

Phytochemical and Antibacterial Potential of *Vernonia amygdalina* Leaf Extracts Against Two Bacteria

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Abstract: The study aims to evaluate the phytochemical screening and antibacterial activity of *Vernonia amygdalina* leaves extracts against microorganism *Staphylococcus aureus* and *Escherichia coli*. Phytochemical study revealed that numerous bioactive components were found in the cold and hot water and ethanol extracts of the plant. Ethanol and water extractions were used to analyze the effects of *V. amygdalina* leaf extracts, and the well diffusion method was used to assess antibacterial activity. *E. coli* had the maximum zone of inhibition (23.66 mm) for ethanol extract followed by cold water (23 mm) and least for hot water (19.33 mm), while *S. aureus* had the maximum zone of inhibition (21.33 mm) for cold water followed by ethanol (20.33 mm) and least for hot water (19.60 mm). Additionally, it demonstrates that *Vernonia amygdalina* has antibacterial properties and may be used to treat MDR bacterial infections instead of manufactured medicines. To standardize its use and investigate other therapeutic alternatives for *Vernonia amygdalina* extracts, more research is needed.

Keywords: *Vernonia amygdalina*, Aqueous Extract, Antimicrobial Resistances, Ethanol Extract, Zone of Inhibition, Well Diffusion Method.

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I. INTRODUCTION

For a very long time, a wide variety of medications have been made from plant materials to cure a variety of illnesses. It is anticipated that this trend would continue, especially in rural areas of developing countries. Approximately 80% of the world's population relies on traditional medicines as their primary source of basic healthcare, with the majority of these treatments involving the use of plant extracts (Singh and Jain, 2011). Uses of medicinal plants has significantly increased in recent decades as a substitute method for improving the standard of living and maintaining excellent health (Bello *et al.*, 2024). Traditional medicine is mainly based on medicinal plants, which have been shown to be successful in treating human ailments all around the world. The use of alternative and complementary medicine to treat a variety of diseases and illness is gaining popularity. The key components of plants' therapeutic efficacy are their secondary metabolites and phytochemicals. However, it is still a lot to clarify about the mechanisms underlie the majority of medicinal plant-related treatments. The efficacy of plants in curing various fungal, bacterial, and other illnesses is demonstrated by the success of alternative and complementary medicine in treating infectious disorders (Ofodile, 2025).

Vernonia amygdalina is widely recognized for its several use in conventional medicine. *Vernonia amygdalina*, is a member of the Asteraceae family and also referred to as olubo in Igbo, ewuro in Yoruba, Shiwaka in Hausa, and bitter leaf in English. Bitter leaf is a shrub plant that grows to a height of 2 to 5 meters and is found in many different ecological zones across Africa. It can withstand drought and yields massive volumes of fodder. The leaf is green, tastes harsh, and has a strong smell. The bitter test is caused by non-nutritional substances like alkaloids, saponins, tannin, terphenoids, glycosides, and steroids. The shrub *V. amygdalina* is a perennial. This plant is commonly practised in folk medicine and tropical African cuisine (Jarmai *et al.*, 2022). In many families in both Eastern and Western Nigeria, *Vernonia amygdalina* is used as food, particularly when making soups. It is thought that the distinctively bitter taste has a pleasant aftertaste. The peeled stem works wonders as an anticary and is frequently used as a chewing stick to clean teeth. Nursing moms frequently take use of the leaves' bitterness to help wean their infants by applying the juice to their breasts. Because it facilitates better lactation, it is also thought to be helpful for nursing mothers. The plant's leaves and other materials have been used to treat a variety of suspected ailments, either alone or in combination with other plants. Interaction with some traditional medicine healers in

Ilorin city revealed that the leaves are extracted raw in aqueous solution and taken as anti-dysentery diction. It is also said to be helpful for controlling blood sugar levels in diabetics; in severe cases, patients are prescribed a cup full every day (Anibijuwon *et al.*, 2012).

Phytochemical study has revealed that *V. amygdalina* contains alkaloids, flavonoids, glycosides, phlobatannins, phenols, saponins, and steroids. Its physiological effects, which include hypolipidemic, anti-diabetic, anti-microbial, anti-allergic, antibacterial, antimalarial, anti-fungal, anti-cancer, anti-leukemia, anti-inflammatory, analgesic, antipyretic, antioxidative, anthelmintic, and antimicrobial properties, may be caused by these phytochemicals (Jarmai *et al.*, 2022). Research has shown that *V. amygdalina* has strong antibacterial action against a variety of bacterial and fungal diseases. Research has shown that it is effective against both Gram-positive and Gram-negative bacteria, including *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Clostridium* species, as well as *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, and *Shigella dysenteriae* (Swee *et al.*, 2010). *V. amygdalina*'s bioactive compounds, which disrupt microbial cell wall integrity, protein synthesis, and metabolic pathways to prevent pathogen growth and proliferation, that are primarily responsible for its antimicrobial effectiveness of plant (Jin *et al.*, 2017 and Raji *et al.*, 2025).

The objective of this study was to assess the available data from the literature about *V. amygdalina*'s antibacterial activities on bacteria. Therefore, the main aim of work is to ascertain the preliminary phytochemical composition and antibacterial activity of *Vernonia amygdalina* on *Escherichia coli* and *Staphylococcus aureus*.

II. METHODOLOGY

- Collection of Plant Leaf sample: Fresh leaf samples of *V. amygdalina* were collected from Indira Priyadarshini College, Chhindwara, Madhya Pradesh campus in the month of February, 2025. The leaf were identified by local herbal practitioners and the sample were then brought to the laboratory for further processing.
- Processing of Plant sample : After cleaning the leaf sample with tap water to remove any dirt and dust, it was left out on a table for several days to naturally dry in the shade. Using a mixer, the sample was then ground into a fine powder. For additional investigation, the finely powdered material was kept in plastic bags (Bello *et al.*, 2024).
- Solvent Extraction : For twenty-four hours, ten grams of dried, ground leaf powder were dissolved in one hundred milliliters of ethanol, cold water, and hot water, respectively. After that, the mixture was filtered through Whatman's filter paper No. 1 to produce a solid-free solution. The filtrate was utilized to assess phytochemical screening and antibacterial activity while being kept at 4°C (Unegbu *et al.*, 2020 and Choudhary *et al.*, 2026).
- Phytochemical Screening: Phytochemical screening of the *V. amygdalina* leaves extracts were performed to ascertain

bioactive components such as steroids, terpenoids, flavonoids, phlobatannins, alkaloids, tannins, glycoside and phenolic compounds. The different tests performed are as follows (Ofodile, 2025; Choudhary *et al.*, 2026).

- Saponins: Froth Test- In a test tube, a portion of the crude extract was diluted to 5 mL with distilled water and agitated for approximately five minutes. Saponins are present when honeycomb froth forms.
- Alkaloids: Wagner's test- In a test tube, one mL of extract was mixed with a few drops of Wagner's reagent along the tube's sidewalls. Development of reddish-brown precipitate indicates presence of alkaloids (Galadima *et al.*, 2025).
- Phenolic compounds: Ferric chloride test - A few drops of 5% FeCl₃ were added to one mL of extract. Presence of phenolic compounds were confirmed by the development of a blue-black precipitate (Magaji *et al.*, 2023).
- Tannins: Lead acetate test - A few drops of 10% lead acetate were added to one mL of test solution. A white or yellowish precipitate formation indicates the presence of tannins in the extract.
- Steroids: Salkowski test - In a test tube, one mL of chloroform was added to one mL of the extract, along with a few drops of strong H₂SO₄ by the tube's side. Shaken and allowed to stand for the development of a red hue that indicates the presence of steroids (Bello *et al.*, 2024).
- Phlobatannins: HCL test - One mL of extract and one mL of 1% aqueous HCl were heated together. The presence of phlobatannins indicated by development of a crimson precipitate (Jarmai *et al.*, 2022).
- Flavonoids : Sodium hydroxide test - 3 to 4 drops of 10% aqueous NaOH were added to one mL of extract in a test tube, to create a yellow hue. Then strong HCl was added, the hue changed from yellow to colourless, indicating the presence of flavonoids (Jarmai *et al.*, 2022).
- Glycoside: Borntrager's Test - 3 mL of chloroform was added to 2 mL of extract, agitated to separate the chloroform layer, and then 10% ammonia solution was added. The pink colour indicated the presence of glycosides in the extract (Tura *et al.*, 2024).
- Test Organisms: In this study, Gram-negative bacteria i.e. *Escherichia coli* and gram-positive bacteria i.e. *Staphylococcus aureus* were used. Pure bacterial species were obtained and maintained from the Microbiology Laboratory, of Indira Priyadarshini College, Chhindwara, Madhya Pradesh.
- Standardization of the bacterial strains : Nutrient broth was used to inoculate a loop full of test microorganisms, which were then cultured for a full day. To standardize the culture to 0.5 McFarland standards (10⁶ cfu/ml) before use, precisely 0.2 ml of the organisms' 24-hour-grown culture was added to 20 ml of sterile nutrient broth and cultured for three to five hours (Akinyele *et al.*, 2014).
- Antibacterial testing: The leave extract of *V. amygdalina* were screened for antibacterial activity by Well diffusion assay. The antibacterial activity were carried out on gram-negative bacteria i.e. *Escherichia coli* and gram-positive bacteria i.e. *Staphylococcus aureus*. MHA plates were prepared for the test. Using the spread plate approach, 25 µl of bacterial suspension was put to the prepared Mueller

Hinton Agar plates under laminar air flow. Using a sterile cork borer, wells were created on the plates. A micropipette (100 μ l) was used to fill the wells with the filtrate. Pure solvent and Tetracycline (30 μ g) was used as a reference standard control. These plates were stored for 24 hours at 37°C in a bacteriological incubator. Following incubation, the activity was measured in millimeters of the zone of inhibition. Three iterations of the experiment were conducted, and the average outcomes were computed.

III. RESULT AND DISCUSSION

➤ Preliminary Phytochemical Screening:

The colour of the filtrate (Aqueous and Ethanol extract) is reddish - brown and greenish in colour. Table 1 revealed

the phytochemical screening of *Vernonia amygdalina*. According to the phytochemical study, several bioactive components were found in the water (hot and cold) and ethanol leaf extracts of the *Vernonia amygdalina* plant. Alkaloids and glycosides were present in hot water, cold water and ethanolic extracts but devoid of tannin. Cold and Hot water extract was found to contain saponins while ethanol extract were found to contain phlobatannins. Phenolic compound and steroids were found in both hot water and ethanol extract but not in cold water extract. Flavonoids were found only in hot water extract. These phytochemicals are the main substances that provide the plant its therapeutic benefit.

Table 1 Preliminary Phytochemical Screening of Different Leaf Extract of *V. amygdalina*.

S. No.	Test Name	Cold Water	Ethanol	Hot Water
1.	Saponins	+	-	+
2.	Alkaloids	+	+	+
3.	Phenolic Compound	-	+	+
4.	Tannins	-	-	-
5.	Steroids	-	+	+
6.	Phlobatannins	-	+	-
7.	Flavonoids	-	-	+
8.	Glycosides	+	+	+

“+” Presence, “-” Absence

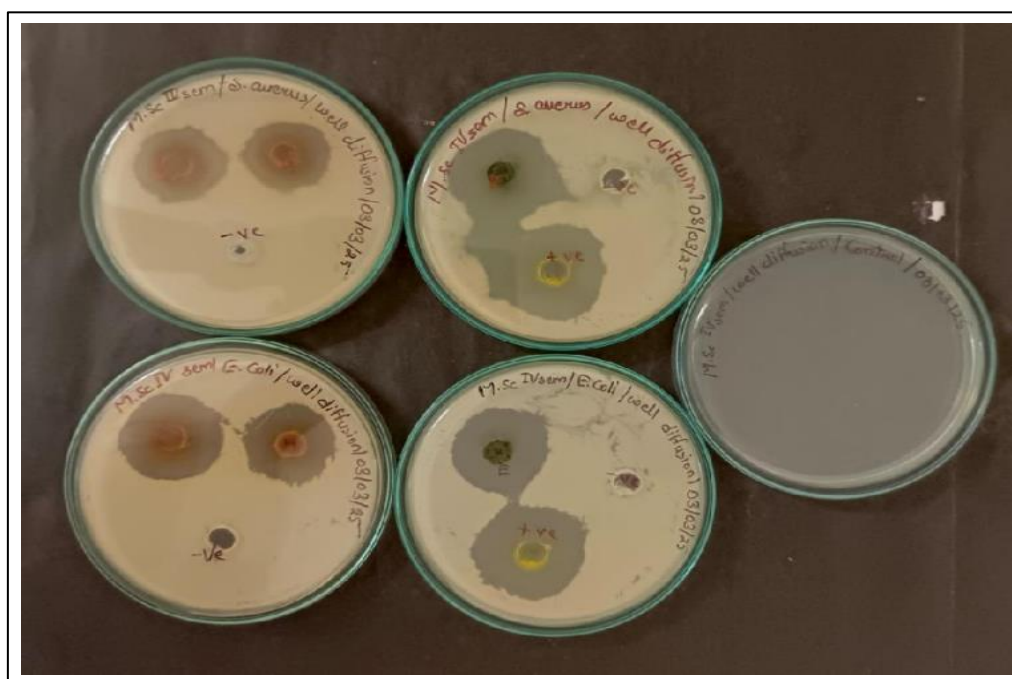


Fig 1 Showing the Antibacterial Activity of *V. amygdalina* Leaf Extract Against *E. coli* and *S. aureus*.

[H = Hot Water extract, C = Cold Water Extract and E = Ethanol Extract, Negative Control = Pure solvents (Distilled Water and Ethanol) and Positive control = Tetracycline]

Table 2 Antibacterial Activity of Different Leaf Extract of *V. amygdalina*.

S. No.	Test Organism	Zone of inhibition (mm)			
		Hot Water	Cold Water	Ethanol	Tetracycline
1.	<i>Staphylococcus aureus</i>	19.60	21.33	20.33	27.00
2.	<i>Escherichia coli</i>	19.33	23.00	23.66	27.00

The antibacterial activity of leaf extracts was studied against microorganism *Staphylococcus aureus* and *Escherichia coli*. Table 2 and Fig 1 revealed the inhibition effect of Hot water, Cold water and Ethanolic Leaf extract of *V. Amygdalina* on test bacterial isolates (*S. aureus* and *E. coli*). *E. coli* had the maximum zone of inhibition (23.66mm) for ethanol extract followed by cold water (23mm) and least for hot water (19.33 mm), while *S. aureus* had the maximum zone of inhibition (21.33 mm) for cold water followed by ethanol (20.33 mm) and least for hot water (19.60 mm). The result obtained from the study suggested that these plant extract possess significant antibacterial property.

IV. CONCLUSION

This investigation confirmed the folkloric use of *V. amygdalina* leaf extracts by highlighting their antibacterial properties on certain bacterial isolates. This study demonstrates that the antibacterial qualities of *Vernonia amygdalina* are caused by bioactive phytochemicals. *Vernonia amygdalina* was subjected to phytochemical screening in hot water, cold water, and ethanol extract, which revealed the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins and steroids. According to the study, *Vernonia amygdalina* extract has medicinally significant chemicals that can stop bacterial development. The results of antibacterial activity of leaf of *Vernonia amygdalina* against the bacterial strains are investigated. Its activity against *Escherichia coli* and *Staphylococcus aureus* demonstrates its promise as a natural antibacterial agent; variations in bacterial sensitivity are ascribed to variances in cell wall composition. In ancient time, in practice water is primarily used as the solvent but we found in this study the plant extract by ethanol provided more consistent antibacterial activity. The pharmacologically active phytochemicals found in the extract may be the cause of the plant leaf extract's many therapeutic qualities. It also has great promise as an antibacterial agent for the treatment of pathogens that cause infections. To isolate the active chemicals for additional pharmacological, clinical, and antibacterial testing, more work needs to be done.

➤ Conflict of Interest

No conflicts of interest to disclose.

➤ Funding

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➤ Authors Contribution

Akansha Langiwar carried out the research drafted, Annu Choudhary help in drafting and reviewing the research and Randhir Kumar supervised the research and guided the researchers.

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