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COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFICACY AND PHYTOCHEMICAL PROFILE OF METHANOL AND AQUEOUS EXTRACTS OF *Vernonia amygdalina* AGAINST GASTROENTERITIS CAUSING BACTERIA

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ABSTRACT

Vernonia amygdalina, commonly known as bitter leaf, is a shrub recognized for its bioactive compounds with documented antibacterial properties against gastroenteritis. However, the efficacy of methanolic and aqueous extracts of *Vernonia amygdalina* leaves has not been extensively evaluated. This study aimed to assess the efficacy of these extracts and determine their chemical composition, as well as their potential to inhibit the activity of selected bacteria associated with gastroenteritis. *Vernonia amygdalina* leaves were procured from Gombe central market, and the extracts were subjected to phytochemical screening. Three distinct bacterial isolates (*Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*) associated with gastroenteritis were obtained from the microbiology laboratory at Gombe State University and used in an antibacterial assay of the leaf extracts at varying concentrations (12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL), conducted using the agar well diffusion method. The results confirmed the presence of tannins, alkaloids, flavonoids, steroids, and saponins, and their antimicrobial action on the isolates. The methanol extract demonstrated a significantly higher inhibition zone of 15.1 mm at 100 mg/mL against E. coli, compared to 14.0 mm for the aqueous extract against S. typhi, suggesting methanol as a more effective solvent for extracting antibacterial compounds from *Vernonia amygdalina*.

Keywords: Antimicrobial activities, Leaf extract, Methanol extract, Phytochemicals, *Vernonia amygdalina*

INTRODUCTION

Phytochemicals are naturally occurring biochemical metabolites in plants, characterized as non-nutritive compounds with preventive properties, including anti-inflammatory, antioxidant, and enzyme-stimulating effects (Berkovich *et al*., 2013; Sharma *et al*., 2018). Additionally, they exhibit antibacterial, anti-cancer, and hormonal activities. Plants with a high concentration of these phytochemicals are often referred to as therapeutic plants. Specific chemical substances with distinct physiological effects on the human body have been identified in medicinal plants (Yadav and Agarwala, 2011). The antioxidant potential and therapeutic properties of plants and their extracts are closely associated with the presence of phytochemicals in their leaves, stems, bark, and roots (Arawande *et al*., 2012).

Researchers are looking more closely for potential substitutes because of the current trend of a high percentage of resistance among microbial strains resistant to multiple medications (Adetunji *et al.*, 2013). In West Africa, medicinal herbs and traditional preparations with antibacterial properties have been widely employed, and studies have demonstrated the remarkable efficacy of these medicinally significant herbs in situations where antibiotic therapies were ineffective (Oshim *et al.*, 2016).

Vernonia amygdalina, a shrub native to tropical Africa, is now cultivated in various West African countries. It can grow up to 10 meters in height and features oval leaves approximately 6 mm in diameter (Etim *et al*., 2012; Habtamu and Melaku, 2018). In Nigeria, the leaves are traditionally utilized in the preparation of vegetable soups and in folk medicine to address various health issues (Mohammed *et al*., 2019). The leaves possess a bitter taste, which can be mitigated by soaking or boiling in fresh water. This bitterness is attributed to the presence of alkaloids, glycosides, saponins, and tannins (Habtamu and Melaku, 2018). Due to this characteristic bitterness, the leaves are employed as a bittering agent and as a substitute for hops in beer brewing, without compromising the quality of the malt (Farombi *et al*., 2011).

Moreover, the leaves and roots of the plant are utilized in ethnomedicine to address a range of health conditions, including fever, hiccups, kidney disorders, and stomach pain (Mohammed et al., 2019). Izevbigie et al. (2004) reported that aqueous and methanolic extracts from the roots, stems, and leaves of the plant are extensively employed in the treatment of malaria, purging, and eczema. Additionally, in human medicine, the plant exhibits significant anti-tumorigenic properties.

Numerous investigations conducted on this plant have indicated the presence of bioactive substances, such as alkaloids, flavonoids, phenolics, saponins, steroidal glycosides, tannins, terpenes, and triterpenoids (Luo *et al.*, 2017), which confer a variety of pharmacological properties, such as antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, antihelmintic, anti-inflammatory, cathartic, anticancer, antifertility, antifungal, and antibacterial properties (Alara *et al.*, 2017).

While previous studies have demonstrated the antibacterial properties of *Vernonia amygdalina*, there is limited research on the comparative effectiveness of different extraction solvents. This study aimed to address this gap by evaluating the phytochemical composition and antibacterial activity of methanol and aqueous extracts against gastroenteritis-causing bacteria.

MATERIALS AND METHODS

Bitter leaf Collection and identification

Fresh leaves of *Vernonia amygdalina* were procured from the Gombe main Market in Gombe local government area of Gombe State, Nigeria. Leaves were identified at the Department of Plant Sciences, Gombe State University (GSU). The leaves were washed severally and dried in the shade at room temperature, grounded to powder, and stored in a sterile air-tight container

Extraction of Bioactive Compounds

Following a two-week period of air drying, the leaves were pulverized using a laboratory blender, as recommended by Alara et al. (2017). Fifty grams (50 g) of powdered bitter leaf was finely ground and macerated (cold maceration) for five days using distilled water and methanol. Whatman No. 2 filter paper was used to filter the extracts, and the aqueous and methanol extracts were concentrated to crystal solids in a water bath and rotary evaporator. Two separate beakers marked "Methanol" and "Aqueous" were used to collect the extracts.

Isolates of Bacteria

Three (3) different bacterial isolates (*Salmonella typhi*., *Escherichia coli*, and *Staphylococcus aureus*) associated with gastroenteritis were obtained from the pathology department of the Federal Teaching Hospital, Gombe. The isolates were characterized at the species level at the Microbiology Laboratory of Gombe State University. Colony morphology, Gram staining, and biochemical assays (Voges Proskauer, Indole, Methyl Red, Catalase, Citrate Utilization, and Coagulase assays) were among the methods used to characterize the isolates (Prescott *et al.,* 2011). The isolates were kept on nutrient agar slants in a refrigerator at 4^{o}C until needed.

McFarland (0.5) Standard for Turbidity Preparation

A beaker containing 1.17 g of barium chloride was filled with 100mL of distilled water to dissolve it.1 milliliter of sulfuric acid and 100 milliliters of distilled water were mixed in a different beaker to yield 1% sulfuric acid. One milliliter (1 ml) of 1% sulfuric acid solution and a volume of barium chloride solution (0.5 mL) were mixed, resulting in McFarland Benchmark solutions. Standardized bacterial isolates were obtained by culturing the isolated colonies in 4 mL of sterile normal saline and comparing the turbidity to the McFarland standard after 24 h. This was performed for every isolate (Robinson *et al.,* 2023).

Stock Preparation

A single gram (1 g) of the methanolic and aqueous extract of bitter leaf was placed in each of two "stock" sample vials. Dimethyl sulfoxide (DMSO) (2 ml) was added to each sample and thoroughly mixed. One milliliter of dimethyl sulfoxide (DMSO) was serially diluted in eight separate sample bottles labeled Aq12.5, Aq25, Aq50, Aq100, and Met12. 5, Met 25, Met 50, and Met 100 using one milliliter of aqueous and methanolic extracts of bitter leaves from the corresponding stocks. Following Robinson *et al.*(2020), serial dilutions were used to achieve concentrations of 12.5, 25, 50, and 100 mg/ml.

Antimicrobial screening of extract by agar well diffusion method

The antibacterial efficacy of the leaf extract of *V. amygdalina* was evaluated against the test organisms using the agar-well diffusion method. Sterile Mueller-Hinton (MH) agar (25 mL) was aseptically dispensed into each labeled petri dish and allowed to solidify at room temperature. A sterile cotton swab was immersed in a bacterial suspension standardized to a 0.5 McFarland standard (containing 1.5×10^{8} CFU/mL). The excess fluid was removed by rotating the swab against the inner wall of the test tube. This swab was then used to inoculate the surface of Mueller-Hinton agar plates by rotating the plate anticlockwise until the entire surface was covered. Five wells were created in each MH agar plate using a cork borer of 8 mm diameter, and the wells were labeled with different concentrations of the extract (100, 50, 25 mg/mL, 12.5 mg/mL). Subsequently, 0.1 mL of each extract concentration was introduced into the appropriately labeled wells. The plates were left to stand at room temperature for 1 h to allow adequate diffusion, followed by incubation at 35°C for 18-24 hours. Diluent served as a negative control. After incubation, the plates were examined for zones of inhibition, and the diameters of these zones were measured using a transparent ruler and recorded in millimeters (Ghamba *et al*.,2014).

Phytochemical Qualitative Analysis

After dissolving two grams (2 g) of the *V. amygdalina's* aqueous and methanolic extracts in ten milliliters (10 ml) of distilled water, two milliliters of these mixtures were put into ten distinct test tubes (5 for methanol and five for aqueous), and the following tests were conducted as described by Robinson *et al.*(2023).

i. Test for Alkaloids Wagner's reagent (2 ml of) was added to an aqueous and a methanol extract sample, and a reddish brown coloration was formed.
ii. Test for Saponins: Two of the test tubes containing the aqueous and methanol extracts were collected and shaken well, and long-lasting persistent lather froths were formed on top.
iii. Test for Tannins: Ferric chloride was added to two sample tubes(one aqueous and one methanol), and a brownish green coloration was formed.
iv. To test for flavonoids, 2% sodium hydroxide was added to two sample tubes (one aqueous and one methanol), and an intense yellow coloration was formed.
v. Test for Steroids: To the last two sample tubes, 2 ml of chloroform was added to each tube, giving a pale green color, followed by 2 ml of sulfuric acid, which yielded rush bubbles. A dark brown layer was formed beneath, with yellowish florescence on top.

RESULTS AND DISCUSSION

Results

Phytochemical Screening

Table 1 presents the findings from the qualitative phytochemical analysis of the *V. amygdalina* leaf extract. The screening identified the presence of flavonoids, alkaloids, steroids, saponins, and tannins within the extract. Notably, steroids were absent in the aqueous extracts, whereas alkaloids, tannins, saponins, and flavonoids were detected. All five bioactive constituents were identified in the methanolic extract.

The symbols of single sign (+), double sign (++), and triple sign (+++) denote the intensity of effect or coloration observed during the phytochemical screening. Saponins and tannins exhibited a very high effect (+++), characterized by a persistent froth for saponins and a pronounced brownish-green coloration for tannins. Alkaloids and flavonoids demonstrated a moderately strong effect, indicated by the double sign (++), with their resultant coloration (reddish-brown and intense yellow, respectively) diminishing over time. Steroids exhibited a single sign (+), indicating a lesser

effect, with a dark brown layer beneath and yellowish fluorescence above, which also faded after a period.
The findings indicate that the aqueous and methanolic extracts exerted distinct effects on the isolated ethanol, with the latter demonstrating a significant impact. This disparity can be attributed to differences in the solvents' capacities to dissolve substances, which subsequently influences the quantity of phytochemicals extracted. Consequently, it can be inferred that methanolic extracts contain a higher concentration of phytochemicals compared to aqueous extracts.

Table 1: Qualitative phytochemical screening of solvent-extracts of bitter leaf

	Aqueous Extract	Methanolic Extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Steroids	-	+

Key: + = Presence of phytochemicals, - = Absence of phytochemicals

Table 2: Sensitivity test result of aqueous extract of *Vernonia amygdalina* leaf Concentration (mg/ml)/zone of inhibition(mm)

Isolates	12.5	25	50	100	Control (10 µg)
E. coli	2.5±0.56	9.8±1.20	10.8±0.50	12.0±1.15	21.0±0.25
Salmonella typhi	9.8±0.21	10.5±0.02	12.0±0.06	14.0±0.65	22.0±0.07
Staphylococcus aureus	1.0±0.78	8.5±0.87	10.5±0.37	11.2±0.75	20.2±0.01

Table 3: Sensitivity test result of methanolic extract of *Vernonia amygdalina* leaf Concentration (mg/ml)/zone of inhibition(mm)

Isolates	12.5	25	50	100	Control (10 µg)
E. coli	8.0±0.55	13.5±0.95	14.0±0.45	15.1±0.75	22.0±0.04
Salmonella typhi	8.8±0.51	11.5±1.65	14.0±0.25	13.8±0.45	21.0±0.55
Staphylococcus aureus	8.0±0.75	10.0±0.55	12.5±0.78	13.0±1.45	20.0±0.25

Sensitivity Test

Aqueous Extract

Figures 1 and 2 illustrate the antibacterial activity of aqueous extracts from bitter leaves. The results indicate that the zones of inhibition observed in the isolates varied depending on the bacterial species and the concentration of the extract. Notably, *Salmonella typhi* exhibited the largest zone of inhibition at 14.0±0.65 mm with a concentration of 100 mg/ml, whereas *Staphylococcus aureus* demonstrated the smallest zone of inhibition at 1.0±0.78 mm with a concentration of 12.5 mg/ml. In comparison, ciprofloxacin (10 µg, control) produced an inhibitory zone ranging from 19 to 22 mm.

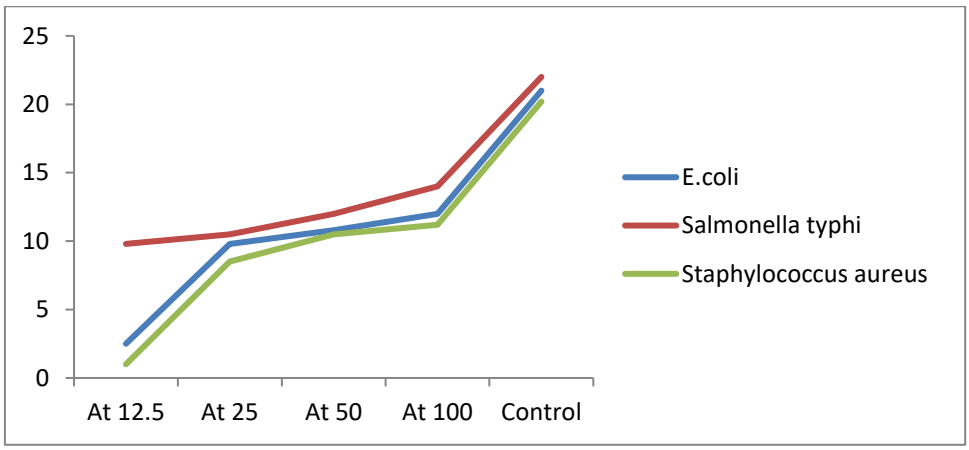

Figure 1: Sensitivity Test Result of Aqueous Extract of Bitter leaf

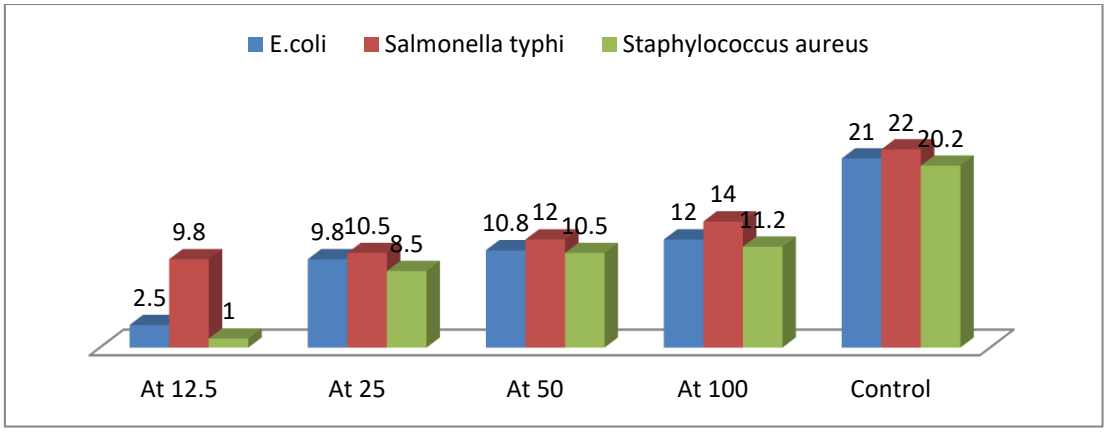

Figure 2: Sensitivity Test Result of Aqueous Extract of Vernoniaamygdalina Leaf Concentration (mg/ml) / Zone of Inhibition (mm)

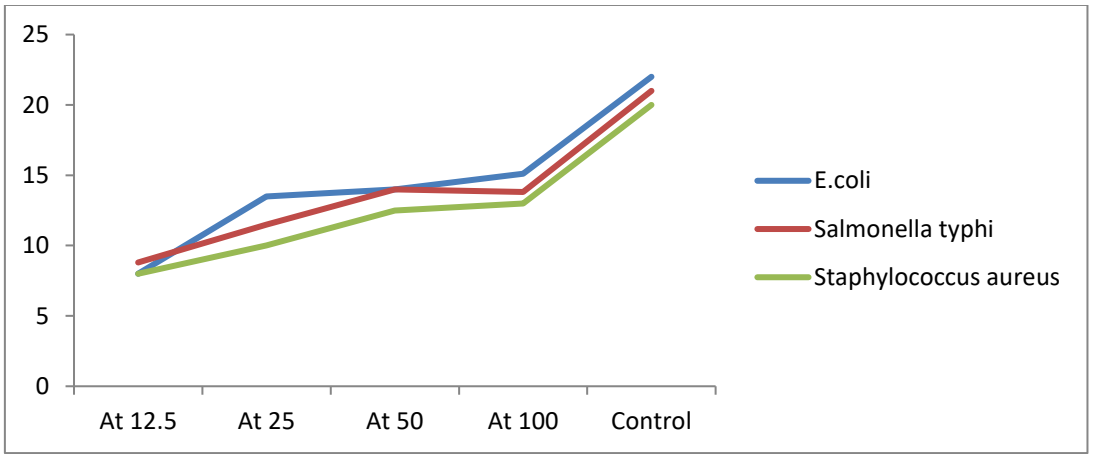

Figure 3: Sensitivity Test Result of Methanolic Extract of Bitter leaf

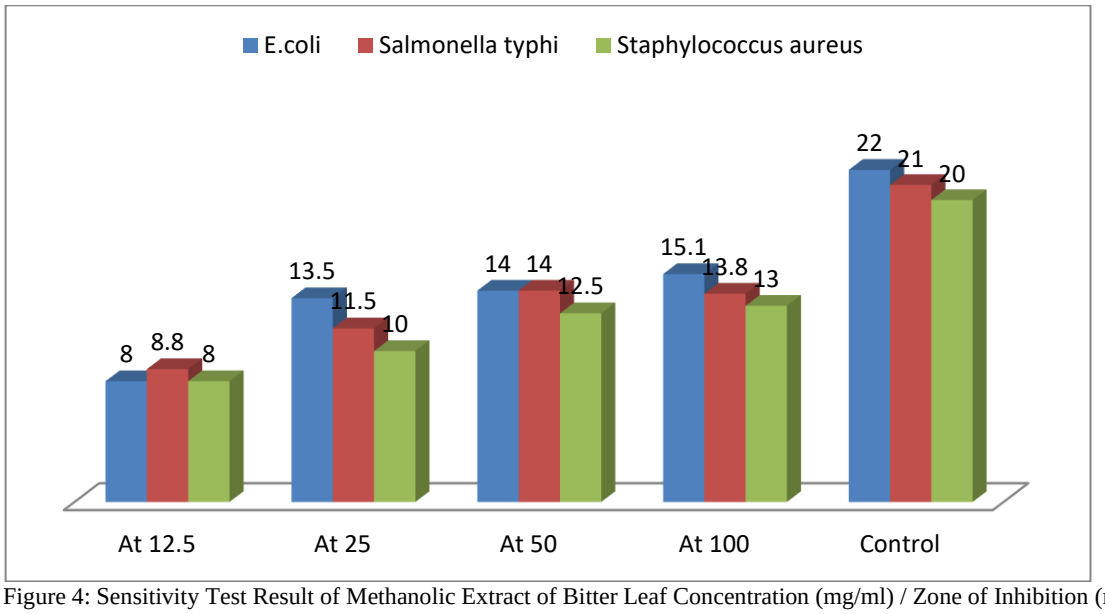

Figure 4: Sensitivity Test Result of Methanolic Extract of Bitter Leaf Concentration (mg/ml) / Zone of Inhibition (mm)

Discussion

Using ethanol and water as extraction solvents, this study aimed to determine the effects and efficacy of *Vernonia amygdalina* leaf extract on *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* in vitro. *V. amygdalina's* phytochemical research showed the existence of alkaloids, flavonoids, saponin and tannins in both the ethanol and cold water extracts while steroid was only present in the methanolic extract. These findings are consistent with prior research conducted by (Ali *et al.*, 2019). Several studies have documented the significance of these phytochemicals, each having unique properties and when combined, providing antibacterial, anti-inflammatory, and antioxidant advantages (Ali *et al.*,2017).

The phytochemical composition of the extracts further supported the observed antibacterial activities, as both extracts contained flavonoids, saponins, tannins, alkaloids, and steroids. Notably, the methanol extract exhibited a richer phytochemical profile, which could explain its enhanced antibacterial activity. This finding is consistent with the known antimicrobial properties of these bioactive compounds (Alara *et al*., 2017). The results of this study align with previous findings, which highlighted a correlation between the concentration of these phytochemicals and the strength of their antibacterial effects (Izevbigie *et al*., 2004). The difference in effectiveness between the two solvents underscores the importance of selecting an appropriate extraction method to maximize the therapeutic potential of plant-based antibacterial agents.

In this study, extracts derived from *V. amygdalina* leaves demonstrated a range of antimicrobial activities against bacterial isolates. The results of this investigation are consistent with those reported by Evbuomwan et al. (2018). The antibacterial efficacy of the solvent used for extracting *V. amygdalina* leaves varied according to the isolates; ethanol extracts exhibited superior activity compared to cold water extracts. It was also observed that the effectiveness of both cold-water and ethanolic extracts was concentration-dependent, as evidenced by an increase in the zone of inhibition with higher concentrations. The volatility of ethanol may account for its superior performance as a solvent for extracting the active component compared to cold water. Similar findings were also documented by Zubairu et al. (2019).

The ethanol and cold-water extracts of *V. amygdalina* showed the lowest inhibitory concentrations of 12.5 mg/mL and 25 mg/mL, respectively, for both isolates. The results of this study demonstrated that, at a lower concentration, the ethanol extract of *V. amygdalina* was more efficient than the cold water extracts in suppressing *S. typhi* and *Escherichia coli*. This is consistent with the results of Gberikon et al. (2019), who reported that *V. amygdalina* ethanol extracts are useful in preventing the growth of some bacterial isolates.

In this study, the methanol leaf extract of *Vernonia amygdalina* exhibited more pronounced inhibition zones across all bacterial strains, with the largest zone observed against *Escherichia coli* (15.1 mm at 100 mg/mL), surpassing the zone observed with the aqueous extract (14.0 mm against *Salmonella typhi* at 100 mg/mL). This result contrasts with previous research, which reported that ethanol extracts, owing to their superior solvent strength and ability to dissolve more hydrophobic compounds, demonstrated higher antibacterial efficacy (Azuamah *et al*., 2024; Bello *et al*., 2024). In the literature, both Azuamah et al. (2024) and Bello et al. (2024) found that ethanol extracts had more significant inhibition zones compared to methanol, especially against bacterial strains like *Salmonella typhi* and *Pseudomonas aeruginosa*. This contrast highlights the variability in the extraction solvent efficiency and suggests that methanol may be a more effective solvent for certain antibacterial compounds in this study. This observation is consistent with findings suggesting that ethanol extracts from *Vernonia amygdalina* are particularly potent against gram-positive bacteria, such as *Staphylococcus aureus*, although less effective against gram-negative bacteria (Bello *et al*.,2024).

The findings of this study corroborate those reported by Adetunji et al. (2013). Alkaloids are known to regulate growth and perform specific metabolic functions within living organisms. Given their ability to inhibit cell division, the alkaloids present in bitter leaf may account for their utilization as antibacterial agents. Alkaloids serve as beneficial compounds for plants, acting as deterrents against parasites and predators. As noted by Akinpelu et al. (2018), this characteristic likely contributes to their antibacterial efficacy. Furthermore, flavonoids have been associated with antioxidant activity in both healthy and diseased states. For instance, tea flavonoids have been demonstrated to lower blood triglyceride and cholesterol levels and to reduce the oxidation of low-density lipoprotein. Plants infected with microbes also produce flavonoids, which may suggest their antibacterial properties.

Saponins are postulated to interact with cholesterol-rich cancer cell membranes, thereby inhibiting cellular proliferation and viability. The saponins found in medicinal herbs also exhibit anti-inflammatory properties, which are primarily responsible for the biological activities associated with cell division and proliferation. The presence of saponins in bitter leaves enhances the herb's anti-inflammatory capabilities. Steroids hold significant importance in the pharmaceutical industry due to their inclusion of compounds analogous to sex hormones, which can be utilized in drug development. Terpenoids have demonstrated efficacy in both the treatment and prevention of various disorders, including cancer, as reported by Errasto et al. (2015). These terpenoid compounds exhibit antimicrobial, antifungal, antiviral, antiparasitic, antiallergenic, antispasmodic, anti-inflammatory, and immunomodulatory properties.

Phenolics have been shown to have potent anticancer properties, act as free radical elements that stop oxidative cell damage, and possibly even generate mechanisms that influence cancer cells and prevent tumor invasion. Owing to their capacity to scavenge and neutralize free radicals, they also have anti-inflammatory properties and reduce the risk of heart disease. Tannins are recognized to possess antiviral properties in addition to their preventive and therapeutic effects on cancer cells. The current investigation also showed the antibacterial efficacy of *V. amygdalina* leaves against a few bacterial isolates associated with gastroenteritis (Errasto *et al.,* 2015).

CONCLUSION

This study demonstrated that both methanolic and aqueous extracts of *Vernonia amygdalina* possess significant antibacterial activity against *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*, with methanol proving to be a more effective solvent for extracting bioactive compounds. Phytochemical screening confirmed the presence of key antimicrobial agents including alkaloids, flavonoids, saponins, tannins, and steroids, which contributed to the observed antibacterial effects. These results further suggest that the antibacterial efficacy of the extracts is concentration-dependent, with higher concentrations yielding larger zones of inhibition. Given the increasing resistance of pathogenic bacteria to conventional antibiotics, the findings from this study support the potential use of *Vernonia amygdalina* as a natural alternative to combat gastroenteritis-causing bacteria. Future research should explore the mechanisms of action of these bioactive compounds and their potential applications in pharmaceutical and therapeutic formulations.

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