



Synergistic Effect of Alkaloid and Saponin of Bitter leaf (*Vernonia amygdalina*) Against *Escherichia coli*

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ABSTRACT

The work was aimed at studying the Synergistic effect of alkaloid and saponin compound of the *Vernonia amygdalina* against *Escherichia coli*. Ethanol and aqueous were used as solvent for crude extraction. The antibacterial activities of the alkaloid, saponin and their synergistic effect were carried out at varying concentration of 100, 50, 25, 12.5 and 6.25 mg/ml, using agar well diffusion method. The result of this study indicate that, ethanolic extract shows higher anti-bacterial activity at 100mg/ml concentration (11.29 mm) compared to aqueous extract (9.24mm) against the test bacteria. It also showed that the anti-bacterial activity of the alkaloid content of the *Vernonia amygdalina* at 100 mg/ml (11.01mm) was better than that of saponin (9.32mm). The result revealed that synergistic effect of alkaloid and saponin at concentration 100 mg/ml (15.41mm) was better than the individual compounds. The result demonstrates the possibility of using the alkaloid and saponin compounds synergistically in the treatment of disease associated with *Escherichia coli*.

Keywords: *Vernonia amygdalina*, Synergy, Alkaloid, Saponin, *Escherichia coli*.

INTRODUCTION

The herb known as bitter leaf (*Vernonia amygdalina*) is a shrub or small tree that can reach 23 feet in height when fully grown (Atata and Sani, 2003). Bitter leaf has grey or Brown texture and is flask, the herb is an indigenous Africans plants, which is grown in most part of sub Saharan Africa (Farombi and Owoeye, 2011). The North, West, South and Eastern part of Nigeria are traditionally linked with the plant and can be found growing wild along the edge of agricultural field. Fresh bitter leaf is of great importance in human diet because of the presence of vitamins and minerals (Subha *et al.*, 2012).

Medicinal plants have been used for centuries as remedies for human diseases, because they contain chemical components of therapeutic value (Kigigha and Onyema, 2015). More than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Olamide, 2013). According to the World Health Organization, medicinal plants are the best sources to obtain variety of drugs. The antimicrobial properties of many plants have been investigated by a number of researchers worldwide (Farombi and Owoeye, 2011). Nigeria is covered with a large number of plant species, some of which have been used for centuries (Kowero *et al.*, 2016).

Plants generally produce many secondary metabolites which constitute an important source of antimicrobials, pesticides and many pharmaceutical drugs. Ogundipe *et al.* (1998) asserted that, plant products still remain the principal sources of pharmaceutical drugs and agents used in traditional medicine. Studies have been conducted on the antimicrobial potentials of crude extract of leaves, bark, bulbs, stems and roots of various plants (Subha, *et al.*, 2012). A number of phytotherapy manuals have stated various medicinal plants for treating

infectious diseases (Uzoigwe and Agwa, 2011). Among the diseases that have been managed successfully by traditional (herbal) medicine include malaria, infertility, convulsion, diarrhoea, dysentery, gonorrhoea, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections (Uzoigwe and Agwa, 2011). Phytochemical analysis of *Vernonia amygdalina* revealed that it contain bioactive compounds which include Alkaloid, saponin, tannin, glycoside, flavonoid (Saga and Yamaguchi, 2009). This study focuses on the synergistic effect of alkaloid and saponin against *Escherichia coli*.

MATERIALS AND METHODS

Study Area

This study was carried out in Kebbi State University of Science and Technology Aliero, Aliero Local government, Kebbi State, North-Western Nigeria. The Local Government area is situated on the South-East part of the Kebbi on the longitude and latitude 120°c' 160°c' 420°c'N4'27'066E.

Source of Plant Materials

Bitter leaf plant (*V. amygdalina*) was obtained from farm orchard, Faculty of Agriculture, Kebbi State University of Science and Technology, Aliero, Nigeria. The plant was identified and authenticated by plant taxonomist with a voucher number 304 in the Herbarium Unit of the Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero. The plant was collected using clean polythene bag to avoid contamination and transported to the Microbiology laboratory for further use.

Acquisition of Stock Culture

The stock culture of *Escherichia coli* was originally obtained from Usmanu Danfodiyo University Teaching Hospital Sokoto (UDUTHS) Microbial Germ Plasma Culture Collection Unit (MGPCCU), the isolate was

maintained on nutrient agar and stored at 4°C for further use.

Preparation of Plant Extract

The plant leaves were washed with distilled water to remove debris and dried under room temperature, the dried plant leaves were ground into fine powder using a pestle and mortar and then sieved using a sieve (domestic sieve). 100g of the leaves powder was weighed using electronic weighing scale and mixed with 500ml of ethanol in 1000ml conical flask. The mixture was shaken vigorously and was allowed to stand for 24 hours. The mixture was filtered using Muslin cloth and finally using, Whatman (No. 1) filter paper, the filtrate was placed in a water bath at 45°C thereby obtaining crude jelly ethanolic extract and it was stored in the refrigerator for further use. The same procedures were followed for aqueous extraction except that water was used as solvent in place of ethanol.

Phytochemical Screening

Test for Alkaloids

Vernonia amygdalina leaf extract (3.5g) was weighed and mixed with 2ml of Chloroform; 2.0 drops of Dragendorff reagent were added to the solution. The pH of the filtrate was adjusted with ammonia to about 7.0. The test tubes were observed for colour precipitates. A rose red precipitate indicated the presence of Alkaloid (Nostro *et al.*, 2000).

Tests for Tannins

Vernonia amygdalina leaf extract (0.14g) was weighed and mixed with 10 ml of distilled water and filtered. Two drops of Ferric chloride solution were added to the filtrate. Observation for blue-black precipitate was done and result recorded (Kumar and Varela, 2012).

Tests for Steroidal Glycosides

Vernonia amygdalina leaf extract (0.5g) was weighed and mixed with 10 ml of iso-propanol. The solution was transferred to a dried test tube and 2ml of concentrated Sulphuric acid was added (Liebermann-Burkhart's reaction). Observation for the formation of a reddish brown ring was done and result recorded.

Tests for Saponin

Vernonia amygdalina extract (1g) was weighed into a conical flask in which 10ml of distilled water was added and heated at 45°C for 5minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of distilled water in a test tube. The test tube was stopped and then shaken vigorously for about 30seconds. It was allowed to stand for half an hour. Observation for honeycomb froth was done and the result recorded (Obaroh and Nze- Achionye, 2011).

Extraction of Alkaloid

The method of Nostro *et al.* (2000) was employed. The plant extract was treated with 90–95% (v/v) ethanol, previously acidified with tartaric acid. The proportion of crude extract to solvent was maintained as 1Kg to 1Litter. The alcohol was distilled off under vacuum and the resulting aqueous residue was treated with petroleum-

ether (60–80°C) to remove the fatty components completely. Alkaloid removed by the petroleum ether was recovered by treatment with a dilute mineral acid. Thus, the resulting aqueous extract was mixed with the main bulk of aqueous extract. The combined aqueous extract was filtered and evaporated to dryness in a Rotary Thin-Film Evaporator under vacuum. The residue was extracted with absolute ethanol thereby dissolving the total alkaloids.

Preparation of Culture Media

The media used was Mueller Hinton agar and Nutrient broth. The media was prepared according to the manufacturer's instructions.

Antibacterial Activity Test

Plant extract (1.0g) was weighed and mixed with 5ml of distilled water to obtain 100mg/ml concentration; 0.5g of alkaloid and saponin each were dissolved to obtain 100 mg concentrations. The different concentrations of each extract and compound were then dispensed into their respective wells on the inoculated plates. Broad-spectrum antibiotic Ciprofloxacin (125 mg/ml) was used as control. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured in millimeters (mm) using a transparent ruler. Standard susceptibility range was used to classify the zones of inhibition as either sensitive or resistant.

Susceptibility Testing

The susceptibility test of the plant extracts was carried out using the agar well diffusion method as previously described by Olamide (2013). Clinical isolates of *E. coli* were inoculated on the surface of Mueller Hinton agar plates by surface spreading using a sterile swab-stick. The tested organism (*E. coli*) was evenly spread over the entire surface of the agar plate using sterile cotton swabs to obtain uniform inoculum. Six wells of 6mm diameter and 5mm depth were bored on the solid agar on each plate using a sterile glass borer and properly labelled for the different concentrations of the extracts and compounds. 1g of each plant extract and compounds of *V. amygdalina* was dissolved in 5ml of distilled water to obtain 100mg/ml concentrations. The dilution was employed to obtain the other concentration 50, 25, 12.5 and 6.25mg/ml. The different concentration of each extract and compounds were then dispensed into their respective wells on the inoculated plates. Broad-spectrum antibiotic Ciprofloxacin (125mg/ml) was used as control. The zone of inhibition was measured in millimeters (mm) using a transparent ruler. Standard susceptibility range was used to clarify the zone of inhibition as either sensitive or resistant.

Statistical Analysis

The Statistical Analysis was carried out using Special Package of Social Sciences Version 20 (SPSS 20)

RESULTS

The result showed the presence of alkaloid, saponin, phenolic, tannin, steroid, flavonoid and glycosides as Phytochemical components of *Vernonia amygdalina* (Table 1).

Table 1: Phytochemical Analysis of *V. amygdalina* extract

Compounds	Ethanollic	Aqueous
Alkaloid	+++	+++
Saponin	+++	+++
Phenolic	+++	+++
Tannin	++	++
Flavonoid	+++	+
Glycoside	++	+
Steroid	-	-

Key: +++ = present, - = absent.

In terms of solvent, ethanol extract showed a higher level of antibacterial activity against *Escherichia coli* compared to the aqueous extract (Table, 2). The highest zone of inhibition (20.13 mm) was observed at the

concentration of 100 mg/ml using ethanolic extract, while the lowest zone of inhibition (7.68mm) was recorded at the concentration at 6.25 mg/ml with the aqueous extract (Table 2).

Table 2: The Mean zone of inhibition (mm) of *V. amygdalina* extract against *E. coli*

Concentration mg/ml	Ethanol extract	Aqueous extract
Control	8.51±0.16 ^a	7.86±0.24 ^a
6.25	10.67±0.16 ^{ab}	7.68±0.70 ^a
12.5	9.67 ±1.11 ^a	8.32±0.90 ^{ab}
25.0	11.29 ±1.9 ^b	8.47±0.70 ^{ab}
50.0	18.58 ±4.5 ^{cd}	8.63±1.08 ^{ab}
100	20.13 ±3.11 ^d	9.24±1.20 ^b

Values with different superscript letters are significantly different along the column

The synergistic effect of alkaloid and saponin content of *V. amygdalina* extract against *E. coli* showed the highest

zone of inhibition (15.41mm) at 100mg/ml as compared to individual compound (Table 3).

Table 3: The synergistic effect of Alkaloid and Saponin content of *V. amygdalina* extract against *E. coli*

Concentration Mg/ml	Alkaloid	Saponin	Synergy
Control	8.57±0.50 ^a	9.45±0.07 ^b	10.16 ±0.20 ^a
6.25	9.91±0.40 ^{ab}	7.50±0.60 ^a	13.24±0.70 ^b
12.5	10.49 ±0.10 ^b	7.72 ±0.40 ^a	14.32 ±0.40 ^c
25.0	10.64 ±0.40 ^b	7.99 ±0.40 ^a	14.65±0.04 ^c
50.0	10.72 ±0.80 ^b	8.13 ±0.50 ^b	14.89±0.22 ^{cd}
100	11.01 ±1.20 ^b	9.32 ±0.30 ^c	15.41±0.08 ^d

Values with different superscript letters are significantly different along the column

DISCUSSION

The phytochemicals analysis revealed the presence of alkaloid, saponin, phenolic, tannin, steroid, flavonoid and glycosides which have been associated with antimicrobial activities (Aliyu *et al.*, 2008). This study agrees with that of several researchers that demonstrated that *V. amygdalina* extracts contained bioactive components that have antibacterial activity against

several species of bacteria and even fungi. The abundance of bioactive compounds in *V. amygdalina* leaves extracts indicate the possibility of its application in the treatment of diseases associated with antibiotics/anti-fungal resistance organisms. This study indicated that ethanolic extract showed higher antimicrobial activity on *E. coli* compared to that of aqueous extract. this can be as a result of the ability of ethanol to extract more essential secondary metabolites

which are believed to exert antibacterial activity on the test organisms. This corroborates the report of El-Mahmood *et al.* (2010) that the extract ability of phyto-constituents differs from solvent to solvent which is depending on their polarities. This was also observed in similar research conducted by Kumar *et al.* (2006) that ethanolic extract of *V. amygdalina* had a greater zone of inhibition against *Pseudomonas aerogenosa*.

The findings also revealed that crude extract was more active against the test bacteria, this corroborates with an earlier report by Johnson *et al.* (2011). where greater activity was observed against Gram-negative bacteria than the Gram-positive bacteria. This is also similar to the result obtained by Olamide *et al.* (2013) where herba-alba aqueous extracts were reported to have weak antimicrobial activities against *E. coli*. Alkaloid recorded a higher zone of inhibition (11.01mm) at a concentration of 100 mg/ml. This showed alkaloid to be more effective against the test organisms compared to saponin. This finding corroborates the report of Shato (2003) who observed that the alkaloid compound detected from the phytochemical analysis of *Anacardium occidentale* seed and shell had cytotoxic and pharmacological attributes against *E. coli*. The higher zone of inhibition observed from the result obtained signifies the efficacy of the plant extracts and could be an alternative treatment to orthodox medication used as an antibacterial agent. However, the plant could be used to treat illness due to bacterial infections after further toxicity study is been carried out (Singleton *et al.*, 1999).

Furthermore, the alkaloid compound of *V. amygdalina* was reported to have a greater effect against Gram-negative bacteria compared to Gram-positive ones. This was also observed in a study conducted by Ferro *et al.* (2003) who reported that the alkaloid compound detected from the phytochemical analysis of *Anacardium occidentale* seed and shell had cytotoxic and pharmacological attributes against *E. coli*. Moreover, among the effect of alkaloids compounds that they inhibit certain mammalian enzymatic activities such as phosphodiesterase, plunging the action of cyclic AMP. Alkaloid also affects glucagons and thyroid-stimulating hormones (Olusesan *et al.*, 2010).

The synergistic effect of alkaloid and saponin compound of *V. amygdalina* had greater antimicrobial activity against *E. coli* at the same concentration. This finding is in agreement with the result reported by Adwan and Mhanna (2008) of a synergistic effect of plant extract and antibiotics that have the ability to inhibit the growth of microorganisms synergistically depending on the method. Similarly, this result agrees with the work of EL Mahmoud *et al.* (2010) that the synergistic effect of *Artemisia herba-Alba*, and allium showed greater antibacterial effect against *E. coli* than an individual. This study could serve as a good opening for policymakers wishing to promote the use of *V. amygdalina* plant (bitter leaf) as a traditional medicine to fight against infectious diseases in developing countries, particularly Nigeria.

CONCLUSION

The phytochemical analysis revealed the presence of alkaloid, saponin, phenolic, Tannin, flavonoid and glycoside in both ethanolic and aqueous extract. The result of this study indicated that ethanolic extract shows higher anti-bacterial activity compared to aqueous extract

against the test bacteria. It also showed that the antibacterial activity of the alkaloid content of the *Vernonia amygdalina* was better than that of saponin. While the synergistic effect of alkaloid and saponin were better than the individual compounds. It is hereby concluded that potency of the herbal mixture is dependent on the solvent extraction and the dose administered which may vary with target organism.

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