH test, which also reveals the presence of flavonoid and phenolic components in plant extracts (Aryal et al. 2019). Figure 1A shows the DPPH activity values for SAAE, SAME, SAEE and SACE. The highest value was attained for the SAAE (99.45 ± 1.23%), followed by SAME (85.12 ± 3.21%), SAEE (80.32 ± 2.31%), and SACE (50.21 ± 3.22%).

Radical scavenging activity of ABTS

The ABTS cations scavenging activity generated by the interaction between ABTS and $K_2S_2O_8$ indicates the special 1 H/e-donating or lowering capacities of plant extracts. In agreement with the data attained by DPPH, SAAE turned out to be the most active one with an inhibition of 88.45 ± 0.98% (Figure 1C), followed by SAME, SAEE and SACE with values of 45.21 ± 6.23%, 40.15 ± 2.36%, and 30.12 ± 6.52% for SAME, SAEE and SACE, respectively.

Iron chelating ability

Iron chelators with ROS scavenging properties include desferroxamine, caffeic acid, and 2,3-dihydroxybenzoic acid. At a concentration of 20 µg/mL, the SAAE exhibited a higher chelating activity (32.14 ± 5.38%), followed by SAME, SAEE and SACE with values of 25.02 ± 4.52%, 20.15 ± 2.30% and 15.16 ± 5.41%, respectively, Chelation therapy uses synthetic chelators, which also have side effects, to counteract iron overload (thalassemia and anemia) and regulate oxidative damage in neurodegenerative diseases. As a result, plant-derived compounds that chelate metal ions may be a safer treatment option.

Antioxidant enzyme activity

The overall activity values for peroxidase (POD) and polyphenol oxidase (PPO) for SAAE were 70.00 ± 2.89 U/mL/min and 83.38 U/mL/min, respectively. These values are substantially greater than those obtained for SAME, SAEE, and SACE (Table 2). Enzymatic defense systems including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) which help humans to withstand the damaging effects of free radicals. Furthermore, POD and PPO shield plants against biotic and abiotic stresses (He et al. 2011). All extracts good enzyme activity and can be used as a natural source of enzymatic antioxidants.

Antibacterial activity of bark and organic extracts

SAAE, SAME, SAEE, and SACE were studied for their potential bactericidal activity against tested pathogens and results are displayed in Figure 2.

All extracts exhibited effective growth inhibitory activity against both Gram positive and negative bacteria. In particular, the SAAE showed a higher zone of inhibition against *S. aureus* (11.12 ± 0.1), *B. subtilis* (10.21 ± 0.2), *B. megaterium* (9.12 ± 0.1), *B. cereus* (9.52 ± 0.1), *B. ariyapattai* (8.25 ± 0.1), *P. putida* (7.81 ± 0.1), *E. coli* (6.85 ± 0.2), *K. pnemoniae* (8.42 ± 0.31)

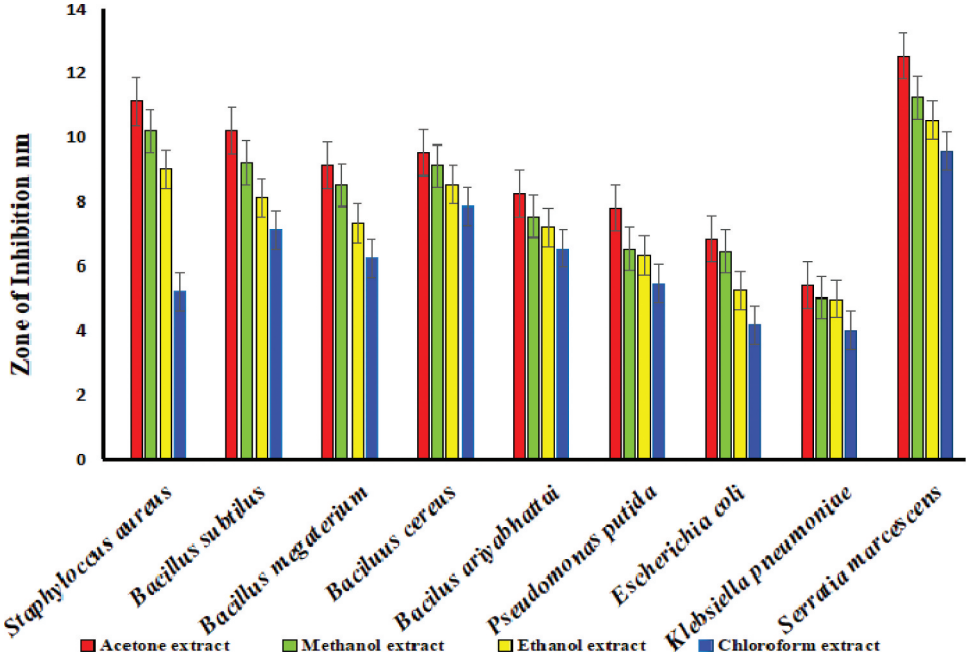

Figure 2. Antibacterial activity of *Senna auriculata* extracts against various microorganisms.

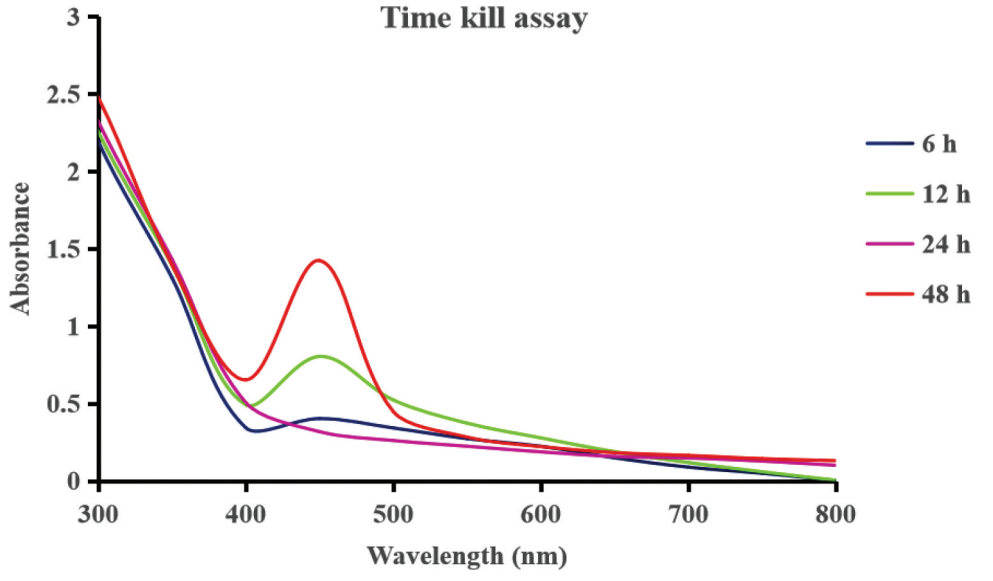

Figure 3. Time-kill assay against *Serratia marcescens*.

S. marcescens (12.54 ± 0.3). SAME showed moderate activity against, *S. aureus* (10.21 ± 0.1), *B. subtilis* (09.21 ± 0.2), *B. megaterium* (8.52 ± 0.1), *B. cereus* (9.12 ± 0.1), *B. ariyapattai* (7.52 ± 0.1), *P. putida* (6.54 ± 0.1), *E. coli* (6.45 ± 0.2), *K. pnemoniae* (5.02 ± 0.31) *S. marcescens* (11.24 ± 0.3). SAEE showed moderate activity against *S. aureus* (9.12 ± 0.1), *B. subtilis* (8.12 ± 0.2), *B. megaterium* (6.32 ± 0.1), *B. cereus* (5.24 ± 0.1), *B. ariyapattai* (4.21 ± 0.1), *P. putida* (7.81 ± 0.1), *E. coli* (6.85 ± 0.2), *K. pnemoniae*,(8.42 ± 0.31) *S. marcescens* (10.54 ± 0.3). However SACE showed lower activity against *S. aureus* (5.21 ± 0.1), *B. subtilis* (7.12 ± 0.2), *B. megaterium* (6.25 ± 0.1), *B. cereus* (7.82 ± 0.1), *B. ariyapattai* (6.54 ± 0.1), *P. putida* (5.46 ± 0.1), *E. coli* (4.15 ± 0.2), *K. pnemoniae* (4.01 ± 0.31) *S. marcescens* (0.956 ± 0.3). The efficacy of the antimicrobial activity of the bark extracts was quantitatively assessed using agar well diffusion method and the results attained were in the order of acetone> methanol > ethanol and chloroform extract. This is probably due to the higher content of bioactive compounds occurring in the *Senna auriculata* extracts. Shanawany Ma (1996) studied the ethanol extract of *S. auriculata* bark against *Bacillus subtilis* and *Klebsiella pneumoniae* inferring the results to the compounds identified e.g. flavanoids, polyphenols tannins, glycoside organic acid and cumarins Increase of antibiotic resistance as well as undesirable side effects of synthetic drugs have triggered immense interest in the search for new antimicrobial agents of plant origin (Alves et al. 2012). The antibacterial activity exhibited by SAAE is comparable with amoxycillin as standard antibiotic and consistent with the results of previous studies (Miceli et al. 2009; Quereshi et al. 2010).

Time-kill assay

Time kill assay was performed by using the SAAE to confirm the bactericidal effect of *S. marcescens* along with the killing rates. The significant reduction in the bacterial population suggested that the extract was highly bactericidal after a 6 h incubation period, while the bacterial colonies were almost wiped out after 48 h incubation (Figure 3).

Growth inhibition and efficacy of the SAAE were observed to be time-dependent providing distinct time-kill profiles for the tested bacteria. Time-kill antibacterial study has been used to

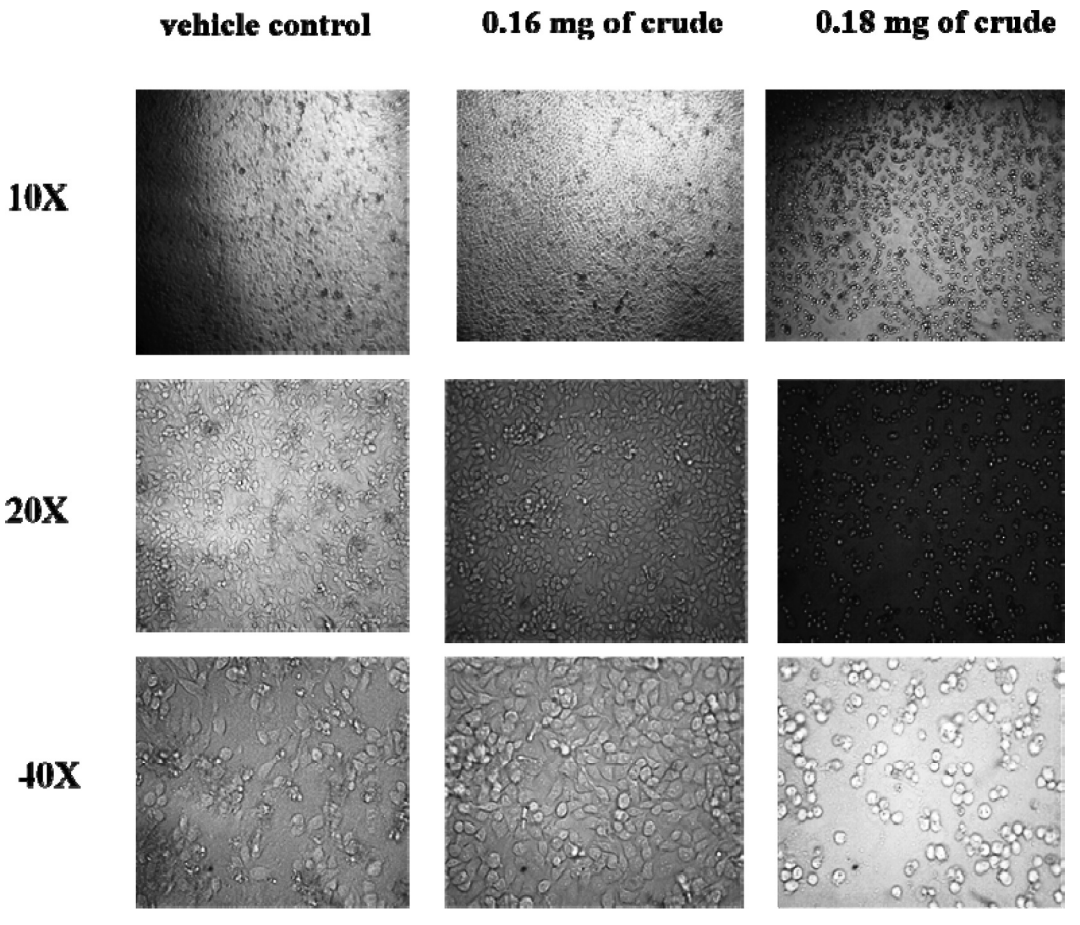

Figure 4. Antiproliferative effect of SAAE on MDA-MB-231 (breast cancer).

investigate numerous antimicrobial agents and they are also often used as the basis for in vitro investigations for pharmacodynamic drug interaction (Ogunwonyi et al. 2010).

In vitro anti-cancer activity

Cancer patients frequently experience bacterial infections and ROS production, thus it is critical to develop drugs that work as antibacterial and antioxidant agents, in addition to their anti-cancer properties. In the current investigation, SAAE and SAME significantly inhibited bacterial growth and scavenged the DPPH radical in comparison to extracts in other solvents. Consequently, the anti-cancer effectiveness of these two extracts on MDA-MB-231 cells (breast cancer) was examined further. After 24 hours of treatment, both were able to elicit MDA-MB-231 cell death (Figure 4) significantly and dose-dependently. In 2009, Prasanna et al. reported the anti-cancer activity in human breast adenocarcinoma MCF-7 and human larynx carcinoma Hep-2 cell lines exhibited by an ethanolic extract of the leaves of this plant. Because of their increased metabolic rate and other unique characteristics that set them apart from normal cells, cancer cells have greater basal levels of ROS (Liou and Storz 2010). A high ROS level is necessary for cancer cells to multiply, develop, and spread. However, an excess of ROS is necessary might cause oxidative stress and even death in cancer cells (Qian et al. 2019). The secondary metabolites present in

plant extracts might inhibit cancer cells from causing DNA damage and activating apoptosis-inducing enzymes leading to desirable cancer suppression.

Conclusion

The present study demonstrated that *S. auriculata* bark extracts and especially acetone do possess considerable antioxidant and antimicrobial activity. GC-MS analysis of the acetone extract highlighted the presence of many volatile bioactive compounds that could contribute to antimicrobial activity. A positive correlation between the bioactive content and free radical scavenging activity was noted. In vitro assays indicated that the acetone bark could act as a direct antioxidant agent through free radical scavenging, or as indirect antioxidants through the induction of enzymes system accountable for antioxidant activity. Also, acetone bark extract showed the capability to suppressing the growth of phytopathogens. Acetone and methanol extracts were also capable to induce MDA-MB-231 cell death significantly in dose dependent manner after 24 h of treatment. However, more investigation is required to ascertain the molecular mechanism of the cytotoxicity exhibited by the extract.

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Data availability statement

Samples will be provided upon reasonable request.

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