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ANTIMICROBIAL ACTIVITY OF AMARANTHUS CAUDATUS EXTRACT AGAINST MULTIDRUG RESISTANT ACINETOBACTER BAUMANNII AND KLEBSIELLA PNEUMONIAE

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Abstract

Nowadays, new and effective antifungal or antimicrobial medications are required due to the rise of drug-resistant organisms. Most people have thought about using medicinal herbs to naturally treat illnesses with bacterial origins. In this study, two multidrug resistant (MDR) bacteria were used as in vitro models to compare the effects of antimicrobial medicinal plant extracts. The antibacterial effects of a Amaranthus caudatus seed extract was elicited by different solvents on Acinetobacter baumannii and Klebsiella pneumoniae were examined in this experimental study using the disc diffusion assay and the minimum inhibitory concentration (MIC) method. GC-MS analysis identified several antibacterial compounds, viz. hexadecane, dodecane, undecane, heptacosane, etc. The extracts' effectiveness was examined through their anti-bacterial and antioxidant activity. Plant extracts with various compounds demonstrated antibacterial activity in the current study. They can therefore serve as a new source of antibacterial compounds.

Keywords: *Amaranthus caudatus*, MDR- *Acinetobacter baumannii, Klebsiella pneumoniae*, GCMS, Antibacterial activity, Anti-oxidant activity.

Introduction

The plant kingdom is one of the most productive ecosystems on the planet and plays a significant role in our daily lives. They provide a variety of nutritional, medicinal, and purifying benefits to the people of the earth. Plants have historically been employed by people for their therapeutic and antibacterial effects [*Jani M et al., 2012*]. Antibiotics are now used more frequently to treat a variety of illnesses as microbial and plant compounds. Frequent use of antibiotics can have negative effects, and individuals may even develop resistance to them [*Sevindik M et al., 2018*]. Antibiotic overuse is harmful to the environment, ecosystem, and health of people. Furthermore, the occurrence of drug-resistant pathogens may increase [*Bandow J et al., 2003*]. Antibiotic resistance is a huge issue that is spreading quickly throughout the world, affecting hospitals as well as society at large when it comes to morbidity, mortality, and healthcare [*Cunha B, 2001*]. The immune system depends heavily on a healthy gut microflora, and taking antibiotics harms and interferes with the good bacteria's ability to do their jobs. Antibiotics are used to treat a variety of bacterial diseases, but doing so stresses the body and damages the environment [*Ahmed E, 2016*]. Plants and their extracts are a good substitute for this.

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Plants, their components, and their products have been used to treat illnesses in both humans and animals since the dawn of time [*Dadasoglu F et al., 2016; Yilar M et al., 2018*]. There is a growing need for new medications to maintain animal health due to the intensive growth of various animal production methods [*Jani Y et al., 2012*].

The use of herbal extracts has become more popular in recent years due to their significant potential as a source of naturally occurring chemicals with anti-inflammatory, anti-bacterial, and antifungal activities [*Hussain A et al., 2008; Naili M et al., 2010*]. The amino acids lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, and tyrosine, among others, are found in species of the genus Amaranthus [*Reyad-ul-Ferdous M et al., 2015*]. Certain antibacterial properties of the Amaranthus species have been described by some writers [*Maiyo Z et al., 2010; Tharun K et al., 2012; Jin Y et al., 2013; Zhang Y et al., 2013*].

In all parts of Venezuela and other tropical and subtropical American nations, *Amaranthus caudatus* is widely dispersed and is regarded as a weed of many subsistence crops, including corn, sorghum, and other legumes [*Olivares E, Peña E, 2009*]. Additionally, it has become localised across Europe, Asia, and Africa. Although this species is underutilised as a crop, its seeds are used as a grain and its leaves and stems as a vegetable. It also has a high output of green matter and an outstanding nutrient content [*Arellano M et al. 2004; Montero-Quintero et al. 2011*].

As a result, multiple drug-resistant bacteria were the leading cause of treatment failure in infectious diseases [*Hang C et al., 2020: Olivares et al., 2009*]. Therefore, it is essential to look for and develop other strategies to control resistant microorganisms. The rational localisation of bioactive phytochemicals with antibacterial activity is one of the ways that may be used [*Arellano M et al., 2004; Montero-Quintero et al., 2011*]. Currently, scientists are looking at plants that contain a wide range of secondary chemicals that can serve as a possible source for different antimicrobial medicines.

The growth of multi-drug resistant (MDR) infections, the recent introduction of strains with lower susceptibility, as well as unfavourable side effects of some antibiotics, pose a danger to the therapeutic efficacy of many currently available antibiotics [*Al-Farsi M et* al., 2005]. Multiple drug resistance (MDR) to diseases is brought on by widespread use of antibiotics and poses a significant mortality risk. Nearly 25 years ago, scientists discovered acquired resistance in *Acinetobacter baumannii* to several routinely used antimicrobial medications, including cephamycin, most aminoglycosides, chloramphenicol, ureidopenicillins, first and second-generation cephalosporins, and aminopenicillins [*Murray B et al., 1929*]. Since then, Acinetobacter baumannii strains have also evolved a resistance to recently created antimicrobial medications. Despite being uncommon in community isolates, multidrug-resistant (MDR) *Acinetobacter baumannii* has become common in several hospitals [*Zeana C et al., 2003*].

One of the most common causes of hospital outbreaks globally is *Klebsiella pneumoniae.* Additionally, *Klebsiella pneumoniae* that is resistant to antibiotics is becoming more and more common in invasive infections that have significant morbidity and fatality rates. The goal of the current study was to determine the antibacterial activity of *Amaranthus caudatus* seed extract against MDR, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*.

Materials and methods

***Collection of samples*:** The seeds of *Amaranthus caudatus* were procured from the local market, and stored at room temperature, purely grounded and stored in sterile containers for extraction (Fig. 1).

Extraction of bioactive compounds using Soxhlet apparatus: *Amaranthus caudatus* seed powder

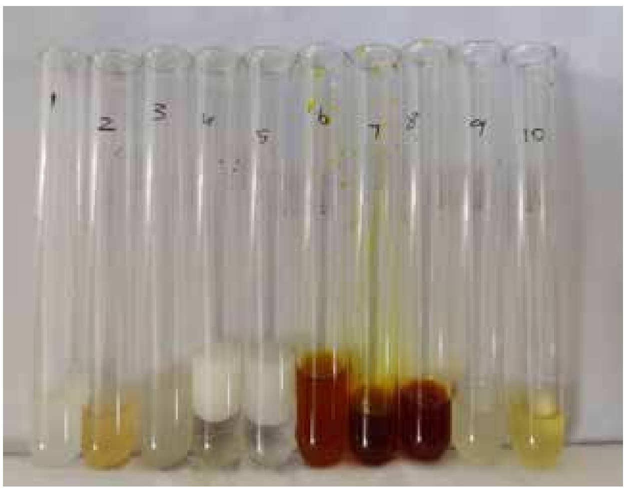

Figure 1. *Phytochemical analysis test*

was contained in a porous cellulose paper bag, which was positioned in the Soxhlet apparatus' thimble chamber. A methanol solvent solution was added to the extractor, which was then heated to 60°C and left for 6 hours. The extracts were collected, the solvent was evaporated, and they were stored in sterile containers (Fig. 2).

***Phytochemical analysis*:** A preliminary qualitative phytochemical analysis was conducted to determine the secondary metabolites present in the various alcoholic and aqueous extracts of *Amaranthus caudatus* seeds.

Gas Chromatography Mass Spectrometry (GCMS) Analysis: GC analysis: A GC-MS system with a gas chromatograph interfaced to a mass spectrometer (GC-MS) was used to conduct the analysis under the following circumstances: 100% Dimethyl polysiloxane-based column Elite-1 fused silica capillary column operating in electron impact mode at 70 eV; helium (99.999%) was utilised as the carrier gas with a continuous flow of 1 ml/min and an injection volume of 0.5 EI was used (split ratio of 10:1) The ion source temperature is 280 °C, and the injector temperature is 250°C. The oven temperature was set to begin at 110°C (isothermal for 2 minutes), increase at a rate of 10°C/min to 200°C, then decrease at a rate of 5°C/min to 280°C, and terminate with a 9-minute isothermal at 280°C.At 70 eV, a scan interval of 0.5 s, and fragment sizes ranging from 40 to 550 Da, mass spectra were recorded [*Zeana C et al., 2003*].

***Antibiotic sensitivity testing (ABST)*:** Using the dehydrated medium, prepare MHA as directed by the manufacturer. Distilled or deionized water should be used to prepare the media. The medium must boil and be heated while being stirred frequently. Sterilize for 15 minutes at 121°C in an autoclave. The agar medium should be chilled to 40-50°C. Pour the agar into a clean, flat petri dish made of glass or plastic to a uniform depth of 4 mm, then wait for it to set. A sterile cotton swab should be dipped into the predetermined bacterial suspension. By streaking the agar with the inoculum-containing swab, you inoculate it. Repeat the rubbing process after rotating the plate by 60 degrees. Repetition twice. As a result, the inoculums will be distributed evenly. Placing an antibiotic disc on the inoculated and dried plate's surface requires the use of sterile forceps or a disc dispenser. As soon as possible, lightly press it down with the tool to make sure the disc is completely in touch with the agar surface. Once a disc has made contact with the agar surface, do not move it again because instantaneous drug diffusion happens. Plates should be incubated upside-down at 30°C or at another temperature that promotes growth. The zone of inhibition should be visible after 16 to 18 hours. Longer incubation periods may be necessary for species with slow growth.

***Antibacterial activity (ABA)*:** By using the well diffusion method, the antibacterial sensitivity rate of the crude extracts was examined against MDR *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Muller Hinton Agar was made, sanitised, and then placed into plates. The test pathogens were developed in overnight cultures, and 0.1% of each test organism's liquid culture was streaked across the Petri plate using a cotton swab while the plate was rotated at various angles. Each plate's agar surface had 6mm borer holes drilled into it. *Amaranthus caudatus* crude extracts were extracted from the seeds and placed into the wells at various concentrations. The plates were then incubated in an incubator at 37°C for 48 hours. On each plate containing the test pathogens, the inhibitory zones around the wells were measured to evaluate the antibacterial activity. While observing the development of clear zones (Dhanasekaran *et al*., 2005), millimetre measurements were taken.

***Determination of minimum inhibitory concentration (MIC)*:** The broth dilution method was used to estimate the Minimum Inhibitory Concentration of the crude ethyl acetate extract of *Amaranthus caudatus* seeds [*Clinical and Laboratory Standard Institute, 2012*]. In sterile 96-well plates with Muller-Hinton broth medium, the crude ethyl acetate extract of *Amaranthus caudatus* seeds were prepared. Following that, each well received 50 L of bacterial inoculum at a density of 10^5 CFU mL-1 and was incubated overnight at 37°C. The extract's minimum inhibitory concentration (MIC), which prevents the growth of test organisms, was noted.

Anti-oxidant activity

***DPPH*:** Different extracts' capacities to neutralise free radicals were assessed using Kulisic's method [*Kulisic T et al., 2004*] with a few changes. 50 mL of sample solution at various concentrations (ranging from 25 to 400 g/mL) were combined with 950 mL of a DPPH methanolic solution (3.4 mg/100 mL). The reaction mixture was left to sit at 37°C in the dark for one hour. The extracts' ability to scavenge free radicals was demonstrated by the initial purple colour's removal. The reaction mixture's absorbance was measured with a UV-visible spectrophotometer at 517 nm (Agilent 8453, Germany). The positive control used was ascorbic acid. DPPH scavenging capacity was calculated by using the following formula

$$Sa = Ac - \frac{As}{Ac} \times 100$$

where Sa -Scavenging activity (%); Ac - (Absorbance control) and As - Absorbance sample.

***Ferric reducing antioxidant power (FRAP) assay*:** Incubate FRAP solution (3.6 mL) in distilled water (0.4 mL) for 5 minutes at 37 °C. This solution was then combined with an 80 mL concentration of plant extract, which was then incubated at 37°C for 10 min. At 593 nm, the reaction mixture's absorbance was gauged. The five concentrations of FeSO4, 7H2O (0.1, 0.4, 0.8, 1, 1.12, and 1.5 mM) were used to form the calibration curve, and the absorbance values were determined as for sample solutions [*Al-Farsi M et al., 2005*].

***Phosphomolybdenum assay*:** The Prieto *et al.* technique was used to carry out the phosphomolybdenum assay [*Prieto P et al., 1999*]. 0.1 mL of sample solution at various concentrations (ranging from 25 to 400 g/mL) was treated with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). For 90 minutes, the tubes were incubated in a water bath at 95°C. The samples' absorbance at 765 nm was measured after they had been cooled to room temperature. Ascorbic acid was used as the positive control. Antioxidant capacity was estimated by using following equation:

$$Aa = Ac - \frac{As}{Ac} \times 100$$

where A -Antioxidant activity (%); Ac - (Absorbance control) and As - Absorbance sample.

Anti-inflammatory activity

***Albumin denaturation assay*:** Egg albumin denaturation was used to measure the anti-inflammatory effects of *Amaranthus caudatus* seed extracts. A reaction mixture containing egg albumin (0.2 ml), phosphate saline buffer (PSB, pH 6.4), and *Amaranthus caudatus* seed extract (with varying concentrations of 100 g/ml, 250 g/ml, 500 g/ml, 750 g/ml, and 1000 g/ml) was prepared. The reaction mixture contained these components: 100 g, 2.8 mL PBS The control was made with the same amount of sterile distilled water and incubated for 15 minutes at 37.2°C. After the incubation period, it was heated for 5 minutes at 70°C. The absorbance was then determined at 660 nm. Aspirin was utilised as a reference at the same concentrations and absorbance.

The following formula was used to estimate the protein denaturation inhibition:

$$Inh = (1-\frac{OD2- OD1}{OD3- OD1}) \times 100$$

where Inh - % inhibition of egg albumin denaturation; OD1=Unheated test sample; OD2=heated test sample and OD3=heated control sample.

***Heat induced haemolysis*:** The reaction mixture (two millilitres) contained one millilitre of 10% RBC suspension and one millilitre of test sample at various concentrations (ranging from 100 to 500 g/ml). In the control test tube, saline was used in place of the test sample. Aspirin was a widely prescribed medication. All centrifuge tubes containing reaction mixtures underwent a 30-minute incubation in a water bath at 56°C. The tubes were cooled under running water after the incubation period. Centrifuging the reaction mixture at 2500 rpm for 5 minutes allowed the supernatants' absorbance to be measured at 560 nm. For each test sample, the experiment was run in triplicate.

RESULTS

***Phytochemical analysis*:** The results of phytochemical analysis (Table 1) revealed the presence of Tannins, flavanoids, phenol and proteins, whereas other phyohemicals like saponins, steroids, terphenoids, glycolides, alkaloids, quinons shows negative result. (Figure 1)

***Antibiotic sensitivity test (ABST)*:** After the incubation period (up to 24 hours), during which the antibiotic diffuses across the plate and creates a gradient of concentrations around the disc, the test findings were analysed. Bacteria are more likely to proliferate as the antibiotic concentration falls. According to CLSI (Clinical and Laboratory Standards Institute) guidelines, the width of the area showing no growth is evaluated to indicate suscep-

TABLE 1.

Phytochemical screening of *Amaranthus caudatus* seed extract

Sl. No.	Phytochemicals	Result
1	Tannins	+
2	Flavanoids	+
3	Saponins	-
4	Steroid	-
5	Terphenoids	-
6	Glycolides	-
7	Phenol	+
8	Alkaloids	-
9	Quinons	-
10	Proteins	+

tibility. Greater antibiotic susceptibility is associated with decreased bacterial growth in larger zones, whereas reduced antibiotic susceptibility is associated with higher bacterial growth in smaller zones. Hence, there is no zone of inhibition if the antibiotic does not prevent growth, hence the bacteria are resistant.

By observing the zone of inhibition, the organism *Klebsiella pneumoniae* shows larger zone around the antibiotic cefmetazole. Thus, this organism is more susceptible to bacterial growth (Figure 2).

Bactericidal activity: After overnight incubation, the growth of test organisms such as *Acinetobacter baumannii* and *Klebsiella pneumoniae* were inhibited at 50 mg and 100 mg concentration range respectively. The bacterium is overnight cultured and diluted between 1 x 10^5 and 1 x 10^6 cfu/ml in Mueller Hinton Broth. The test chemicals are prepared using 2-fold serial dilutions. The preparation of the control tubes or wells, positive and negative, the development of the microbe is shown by turbidity following the appropriate temperature and time-based incubation of the tubes or microtiter plates. Two ad-

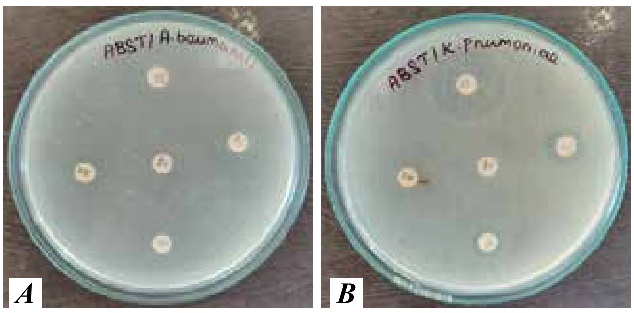

FIGURE 2. *Antibiotic sensitivity testing of Acinetobacter baumannii (A) and of Klebsiella pneumoniae (B)*

TABLE 2.

Result for bactericidal activity of the two organisms for four different concentrations

	Concentrations			
Organisms	**1 g**	**0.5 g**	**0.25 g**	**0.125 g**
Acinetobacter baumannii	22 mm	17 mm	14 mm	-
Klebsiella pneumoniae	24 mm	18 mm	15 mm	-

ditional concentrated test product dilutions are plated and counted to determine viable CFU/ml, and the dilution corresponds to the MIC. The organism *Klebsiella pneumoniae* (Fig 3A) shows the presence of clear zone around three different concentrations such as 0.25 g, 0.5 g, and 1g. Absence of zone in 0.125 g. Whereas in *Acinetobacter baumannii* (Fig. 3B), the presence of clear zone is seen and measured in three different concentrations such as 0.25 g, 0.5 g, and 1 g. Zone is absent in 0.125 g. The diameters for both the organisms were measured and shown in the Table 2.

Anti-oxidant activity

Ferric reducing antioxidant power (FRAP). Table 3 presents the findings of the FRAP assay. The amounts of antioxidants with a ferric reducing capacity equal to that of 1 mM of $FeSO_4$ were used to express the antioxidant activity.The anti-oxidant activity for 25 mg results in 19.20%, for 50 mg the activity was found to be 21.94%, for 75 mg the FRAP percentage was found to be 28.72 %, for 100mg it results in 35.38 %, whereas for 250 mg the percentage was 52.64%, 500 mg the percentage was 63.32%, for 750mg the percentage was found to be 68.49%, and for 1000 mg, the anti-oxidant activity was found to be 80.17% (Table 3, Figure-4).

DPPH (2, 2diphenyl1picrylhydrazyl) free radical scavenging assay: The DPPH assay findings were tabulated in the table given below (Table 3, Fig-

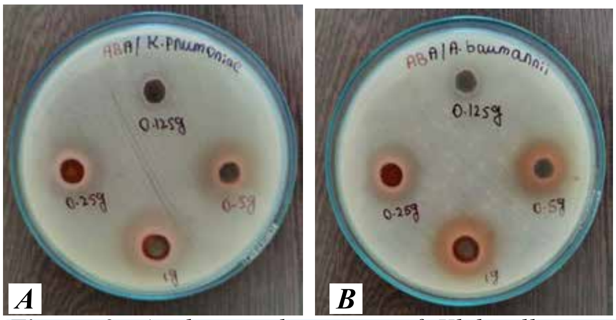

Figure 3. *Antibacterial activity of Klebsiella pneumoniae (A) and Acinetobacter baumannii (B)*

Table 3.

Anti-oxidant and anti-inflammatory activity for various concentration

Concentration (µg/mL)	Anti-oxidant activity (%)			Anti-inflammatory (%)	
	DPPH	FRAP	Phosphomolybdenum	Albumin	Heat-induced
25	0.784 ± 0.2	19.10 ± 3.6	12.98 ± 6.6	23.10 ± 11.2	13.96 ± 6.4
50	3.37 ± 1.2	21.94 ± 7.3	25.16 ± 12.3	32.91 ± 9.6	14.71 ± 3.1
75	3.49 ± 0.7	28.72 ± 11.2	39.02 ± 16.3	39.87 ± 12.4	14.98 ± 5.2
100	21.24 ± 4.2	35.38 ± 13.3	55.88 ± 22.2	48.90 ± 14.6	15.07 ± 6.5
250	26.24 ± 5.1	52.64 ± 16.8	78.76 ± 17.2	56.13 ± 10.8	15.43 ± 8.3
500	48.68 ± 7.3	63.32 ± 15.5	79.10 ± 17.2	64.14 ± 7.3	16.60 ± 4.4
750	50.32 ± 8.2	68.49 ± 12.9	92.59 ± 25.5	71.57 ± 11.6	29.68 ± 11.7
1000	51.64 ± 6.7	80.17 ± 17.1	98.67 ± 23.7	89.65 ± 15.2	36.17 ± 13.3

Experiments were carried out in triplicates and expressed in Average ± SD

ure-7). The anti-oxidant activity for different concentrations such as 25 mg, 50 mg, 75 mg, 100 mg, 250 mg, 500 mg, 750 mg, and 1000 mg were found to be 0.784%, 3.37%, 3.49%, 21.24%, 26.24%, 48.68%, 50.32%, and 51.64% respectively.

Phosphomolybdenum assay**:** The sample was absorbed at 765 nm. The anti-oxidant activity for eight different concentration levels such as 25,50,75,100,250,500,750,1000µg resulted in 12.98%, 25.16%, 39.02%, 55.88%, 78.76%, 79.10%, 92.59%, 98.67% respectively. This is shown in the table (Table 3, Figure-5).

Anti-inflammatory activity

Albumin denaturation assay**:** According to the albumin denaturation assay, protein denaturation produces inflammatory and arthritic conditions, according to the albumin denaturation assay. Consequently, phytochemicals with the potential to decrease protein denaturation can be used in the creation of painkillers. Eight different concentrations of *Amaranthus caudatus* seed extracts were tested for their ability to reduce inflammation and Aspirin is used as the standard. Results are tabulated (Table 4). The percentage of antioxidant activities for various concentrations such as 25 mg results in 23.10 %, 50 mg results in 32.91%, 75 mg results in 39.87%, 100 mg results in 48.90%, 250 mg shows 56.13%, 500 mg results in 64.14%, 750 mg results in 71.57%, and 1000 mg results in 89.65% (Table 3, Figure-6).

Heat induced haemolysis**:** The extract was effective in inhibiting the heat induced haemolysis at different concentrations. The anti-inflammatory activity for the given extracts for various concentrations such as 25mg, 50mg, 75mg, 100mg, 250mg, 500 mg, 750 mg,1000mg resulted in 13.96%. 14.71%, 14.98%, 15.07%, 15.43%, 16.60%, 29.68%, 36.17% respectively (Table 3).

GCMS analysis**:** Gas Chromatography Mass Spectroscopy (Graph 1) use for deduction of bioactive metabolites in the volatile samples. *Amaranthus caudatus* seed extract was given for the GCMS analysis to TUV SUD South Asia Private limited.

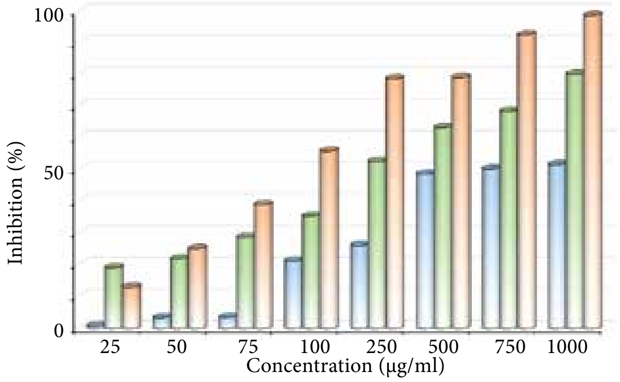

Figure 4. *Anti-oxidant activity analysis*

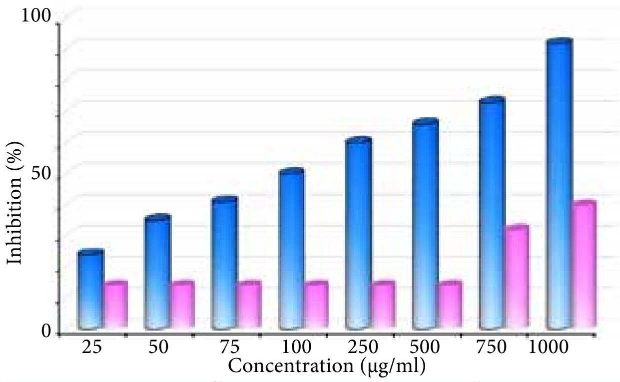

Figure 5. *Anti-inflammatory activity analysis*

According to the results shown in the table below, the compound undecane, 4,7-dimethyl, has a retardation time of 6.642 seconds and a percentage area of 2.68%. The chemical hexane, 3,3-dimethyl, has an area of 0.98% and a retardation time of 6.731. Heptacosane has a retardation time of 9.631 and an area of 1.13%. The retardation time for the chemical dodecane, 2,6,11-trimethyl, was 9.786 seconds, and the area percentage was 2.39%. The compound dodecane, 4-methyl, was subjected to a retardation time of 10.008 with an area of 0.93%. (Table 4, Figure-6)

DISCUSSION

Due to their anti-cancer, anti-inflammatory, and anti-diabetic qualities, phytochemicals have been said to provide protection against diabetes, cardiovascular disease, and cancer. According to epidemiological research, eating vegetables can help avoid degenerative diseases [*Li B et al. 2015*], and research has demonstrated a strong link between vegetable phytochemicals and antioxidant activity [*Olajire P, Azeez E, 2011*]. However, a variety of parameters, including soil quality (chemical form,

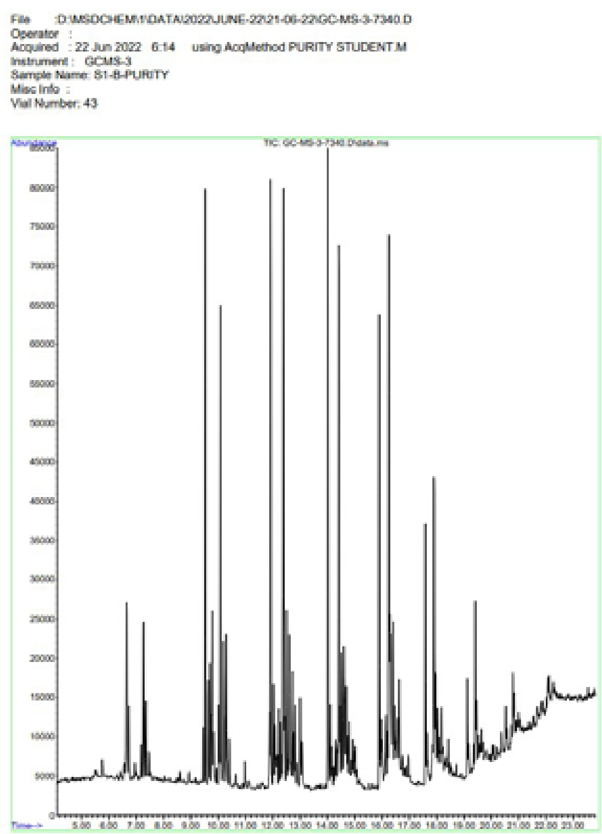

***FIGURE 6.** Gas chromatography mass spectrometry data of the bioactive metabolites*

TABLE 4.

GCMS results for different compounds along with RT and area percentage

Sl. No.	RT	Area %	Compounds
1.	6.642	2.68	Undecane, 4,7- dimethyl-
2.	6.731	0.98	Hexane, 3,3-dimethyl-
3.	7.275	2.01	Undecane, 5- methyl
4.	7.353	0.91	Heptadecane, 2,6,10,14-tetramethyl
5.	9.519	5.18	Octane, 6-ethyl-2-methyl
6.	9.631	1.13	Heptacosane
7.	9.697	1.05	Hexane, 3,3-dimethyl-
8.	9.786	2.39	Dodecane, 2,6,11-trimethyl
9.	10.008	0.93	Dodecane, 4-methyl-
10.	10.075	4.32	Dodecane, 2,6,11-trimethyl-
11.	10.186	2.25	Dodecane, 2,6,11-trimethyl-
12.	10.286	2.16	3-Ethyl-3-methylheptane
13.	11.919	6.75	Hexadecane
14.	12.019	1.05	Docosane, 11-butyl-
15.	12.397	7.59	Decane, 2,3,7-trimethyl-
16.	12.497	1.61	Hexadecane
17.	12.597	2.26	Docosane, 11-butyl-
18.	12.719	1.55	Tridecane, 1-iodo-
19.	12.819	0.89	2-Bromo dodecane
20.	12.997	1.24	Diethyl Phthalate
21.	14.019	5.97	Heneicosane
22.	14.419	6.64	Octacosane
23.	14.508	1.62	Dodecane
24.	14.597	2.20	Heptadecane, 9-octyl-
25.	14.685	0.83	2-Bromo dodecane
26.	15.896	4.38	Decane, 2,3,5-trimethyl-
27.	15.963	1.06	Pentadecane
28.	16.152	1.20	Octacosane
29.	16.252	9.17	Tetratriacontane
30.	16.396	2.01	2- Bromo dodecane
31.	16.463	1.51	Heptacosane
32.	16.574	1.34	Octadecane, 2-methyl-
33.	16.630	1.47	Hexadecane
34.	17.585	2.52	Elcosane
35.	17.896	3.57	Octadecane,1-iodo-
36.	17.952	0.85	Disulfide, di-tert-dodecyl
37.	18.029	0.83	Eicosane
38.	18.174	0.97	Heptadecane
39.	19.118	1.11	Tetradecane
40.	19.407	1.83	Tetratriacontane

bioavailability, and mobility), photosynthetic pigment content, and climatic circumstances, affect the composition and quantity of phytochemicals in vegetables [*Chandra C et al. 2014; Leonov I et al. 2015; Li I et al. 2015; Schlich and Hund-Rinke 2015*].

Phytochemical examination of the *Amaranthus caudatus* seed extracts revealed the presence of several metabolites, including tannins, flavonoids, phenols, and proteins. The bioactive metabolites in the volatile samples are established using GCMS techniques (Graph 1). Antibiotic sensitivity testing was carried out using five different gradient concentrations against two MDR pathogens, such as *Acinetobacter baumannii* and *Klebsiella pneumoniae*. The zone of inhibition was seen, and its resistance and susceptibility were deciphered. A greater zone around the drug Cefmetazole (CMZ 30) is visible in *Klebsiella pneumoniae*. This demonstrates that *Klebsiella pneumoniae* is more prone to bacterial development. Four concentrations were used to demonstrate antibacterial activity. The clear zone is visible in this test in three distinct concentrations in both *Klebsiella pneumoniae* and *Acinetobacter baumannii.*

The zone's diameter is then measured and evaluated. The minimal inhibitory concentration of the extract from *Amaranthus caudatus* is demonstrated by the test organisms *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Both organisms' MIC values were discovered to be 50 mg and 100 mg, respectively. MBC works well for screening purposes. Additionally, it displays the inhibitory range for *Acinetobacter baumannii* at 25 mg and 50 mg (*Klebsiella pneumoniae*). Then, using various concentrations, the FRAP assay finds the antioxidant activity. The spectrophotometer was used to read the concentration ranges. Results from DPPH measurements were read using a spectrophotometer at various concentration levels (Table 3). Inflammatory and arthritic diseases are brought on by protein denaturation. *Amaranthus caudatus* seed extracts were tested at eight different concentrations to see if they might lessen inflammation. At various concentrations, the extract proved efficient in preventing heat-induced hemolysis.

Conclusion

The antibacterial effects of a *Amaranthus caudatus* seed extract was elicited by different solvents on *Acinetobacter baumannii* and *Klebsiella pneumoniae* were examined in this experimental study using the disc diffusion assay and the minimum inhibitory concentration (MIC) method. GC-MS analysis identified several antibacterial compounds, viz. hexadecane, dodecane, undecane, heptacosane,etc. . The extracts' effectiveness was examined through their anti-bacterial and anti-oxidant activity. Plant extracts with various compounds demonstrated antibacterial activity in the current study. They can therefore serve as a new source of antibacterial compounds.

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