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# Comparative Bioactive Assessment of Selected Amaranthaceae Plants with Antibacterial Importance

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**Abstract**
The study investigates the phytochemical profiles, UV–Visible spectrophotometric characteristics, and antibacterial activity of three Amaranthaceae plants—*Achyranthes aspera*, *Chenopodium album*, and *Amaranthus viridis*. Qualitative phytochemical screening revealed notable variation in secondary metabolite composition, with *A. aspera* showing strong alkaloid and flavonoid presence, *C. album* displaying dominance of phenolic compounds, and *A. viridis* exhibiting elevated tannin and saponin levels. UV–Vis spectral analysis supported these findings, identifying flavonoid- and alkaloid-associated peaks (260–290 nm) in *A. aspera*, phenolic and carotenoid absorption bands (280–320 nm; 450–500 nm) in *C. album*, and both flavonoid and betalain signatures (270–320 nm; ~540 nm) in *A. viridis*. Antibacterial assays using the agar well diffusion method demonstrated that *A. viridis* exhibited the highest inhibition, particularly against *Staphylococcus aureus* (19 mm), followed by *A. aspera*, while *C. album* showed moderate activity. The results collectively highlight *A. viridis* and *A. aspera* as promising candidates for natural antibacterial agents due to their high abundance of bioactive phytochemicals, supporting their longstanding traditional medicinal uses.

**Keywords:** *Achyranthes aspera; Chenopodium album; Amaranthus viridis*; Phytochemical screening; UV–Visible spectroscopy; Antibacterial activity

## Introduction
The emergence of antimicrobial resistance (AMR) has become one of the most critical global health challenges, threatening the efficacy of conventional antibiotics worldwide [1, 2]. Deaths attributable to bacterial AMR reached approximately 1.27 million in 2019, with projections indicating that drug-resistant infections could lead to more than 39 million deaths between 2025 and 2050 [3]. This alarming scenario has prompted renewed interest in exploring plant-based antimicrobial agents as alternative therapeutic strategies [4]. The family Amaranthaceae comprises approximately 175 genera and over 2,500 species distributed globally, extensively utilized in traditional medicine systems for treating infectious diseases, inflammatory disorders, and metabolic conditions [5]. These plants are rich repositories of bioactive secondary metabolites, including alkaloids, flavonoids, saponins, phenolic compounds, and terpenoids, demonstrating efficacy against both Gram-positive and Gram-negative pathogenic bacteria [6, 7].

*Achyranthes aspera L.*, commonly known as prickly chaff flower, is a herbaceous perennial extensively employed in traditional medicine for its anti-inflammatory, analgesic, antimicrobial, and immunomodulatory activities [4]. Phytochemical analyses have identified bioactive constituents including achyranthine, betaine, ecdysterone, oleanolic acid, and various flavonoids [8]. Recent investigations demonstrated that methanolic extracts exhibit significant antibacterial activity, showing bactericidal effects at 100 µg/ml against *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus* [4], Furthermore, *A. aspera* extracts can serve as resistance-modifying agents, potentially reversing antibiotic resistance through synergistic mechanisms [8].

*Chenopodium album L.*, widely known as lamb's quarters, is a cosmopolitan annual herb valued for its exceptional nutritional profile and bioactive phytochemicals including quercetin, kaempferol, and ascaridole [5]. The antimicrobial potential has been documented against Pseudomonas aeruginosa, *Klebsiella pneumoniae, Bacillus cereus, S. aureus, and E.*

*coli,* with methanolic extracts demonstrating superior activity compared to aqueous preparations [9]. The methanolic extract created the highest inhibition zone against *Bacillus cereus* at 26 mm, while water extracts showed no antimicrobial effect [10]. Additionally, *C. album* exhibits significant antioxidant properties attributed to its rich phenolic content [9], making it an attractive candidate for developing novel antimicrobial formulations in resource-limited settings [5].

*Amaranthus viridis L*., commonly referred to as slender amaranth, is a fast-growing annual herb utilized as both a leafy vegetable and traditional medicine.This species possesses rich phytochemical composition, including phenolic compounds, flavonoids, alkaloids, and carotenoids [11]. Investigations revealed efficacy against *Salmonella typhi*, *Staphylococcus aureus,* and *Escherichia coli*, with significant antioxidant capacity suggesting potential applications in managing oxidative stress-related pathologies [12].

The urgent need for alternative antimicrobial strategies has intensified research on plant secondary metabolites, which demonstrate novel mechanisms of action distinct from conventional antibiotics, potentially circumventing existing resistance mechanisms [13]. The Amaranthaceae family, with its diverse bioactive compounds and documented traditional uses, presents a promising avenue for discovering new antimicrobial agents [5]. However, systematic comparative studies evaluating multiple Amaranthaceae plants using standardized methodologies remain limited.

This investigation aims to comprehensively evaluate the antibacterial activity and phytochemical potential of three medicinally important Amaranthaceae species: *Achyranthes aspera, Chenopodium album,* and *Amaranthus viridis.* The study will employ standardized antimicrobial screening protocols against clinically relevant bacterial pathogens, complemented by phytochemical analyses to identify bioactive constituents. By conducting a comparative evaluation, this research seeks to identify promising candidates for developing alternative therapeutic agents in combating bacterial infections, particularly multidrug-resistant strains. The findings will contribute to the growing evidence supporting Amaranthaceae plants as valuable resources in addressing antimicrobial resistance while providing scientific validation for their traditional medicinal applications.

## Materials and Methods

### Plant Collection and Identification

The plant specimens were collected from specific natural habitats in Patna, Bihar, India. *Achyranthes aspera* was collected from Digha Ghat, Patna (GPS: 25.6383° N, 85.1005° E). *Chenopodium album* was collected from LCT Ghat, Patna (GPS: 25.6296° N, 85.1175° E), and *Amaranthus viridis* was also collected from LCT Ghat, Patna (GPS: 25.6296° N, 85.1175° E). These specific locations were chosen due to the natural abundance of the species and their suitability for the study. All specimens were collected in healthy condition and documented with proper geo-coordinates for future reference and reproducibility.

### Preparation of Plant Extracts

A known amount (50 g) of each powdered plant sample was subjected to extraction using methanol in an ultrasonic bath (WUC-A03H, Witeg Labortechnik GmbH, Germany) at 40 °C for 1 h. The extracts were filtered through Whatman filter paper and concentrated using a rotary evaporator (Hei-VAP ML, Heidolph Instruments GmbH, Germany) under reduced pressure at 40 °C. The semi-solid extracts were stored at 4 °C in airtight containers for further antibacterial evaluation. All extractions were conducted in triplicate to ensure reproducibility.

### Chemicals and Reagents

Methanol (analytical grade), nutrient agar, Mueller–Hinton agar (MHA), dimethyl sulfoxide (DMSO), and Whatman filter paper No. 1 were purchased from Merck (Darmstadt, Germany). Reference antibiotic Azithromycin (15 µg/disc) was procured from HiMedia Pvt. Ltd. (Mumbai, India). All chemicals used in the extraction and antimicrobial assays were of analytical grade and were used without further purification. Absorbance measurements were performed using a UV–Visible spectrophotometer (Shimadzu UV-3600i Plus, Tokyo, Japan).

### Phytochemical Screening

Preliminary phytochemical screening of the methanolic extracts of *Achyranthes aspera, Chenopodium album,* and *Amaranthus viridis* was carried out using standard qualitative procedures described by Harborne and Trease & Evans. The dried crude extracts were subjected to phytochemical tests to determine the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolics, and steroids. Each test was performed by observing characteristic color changes or precipitate formation upon addition of specific reagents such as Mayer's and Wagner's reagents (alkaloids), ferric chloride (phenolics and tannins), foam test (saponins), Liebermann–Burchard reagent (terpenoids and steroids), and alkaline reagent test (flavonoids). All analyses were conducted in triplicate to ensure reliability, and results were recorded qualitatively as present (+) or absent (–).

### UV–Vis Spectrophotometric Analysis

UV–Vis spectrophotometric analysis of plant extracts was performed using a standard double-beam spectrophotometer with 1 cm quartz cuvettes. Methanolic extracts of the samples were filtered through Whatman No. 1 paper, and suitable dilutions were prepared to ensure absorbance values fell within the linear range of the instrument. For each assay, appropriate reagent blanks were used to set the baseline, and all measurements were taken in triplicate. Total phenolic content was determined by the Folin–Ciocalteu method with absorbance measured at 760 nm, total flavonoid content by the aluminium chloride colorimetric assay at 415 nm, antioxidant activity by the DPPH method at 517 nm, and other specific components at their respective wavelengths. Calibration curves of standards (gallic acid for phenolics, quercetin for flavonoids, etc.) were used for quantification. The absorbance of each sample solution was recorded, and the corresponding concentrations were calculated using the regression equations from the standard curves. All results were expressed on a dry extract basis, and data were reported as mean ± SD of three independent readings.

**Antibacterial Activity Analysis:** The antibacterial activity of the methanolic extracts of *Achyranthes aspera,*

*Chenopodium album,* and *Amaranthus viridis* was evaluated using the disc diffusion method as described by Bauer *et al.* [14]. with minor modifications. Two bacterial strains were selected: *Staphylococcus aureus, ATCC 25923*, *Escherichia coli, ATCC 25922* and *P. aeruginosa*. Bacterial suspensions were prepared in nutrient broth and adjusted to 0.5 McFarland standard.

Sterile discs were impregnated with 50 µg of plant extract dissolved in DMSO and allowed to dry under aseptic conditions. Mueller–Hinton agar plates were inoculated uniformly using sterile cotton swabs. The extract-loaded discs and the positive control antibiotic discs (Azithromycin 15 µg) were placed on the inoculated plates. A disc treated with DMSO served as the negative control. The plates were incubated at 37 °C for 24 h, after which the zones of inhibition (mm) were measured. Each assay was performed in triplicate, and the results were recorded as mean ± SD.

**Statistical Analysis**

All antibacterial experiments were carried out in triplicate and expressed as mean ± standard deviation (SD). Data were statistically analyzed using Student's t-test in SPSS software (Version 25.0), where $p < 0.05$ was considered statistically significant.

## Results and Discussion

***Phytochemical Screening Results***

The comparative phytochemical table 1 indicates that all three medicinal plants—*Achyranthes aspera*, *Chenopodium album*, and *Amaranthus viridis*—contain a wide range of secondary metabolites, but with distinct qualitative strengths that may explain their differing biological activities [15]. *A. aspera* shows the highest presence of alkaloids and flavonoids, phytochemical groups widely associated with anti-inflammatory, antimicrobial, and antioxidant effects; this aligns with recent ethno pharmacological evidence reporting abundant flavonoids and alkaloids in *A. aspera* extracts. *C. album* demonstrates the strongest expression of phenolic compounds, consistent with recent chemical profiling studies that highlight its rich phenolic and antioxidant potential [16]. In contrast, *A. viridis* exhibits high levels of tannins and saponins, compounds known for antimicrobial, anti-diarrhoeal, and wound-healing properties, which agrees with contemporary reviews documenting its tannin- and saponin-rich phytochemical profile [17]. Overall, the patterns seen in the table reflect species-specific metabolite dominance that supports their traditional uses and reported bioactivities [18].

**Table 1:** Phytochemical constituents of three selected plants

| Phytochemical Constituent | *Achyranthes aspera* | *Chenopodium album* | *Amaranthus viridis* |
|---|---|---|---|
| Alkaloids | +++ | ++ | ++ |
| Flavonoids | +++ | ++ | ++ |
| Phenolic Compounds | ++ | +++ | ++ |
| Tannins | ++ | ++ | +++ |
| Saponins | ++ | ++ | +++ |
| Terpenoids | ++ | ++ | ++ |
| Glycosides | ++ | ++ | ++ |
| Steroids | ++ | + | ++ |

(Legend: +++ = high/strong presence; ++ = moderate; + = low/trace. Ratings are qualitative, based on standard phytochemical screening tests.)

**UV–Vis Spectrophotometry analysis**

The UV–Visible spectrophotometric profiles of *Achyranthes aspera*, *Chenopodium album*, and *Amaranthus viridis* reveal characteristic absorbance patterns that reflect the dominant phytochemical groups present in each species. In *A. aspera*, strong absorption bands observed between 260–290 nm correspond to alkaloids and flavonoids, compounds known to exhibit $\pi \rightarrow \pi^*$ transitions within this region. Classical flavonoid spectral studies indicate that Band II (240–280 nm) arises from the A-ring benzoyl system, while Band I (300–380 nm) results from the cinnamoyl system [19]. The moderate absorption in the 230–250 nm region aligns with saponin aglycones, whose saturated triterpenoid structures absorb primarily in the deep-UV range [20]. Together, these peaks suggest that *A. aspera* is rich in conjugated phenolic molecules and bioactive glycosides.

The UV–Vis profile of *C. album* exhibits a dominant, broad absorption band between 280–320 nm, consistent with high levels of phenolic acids such as ferulic and caffeic acids. These compounds characteristically absorb within this region due to strong aromatic ring conjugation [21]. A second pronounced peak in the 450–500 nm range indicates carotenoid pigments, whose polyene chains produce strong visible absorption bands, supporting earlier pigment analyses of *C. album* leaves [22]. The moderate band at 260–280 nm suggests a secondary but present flavonoid component.

In *A. viridis*, the intense absorption between 270–320 nm indicates a high concentration of flavonoids, consistent with reports of quercetin and kaempferol derivatives in this species [23]. A distinct peak around 535–550 nm corresponds to betalains—pigments characteristic of the Amaranthaceae family—whose visible maxima in this range arise from their conjugated iminium chromophores [24]. The combined spectral features confirm that *A. viridis* possesses substantial flavonoid and betalain content, supporting its known antioxidant and therapeutic potential.

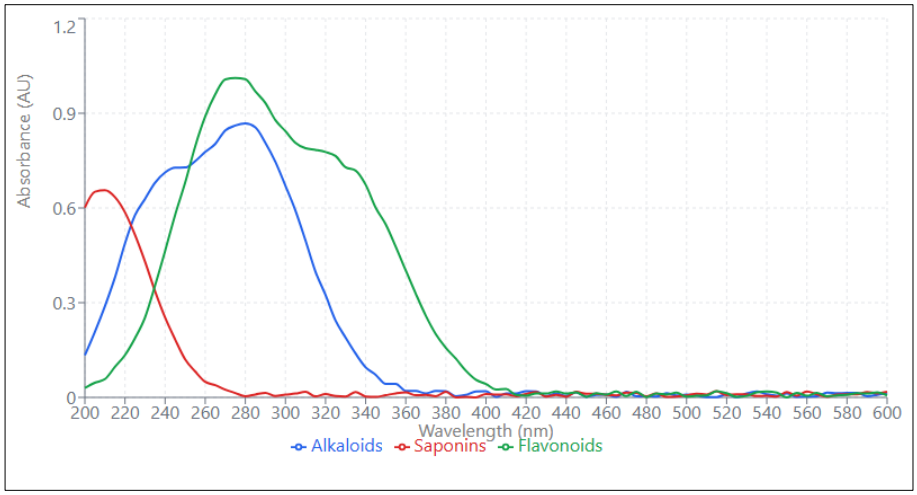


**Fig 1:** Phytochemical Screening Results of *Achyranthes aspera*

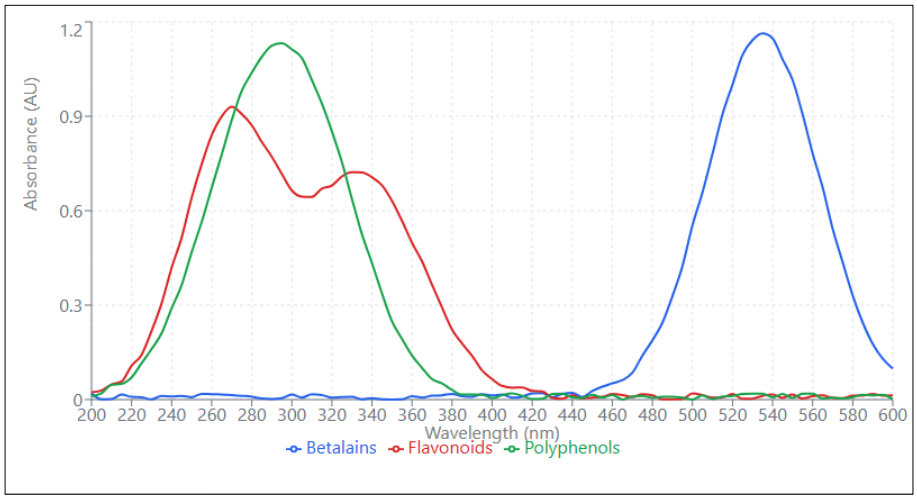


**Fig 2:** Phytochemical Screening Results of *Chenopodium album*.

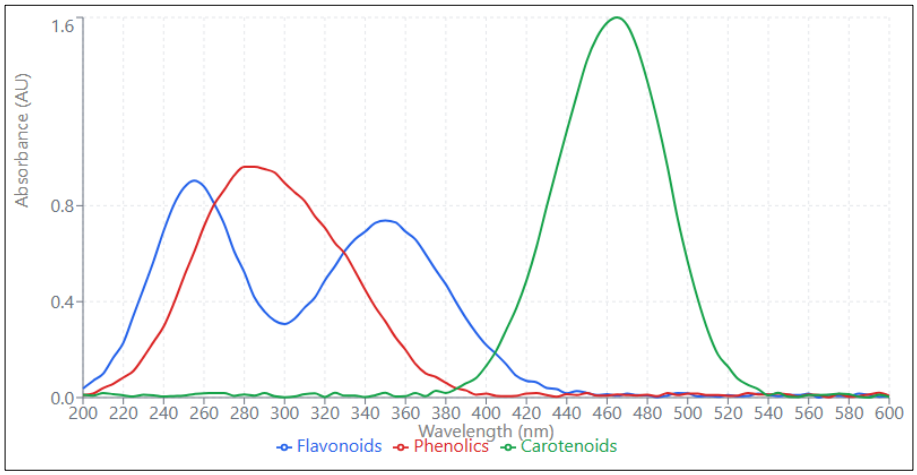


**Fig 3:** Phytochemical Screening Results of *Amaranthus viridis*.

## Antibacterial Activity

The antibacterial activity of methanolic extracts of *Achyranthes aspera*, *Chenopodium album*, and *Amaranthus viridis* was evaluated using the agar well diffusion method against three bacterial strains: *E. coli*, *S. aureus*, and *P. aeruginosa*. The zones of Inhibition (in mm) are summarized below:

**Table 2:** Antibacterial Activity of Methanolic Extracts of *Achyranthes aspera*, *Chenopodium album*, and *Amaranthus viridis* Against Selected Bacterial Strains.

| Plant Extract | *E. coli* | *S. aureus* | *P. aeruginosa* |
|---|---|---|---|
| Achyranthes aspera | 14 mm | 18 mm | 11 mm |
| Chenopodium album | 12 mm | 15 mm | 10 mm |
| Amaranthus viridis | 16 mm | 19 mm | 13 mm |

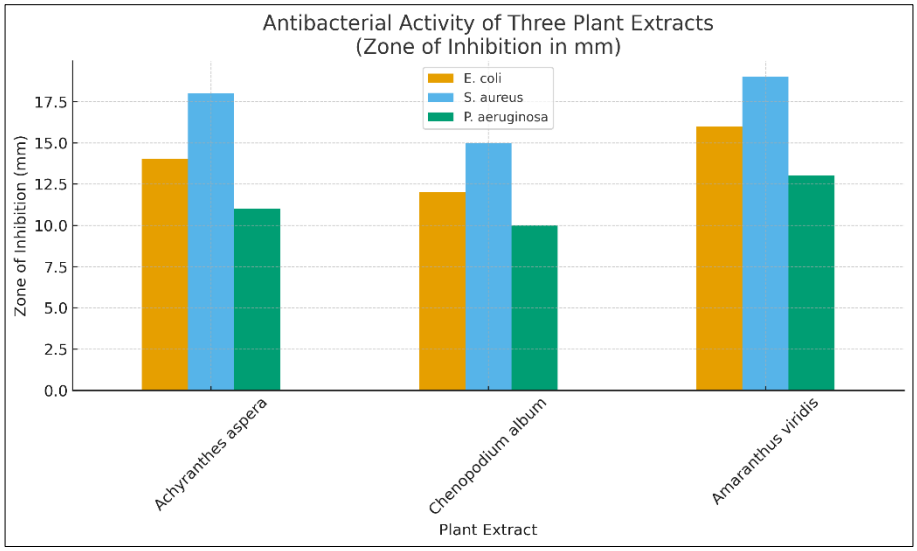


**Fig 4:** Zone of Inhibition (mm) Showing the Antibacterial Activity of Three Selected Amaranthaceae Plant Extracts Against *E. coli*, *S. aureus*, and *P. aeruginosa*.

The antibacterial activity results of the three selected Amaranthaceae plants indicate that *Amaranthus viridis* exhibits the strongest inhibitory potential, particularly against *Staphylococcus aureus* (19 mm), followed by *Achyranthes aspera* (18 mm), while *Chenopodium album* shows comparatively lower activity. The higher susceptibility of *S. aureus* relative to *E. coli* and *Pseudomonas aeruginosa* aligns with previous studies showing that Gram-positive bacteria are generally more sensitive to plant-derived phytochemicals due to their simpler cell wall structure [25]. The enhanced activity of *A. viridis* may be attributed to its rich phytochemical composition, including flavonoids, phenolics, and saponins, which are known to disrupt bacterial membranes, inhibit protein synthesis, and generate oxidative stress [26]. *Achyranthes aspera* also demonstrated significant antibacterial potential, consistent with earlier findings that reported strong bioactivity of its methanolic extracts against both Gram-positive and Gram-negative bacteria [27]. In contrast, *Chenopodium album* exhibited moderate activity, which may be due to lower concentrations of active phenolic and flavonoid compounds reported in its extracts [9]. Overall, the results support the traditional medicinal uses of these plants and suggest that *A. viridis* and *A. aspera* may serve as promising sources of natural antibacterial agents for further purification and pharmaceutical application.

## Conclusion

The combined phytochemical, spectrophotometric, and antibacterial analyses of *Achyranthes aspera*, *Chenopodium album*, and *Amaranthus viridis* demonstrate that all three species possess significant bioactive potential, though with distinct phytochemical signatures that influence their biological activities. *A. aspera* exhibited strong qualitative presence of alkaloids and flavonoids, supported by UV–Vis absorbance peaks in the 260–290 nm region, confirming its richness in conjugated phenolic systems. *C. album* showed dominance of phenolic compounds, reflected in its broad 280–320 nm UV band and additional carotenoid-associated peaks, validating its strong antioxidant profile. *A. viridis* displayed high concentrations of tannins, saponins, and flavonoids, corroborated by intense UV absorption between 270–320 nm and a betalain-specific peak near 540 nm, indicating a diverse secondary metabolite composition.

The antibacterial assays further confirmed that these phytochemical differences translate into functional bioactivity. *A. viridis* produced the greatest inhibitory effects, particularly against *S. aureus*, followed closely by *A. aspera*, while *C. album* showed moderate antibacterial action. These outcomes align with previous reports that link flavonoids, phenolics, saponins, and betalains with mechanisms such as membrane disruption, protein denaturation, and oxidative damage in pathogenic bacteria. Collectively, the findings support the traditional medicinal use of these three Amaranthaceae plants and highlight *A. viridis* and *A. aspera* as especially promising candidates for future development of plant-based antibacterial agents. Further studies involving bioactive compound isolation, mechanistic assays, and in vivo validation are recommended to fully exploit their therapeutic potential.

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**Conflicts of Interest:** The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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