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Research Article

Phytopharmacognostical And Pharmacological Evaluation Of Amaranthus Caudatus Seeds Against Staphylococcus Aureus And Salmonella Typhi

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ABSTRACT

This research explores the potential antibacterial efficacy of *Amaranthus caudatus* seeds against two prominent bacterial strains, *Staphylococcus aureus* and *Salmonella typhi*. Given the rising concern of antibiotic resistance, there is a pressing need to explore alternative sources of antibacterial agents. *Amaranthus caudatus*, commonly known as love-lies-bleeding or tassel flower, has been historically recognized for its medicinal properties. However, its antibacterial potential against clinically relevant pathogens remains relatively underexplored. Soxhlet extraction method has been considered as the most efficient extraction method due to its ability to provide the high yield. Methanol and distilled water were used as the solvent for the extraction of *Amaranthus caudatus* seeds and it was found that methanolic extract have the higher yield in comparison to the aqueous yield. Due to the presence of phytochemicals like, flavonoids, terpenoids, alkaloids, and phenolic compounds, the plant shows the anti-bacterial activity. During the measurement of zone of inhibition, both the extracts showed greater activity towards the *Salmonella typhi* in comparison to the *Staphylococcus aureus*. And 20% concentration was found to be Minimum inhibitory concentration. Through a series of experimental approaches, this study aims to elucidate the antibacterial activity of *Amaranthus caudatus* seeds and provide insights into its mechanism of action against *Staphylococcus aureus* and *Salmonella typhi*.

Keywords: *Amaranthus caudatus*, Pharmacological properties, Anti-bacterial, Zone of inhibition

1. INTRODUCTION

Since bacteria lack additional membrane-bound organelles and an identifiable nucleus, they are classified as prokaryotic-cells, unlike eukaryotic-cells. Rather, the nucleoid region contains a single circular chromosome that contains their genetic material. (Galperin et al., 2018) Bacteria also have ribosomes for protein synthesis and a cell wall to give structural support and protection. (Fromm et al., 2023) Bacterial infections are spread by coming in direct-interaction with diseased people, polluted surfaces, or the ingestion of tainted food or beverages. Airborne transmission can also occur via respiratory droplets produced by coughing or sneezing. Bacterial infections are more likely to occur when people practise poor hygiene and have impaired immune systems. (Roberts et al., 2019) On the basis of cell-wall composition, bacteria can be classified into 2 types-gram +ve and gram -ve bacteria. (Fisher et al.,) Gram +ve bacteria retain the crystal violet stain used in the Gramme staining procedure, which indicates a thick coating of peptidoglycan in the cell-walls. (Pasquina et al., 2020) While Gram -ve bacteria lose their crystal-violet stain and appear red or pink, suggesting a thinner coating of peptidoglycan surrounded by an outer membrane. (Ruhal et al., 2021)
Gram-positive bacteria are a wide group of organisms with varying properties. Here are a few typical types: *Staphylococcus, Streptococcus, Enterococcus, Clostridium, Bacillus,* etc. (Bose et al., 2020)
Staphylococcus aureus is a bacterium that is regularly found on human skin and nasal passages. While it can persist in the body without causing harm, it can also cause a variety of infections, including minor skin ailments and life-threatening illnesses including pneumonia, endocarditis, and septicemia. (Heaton et al., 2020 and Cheung et al.,2021) Its propensity to create toxins and develop resistance to medicines, particularly

methicillin-resistant Staphylococcus aureus (MRSA), presents substantial issues in hospital settings. (Algammal et al., 2020)
Gram-negative bacteria are a complex category that includes numerous species, some of which are harmful to humans and others that are helpful or neutral. Here are a few typical types: *Escherichia coli, Salmonella, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Klebsiella pneumoniae*, etc. (Alav et al., 2021)
Salmonella typhi is the bacteria that causes typhoid fever, a systemic sickness marked by high fever, stomach discomfort, headache, and, in extreme instances, intestinal perforation or death. It spreads by contaminated food and water, particularly in places with inadequate sanitation. Although medicines may effectively cure typhoid fever, prevention through immunisation and sanitary measures remains critical in endemic areas. (Kaluse et al., 2021)
The drugs that are used to kill or inhibit the bacteria are called antibacterial drugs. (Hoffman et al., 2020) Herbal medications provide a natural option for treating bacterial infections, perhaps with fewer side effects than synthetic antibacterial pharmaceuticals, while also boosting the immune system. (Ahmadi et al., 2022) *Amaranthus caudatus* has substantial antibacterial effects, limiting the development of many harmful bacteria, which may be related to its bioactive components like as phenolics and flavonoids. (Jimoh et al., 2020) *Amaranthus caudatus*, commonly known as love-lies bleeding, is a stunning decorative plant native to the Andes that is extensively cultivated across the world, including India. It flourishes in varied conditions across India, from the subtropical regions of the north to the tropical portions in the south. (Martinez et al., 2020) Apart from antibacterial activity, *Amaranthus caudatus* is also responsible for various pharmacological properties like anti-inflammatory, anti-diabetic, antioxidant, anthelminthic, anticancer and wound healing properties. (Sishu et al., 2023) In this research, we evaluated the function of seeds of *Amaranthus caudatus* in preventing infections caused by *Staphylococcus aureus* and *Salmonella typhi*.

Pharmacological property of *Amaranthus caudatus*: *Amaranthus caudatus* contains a wide variety of pharmacological properties like Anti-microbial activity, anthelmintic activity, antioxidant activity, cardioprotective activity, hepatoprotective activity, anti-cancer activity, etc. (Jimoh et al., 2019) which are shown in the following table:

Table no.1: showing pharmacological properties of *Amaranthus caudatus* due to the presence of chemical constituents.

S. No.	Pharmacological property	Chemical constituents
1.	Anti-microbial activity	Phenols, alkaloids and terpenoids
2.	Oxidative stress and degenerative disease	Betalains, flavonoids
3.	Anthelmintic activity	Polyphenols
4.	Antioxidant activity	Squalene
5.	Cardioprotective activity	Poly-unsaturated fatty acids, linoleic acid, vitamin F
6.	Anti-cancer activity	Lectin
7.	Hepatoprotective activity	Amaranthine

2. MATERIALS. AND. METHODS.

2.1 Materials.

2.1.1. Collection. and authentication of seeds of *Amaranthus caudatus*

The Seeds of the plant, *Amaranthus caudatus* were collected from local market of Dehradun (Uttarakhand). The seeds were dried under the shade for 2-3 days. Then the seeds were crushed using the mortar and pestle to coarse powder. The seeds of *Amaranthus caudatus* were identified and certified by botany scientist Dr. Anil Kumar, Department of botany, SDM Government PG College, Doiwala, Dehradun (Uttarakhand) on 29 January 2024.

2.1.2. Solvents used for extraction

The solvents used for the extraction of seeds of *Amaranthus caudatus* were
- Distilled water
- Methanol.

2.1.3. Name of organisms for anti – microbial activity

Gram +ve microorganisms
- *Staphylococcus aureus*

Gram -ve microorganisms
- *Salmonella typhi*

2.1.4. Apparatus and equipment

The following apparatus and equipment were used for the extraction of seeds of *Amaranthus caudatus*:

Apparatus: Round bottom flask, beaker, Soxhlet chamber, condenser, petri dish, china dish.
All the apparatus were then collected and sterilized in hot air oven for 1hour at 60°c.
Equipment: hot air oven, heating mantle, rectangular water bath.

2.2. Methodology

2.2.1. Extraction process

The extraction was done according to the procedure of (López et al., 2014) with slight modifications.

Methanolic extraction:

The collected seeds were shade dried and grinded into coarse powder using the mortar and pestle. Then the coarse powder of seeds filled into a thimble that is made with muslin cloth. Soxhlet method was utilized for the extraction of *Amaranthus caudatus* seeds. Soxhlet extraction was carried out using seeds (50g) with 500 ml of solvent, methanol (ratio 1:10) and performed for 18 hrs at 50°c temperature to get the high yield. After the extraction, the solvent was evaporated using rectangular water bath at 90°c till it becomes semi-solid in nature. Then the semisolid extract kept into the vials in the refrigerator until analysis.

Fig. No. 1: Process of methanolic extraction of *Amaranthus caudatus* seeds.

Aqueous extraction:

Same procedure was utilized for the aqueous extraction of the *Amaranthus caudatus* seeds. The seeds were collected, shade dried and then crushed into coarse powder with a mortar and pestle. The coarse seed powder was then put into a muslin fabric thimble. The Soxhlet technique was used to extract *Amaranthus caudatus* seeds. To get a high yield, Soxhlet extraction was done using 50g seeds, 500 ml of solvent, and distilled water (ratio 1:10) for 18 hours at 50°C. After extraction, the solvent was evaporated in a rectangular water bath at 90°C till it became semi-solid. The semisolid extract was then placed in vials and stored in the refrigerator until analysis.

Fig. No. 2: Process of Aqueous extraction of *Amaranthus caudatus* seeds.

2.2.2. Yield percentage

Yield percentage calculates the percentage of theoretical yield obtained in a reaction. Because two solvents (methanol and distilled water) were employed to extract the seeds of *Amaranthus caudatus*, the % yield for each solvent was computed using the formula below:

Yield %age = Actual yield/ Theoretical yield × 100

2.2.3. Phytochemical-screening:

The phytochemical screening was done for alkaloids, phenols, terpenoids, and flavonoids, (Shaikh et al., 2020) as indicated in the table below:

Table no.2: showing tests for alkaloids, phenols, terpenoids and flavonoids.

S. No.	Tests	Procedure	Observation
1.	Alkaloid-test	(i) Dragendroff's-Test: 2-3 ml of filterate + 1-2ml of dragendroff-reagent. (ii) Hager's-Test: 3-4 ml of filtrate + 1-2 ml of Hager's-reagent.	Formation of Reddish-Brown precipitate Formation of creamy white precipitate
2.	Phenolic-test	(i) Iodine-Test: 1 ml of plant extract + 2-3 drops of dil. Iodine-solution. (ii) Lead-Acetate-Test: Extract of plant in 5ml distilled water + 3ml of 10% lead acetate solution.	Formation of transient red color Formation of white precipitate
3.	Terpenoid-test	2ml-chloroform + 5-ml plant evaporated extract+ 3-ml conc. Sulphuric acid	Formation of grey color solution
4.	Flavonoid-test	Lead-acetate-test: 1-2ml of plant extract + 2-3 drops of 10% lead acetate solution.	Formation of yellow fluorescence

Fig. No. 3: Identification tests for phytochemicals.

2.2.4. Screening of Anti-bacterial activity:

Preparation of the culture:

The culture was generated with two bacteria: *Staphylococcusaureus* (gram +ve bacteria) and *Salmonellatyphi* (gram -ve bacteria). The master culture was produced on an agar slant using nutritional agar medium (beef extract, nutrient agar, peptone, sodium chloride, and distilled water). The culture was then stored in the refrigerator. (Chakraborty et al., 2005) Baird-Parker agar broth was used to cultivate *Staphylococcus aureus* bacteria. (Dong et al., 2024) *Salmonella typhi* culture was prepared using Bismuth sulfite agar broth. (Salzar et al.,2023)

Zone of inhibition:

Disc-diffusion method was used for measuring the zone of inhibition. (Pandey et al., 2011) Firstly, culture media was prepared on the nutrient agar media. As two different culture media was prepared for both of the bacteria (*Staphylococcus aureus* and *Salmonella typhi*). Both of the bacteria were spread on the two-agar media using the swab and then incubated for 24hrs. Then the plant extract (Methanolic and aqueous) containing discs were spread on the surface of agar-plates and kept at 37^{0}c for 24 hrs. The antimicrobial agent diffused and then zone of inhibition was measured using the ruler. (Anantnarayn et al., 1996)

For *Staphylococcus aureus*:

Fig. No.4: Zone of inhibition on *Staphylococcus aureus* (methanolic and aqueous extract).

For *Salmonella typhi*:

Fig. No.5: Zone of inhibition on *Salmonella typhi* using methanolic and aqueous extract.

Minimum inhibitory concentration (MIC):

Turbidity method was used for measuring the Minimum inhibitory concentration. (Teh et al., 2017) First of all, the nutrient media broth was prepared and inoculated into the test tubes containing the bacteria and incubated for 24hrs. The plant extracts (methanolic and aqueous) were then applied to the test tubes at varying ratios. The test sample was then generated at five different concentrations using an aqueous and methanolic extract of *Amaranthus caudatus* seeds (20%, 40%, 60%, 80%, and 100%) in the DMSO by diluting it upto 5ml and incubated for 24hrs. Both aqueous and methanolic extract were used for the determination of MIC against the both bacteria, *Staphylococcus aureus* and *Salmonella typhi*. For *Staphylococcus aureus*, the doses were taken 0.4mg, 0.6mg, 0.8mg, 1mg and 1.2mg having concentration of 20%, 40%, 60%, 80% and 100% respectively into DMSO to make up the volume of 5ml in each test tube. For the *Salmonella typhi*, the doses were taken from 0.2mg, 0,4mg, 0.6mg, 0.8mg, and 1mg having concentration of 20%, 40%, 60%, 80% and 100% respectively into DMSO to make up the volume of 5ml in each test tube along with their respective bacteria. To validate the MIC value, the tube's optical turbidity was measured by visualizing the turbidity in each test tube against the light before and after incubation. (Andrews et al., 2001)

For *Staphylococcus aureus*

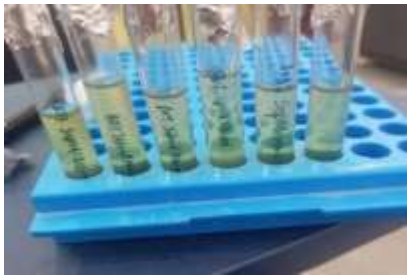

Fig. No.6: MIC of methanolic extract.

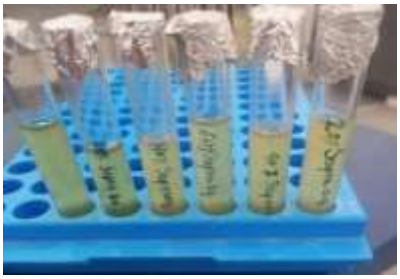

Fig. No.7: MIC of aqueous extract.

For *Salmonella typhi*

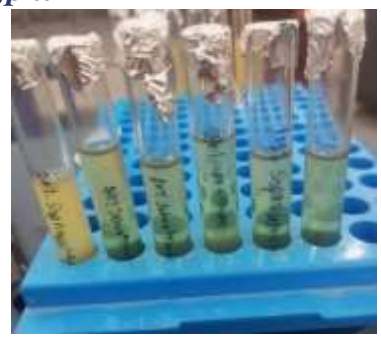

Fig. No.8: MIC of methanolic extract.

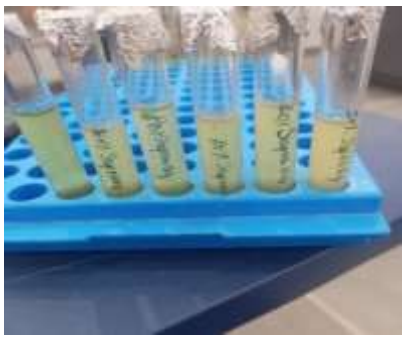

Fig. No.9: MIC of aqueous extract.

3. RESULTS AND DISCUSSION

3.1. Yield percentage:

3.1.1. Yield percentage of Methanolic extract of seeds of *Amaranthus caudatus*:

The yield percentage for the methanolic extract of seeds of *Amaranthus caudatus* was calculated by the formula:

Yield %age = Actual yield/ Theoretical yield × 100

= 3.5/50 ×100

= 7%

3.1.2. Yield percentage of Aqueous extract of the seeds of *Amaranthus caudatus*:

The yield percentage for the aqueous extract of seeds of *Amaranthus caudatus* was calculated by the formula:

Yield %age = Actual yield/ Theoretical yield × 100

= 2.75/50 ×100

= 5.5%

Both methanolic and aqueous extracts of seeds of *Amaranthus caudatus* were found to have great percentage of yield. Among the methanolic and aqueous extract of seeds of *Amaranthus caudatus*, the methanolic extract found to have the high yield percentage in comparison to the aqueous extract of seeds of *Amaranthus caudatus* which means the methanolic extract contains the higher amount of the phytochemical constituents as compared to the aqueous extract of *Amaranthus caudatus* seeds.

3.2. Phytochemical-screening:

During the phytochemical screening, the identification tests were done for the phytochemicals like phenols, flavonoids, alkaloids and terpenoids and the following results were found which are shown in the table:

Table No. 3: showing result of phytochemical screening (Identification tests for alkaloids, phenols, terpenoids, and flavonoids)

S. No.	Tests	Procedure	Result
1.	Tests for alkaloids	(i) Dragendroff's-Test: 2-3 ml of filterate + 1-2ml of dragendroff-reagent. (ii) Hager's-Test: 3-4 ml of filtrate + 1-2 ml of Hager's-reagent.	Positive result was obtained due to the formation of Reddish-brown precipitate Positive result was obtained due to the formation of creamy white precipitate
2.	Tests for Phenolic compounds	(i) Iodine-Test: 1 ml of plant extract + 2-3 drops of dil. Iodine-solution. (ii) Lead-Acetate-Test: Extract of plant in 5ml distilled water + 3ml of 10% lead acetate solution.	The result was negative as the transient red color didn't obtain. Positive result was obtained due to the formation of White precipitate
3.	Tests for Terpenoids	2ml-chloroform + 5-ml plant evaporated extract+ 3-ml conc. Sulphuric acid	The result was negative as the grey-coloured Solution didn't obtain.
4.	Tests for flavonoids	Lead-acetate-test: 1-2ml of plant extract + 2-3 drops of 10% lead acetate solution	Positive result was obtained due to the formation of yellow fluorescence.

3.3. Evaluation of Anti-bacterial activity:

3.3.1. Zone of inhibition

Zone of inhibition of standard plant extracts

Table No. 4: showing the zone of inhibition of standard plant extract.

Micro - Organisms	Concentration	Diameter of zone of inhibition (in mm)	
		Methanolic	Aqueous
Staphylococcus Aureus	1 mg/ml	13mm±0.5mm	12mm±0.5mm
Salmonella Typhi	1mg/ml	14mm±0.5mm	13mm±0.5mm

Zone of inhibition of test plant extracts (seeds of *Amaranthus caudatus*)

Table No. 5: showing the zone of inhibition of test plant extracts.

Micro - Organisms	Concentration	Diameter of zone of inhibition (in mm)	
		Methanolic	Aqueous
Staphylococcus Aureus	1 mg/ml	12mm±0.5mm	11mm±0.5mm
Salmonella Typhi	1mg/ml	13mm±0.5mm	12mm±0.5mm

Aqueous and methanolic extracts of *Amaranthus caudatus* seeds shown superior gram-negative antibacterial activity against *Salmonella typhi* compared to gram-positive bacteria. Among the two extracts- Methanolic and aqueous extract, Methanolic extract demonstrated superior anti-microbial activity against both *Staphylococcus aureus* (gram-positive) and *Salmonella typhi* (gram-negative).

3.3.2. Minimum inhibitory concentration (MIC):

The turbidity method was used for the determination of MIC. Both aqueous and methanolic extract were used for the determination of MIC against the both bacteria, *Staphylococcus aureus* and *Salmonella typhi*. For *Staphylococcus aureus*, the doses were taken 0.4mg, 0.6mg, 0.8mg, 1mg and 1.2mg having concentration of 20%, 40%, 60%, 80% and 100% respectively into DMSO to make up the volume of 5ml in each test tube. For the *Salmonella typhi*, the doses were taken from 0.2mg, 0,4mg, 0.6mg, 0.8mg, and 1mg having concentration of 20%, 40%, 60%, 80% and 100% respectively into DMSO to make up the volume of 5ml in each test tube. For *Staphylococcus aureus*, 0.4mg was the MIC value for both of the plant extract (Methanolic and aqueous). For *Salmonella typhi*, 0.2mg was the MIC value for both plant extract (Methanolic and aqueous).

4. Conclusion:

The potential of *Amaranthus caudatus* seeds as a helpful source of naturally occurring antibacterial chemicals is highlighted in the study's conclusion. These seeds may offer a novel approach to treating bacterial infections in both traditional and alternative medicine, especially those caused by pathogens resistant to antibiotics. Future research should focus on identifying and characterising the specific bioactive compounds, conducting in vivo studies, and looking into the clinical applications of *Amaranthus caudatus* seeds in order to fully grasp the medicinal potential of these seeds. *Amaranthus caudatus* seeds have shown great antibacterial activity towards the both bacteria (*Staphylococcus aureus* and *Salmonella typhi*). As two extracts of *Amaranthus caudatus* seeds (methanolic and aqueous) were used. Among the two, methanolic extract had the high yield percentage in comparison to the aqueous extract. Due to the high yield percentage, methanolic extract was used for the phytochemical screening and shown positive result for the presence of alkaloids, phenolic compounds and flavonoids but shown slight negative result for the terpenoids. During the measurement of zone of inhibition, both the extracts (methanolic and aqueous) have shown greater activity towards the *Salmonella typhi*. And among the both extracts, methanolic extract was found to be more effective. And during the measurement of Minimum inhibitory concentration, the turbidity was measured by visualizing it against the light for the both of bacteria (*Staphylococcus aureus* and *Salmonella typhi*). For *Staphylococcus aureus*, 0.4mg was the MIC value for both of the plant extract (Methanolic and aqueous). For *Salmonella typhi*, 0.2mg was the MIC value for both plant extract (Methanolic and aqueous).

REFERENCE

1. Ahmadi, S., Ahmadi, G., & Ahmadi, H. (2022). A review on antifungal and antibacterial activities of some medicinal plants. *Micro Nano Bio Aspects*, *1*(1), 10-17.
2. Alav, I., Kobylka, J., Kuth, M. S., Pos, K. M., Picard, M., Blair, J. M., & Bavro, V. N. (2021). Structure, assembly, and function of tripartite efflux and type 1 secretion systems in gram-negative bacteria. *Chemical Reviews*, *121*(9), 5479-5596.
3. Algammal, A. M., Hetta, H. F., Elkelish, A., Alkhalifah, D. H. H., Hozzein, W. N., Batiha, G. E. S., ... & Mabrok, M. A. (2020). Methicillin-Resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infection and drug resistance*, 3255-3265.
4. Anantnarayn R., Panikar C. K. Text of Microbiology.5th ed. Orient Longaman Limited; 1996.p.7, 20, 36-40.
5. Andrews J.M. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 2001;48(Suppl. SA):5–16. doi: 10.1093/jac/48.suppl_1.5.
6. Bose, S., Aggarwal, S., Singh, D. V., & Acharya, N. (2020). Extracellular vesicles: An emerging platform in gram-positive bacteria. *Microbial Cell*, *7*(12), 312.
7. Chakraborty P. A., Text Book of Microbiology. 2nd ed. Sen M. (Kolkatta): New central book agency (P) Ltd; 2005.p.9-24, 57-64.
8. Cheung, G. Y., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of Staphylococcus

aureus. *Virulence*, *12*(1), 547-569.
9. Dong, T., Jiao, C., Qiu, Y., & Han, C. (2024). Comparison of Different Media for the Quantitative Detection of Staphylococcus Aureus. *Advances in Food Science and Human Nutrition*, *6*(1), 1-6.
10. Fisher, J. F., & Mobashery, S. (2020). Constructing and deconstructing the bacterial cell wall. *Protein science*, *29*(3), 629-646.
11. Fromm, S. A., O'Connor, K. M., Purdy, M., Bhatt, P. R., Loughran, G., Atkins, J. F., ... & Mattei, S. (2023). The translating bacterial ribosome at 1.55 Å resolution generated by cryo-EM imaging services. *Nature Communications*, *14*(1), 1095.
12. Galperin, M. Y. (2018). What bacteria want. *Environmental microbiology*, *20*(12), 4221-4229.
13. Heaton, C. J., Gerbig, G. R., Sensius, L. D., Patel, V., & Smith, T. C. (2020). Staphylococcus aureus epidemiology in wildlife: A systematic review. *Antibiotics*, *9*(2), 89.
14. Hoffman, P. S. (2020). Antibacterial discovery: 21st century challenges. *Antibiotics*, *9*(5), 213.
15. Jimoh, M. O., Afolayan, A. J., & Lewu, F. B. (2019). Therapeutic uses of *Amaranthus caudatus* L.
16. Jimoh, M. O., Afolayan, A. J., & Lewu, F. B. (2020). Toxicity and antimicrobial activities of *Amaranthus caudatus* L. (Amaranthaceae) harvested from formulated soils at different growth stages. *Journal of Evidence-Based Integrative Medicine*, *25*, 2515690X20971578.
17. Kaluse, P. S., Bhatt, N., & Bankar, N. (2021). Study of Typhoid Fever: A Review. *Journal of Pharmaceutical Research International*, *33*(39A), 286-291.
18. López-Mejía, O. A., López-Malo, A., & Palou, E. (2014). Antioxidant capacity of extracts from amaranth (*Amaranthus hypochondriacus* L.) seeds or leaves. *Industrial Crops and Products*, *53*, 55-59.
19. Martinez-Lopez, A., Millan-Linares, M. C., Rodriguez-Martin, N. M., Millan, F., & Montserrat-de la Paz, S. (2020). Nutraceutical value of kiwicha (*Amaranthus caudatus* L.). *Journal of Functional Foods*, *65*, 103735.
20. Pandey, P., Mehta, A., & Hajra, S. (2011). Evaluation of antimicrobial activity of Ruta graveolens stem extracts by disc diffusion method. *Journal of Phytology*, *3*(3), 92-95.
21. Pasquina-Lemonche, L., Burns, J., Turner, R. D., Kumar, S., Tank, R., Mullin, N., ... & Hobbs, J. K. (2020). The architecture of the Gram-positive bacterial cell wall. *Nature*, *582*(7811), 294-297.
22. Roberts, C. A., & Buikstra, J. E. (2019). Bacterial infections. In *Ortner's Identification of pathological conditions in human skeletal remains* (pp. 321-439). Academic Press.
23. Ruhal, R., & Kataria, R. (2021). Biofilm patterns in gram-positive and gram-negative bacteria. *Microbiological Research*, *251*, 126829.
24. Salazar, J. R. L., Ullauri, V. G. L., Tapia, M. F. B., & Aguirre, S. I. H. (2023). Salmonella Prevalence in Fresh Cheese and its Incidence in Public Health. *Migration Letters*, *20*(S10), 643-648.
25. Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608.
26. SISHU, N. K., THEIVASIGAMANI, P., & SELVARAJ, C. I. (2023). A Review on Phytochemistry and Pharmacology Profile of Pendant Amaranth (*Amaranthus caudatus* L.). *Phytochemistry and Pharmacology of Medicinal Plants, 2-volume set*, 7.
27. Teh, C. H., Nazni, W. A., Nurulhusna, A. H., Norazah, A., & Lee, H. L. (2017). Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly via resazurin-based turbidometric assay. *BMC microbiology*, *17*, 1-8.