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Antibacterial Efficacy of Vernonia Amygdalina (Bitter Leaf) on Some Bacteria Isolated from Locally Produced Soymilk Sold in Different Markets in Enugu Metropolis

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Abstract:
This study aimed to investigate the antibacterial efficacy of Vernonia amygdalina (bitter leaf) on some bacteria isolated from locally produced soymilk in different markets in Enugu metropolis. The test organisms were identified using standard microbiological methods. The antibacterial activities of ethanolic and methanolic extracts of Vernonia amygdalina (bitter leaf) were carried out by agar well diffusion method. Ethanolic and methanolic extracts of the plant were used at varying concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.76 mg/ml, respectively. The two extracts were found to show antibacterial activity. However, the ethanolic extract had more activity than the methanolic extract. The inhibition zone diameter (IZD) of the ethanolic extract varied from 4 – 20 mm, while that of the methanolic extract varied from 4 -16 mm for all the bacteria tested. The minimum inhibitory concentration (MIC) of ethanolic and methanolic extracts varied with bacteria; 1.56 mg/ml respectively for Staphylococcus aureus, 3.125 mg/ml and 6.25 mg/ml for Escherichia coli, 6.25 mg/ml and 25 mg/ml for Salmonella spp, 3.125 mg/ml and 6.25 mg/ml for Klebsiella spp, and 3.125 mg/l for the typed strains (E. coli NCTC 12241, K. pneumoniae NCTC 13368). The ciprofloxacin (50mg/ml) used as a standard antibiotic compared well with the plant extracts at 200 mg/l. The phytochemical analyses revealed the presence of alkaloids, saponins, flavonoids, glycosides, and tannins. This study shows that plant extract can be used as an alternative to conventional antibiotics.

Keywords: *Vernonia amygdalina (bitter leaf), Minimum Inhibitory Concentration (MIC), test organisms, ethanolic and methanolic extracts, phytochemical analyses*

1. Introduction

Soybeans are an excellent source of protein both in quality and quantity (Agboke *et al*., 2012). Approximately 35-40% of the total dry matter content of whole soybeans is protein, and 20% is fat (Ayo *et al*., 2011). Soymilk, also called soybean milk, soy juice, and sometimes referred to as soy drink is a beverage made from soybeans, a leguminous seed nicknamed the gold pop or the miracle seed (Nzelu, 2009). Soybeans contain a factor that inhibits the action of the digestive enzyme - trypsin- but heat can destroy this factor. A generalized method of processing soymilk includes cleaning, soaking, boiling, cooling, dehulling, wet milling, sieving, reconstitution, pasteurization, packaging, cooling, and refrigeration. However, the local producers of soymilk do not undergo detailed processes and still produce and sell under unhygienic conditions, thereby exposing the soymilk to high levels of contamination by pathogenic and other spoilage organisms (Nzelu, 2009). The poor manufacturing practices employed in soymilk production, including the use of contaminated raw materials and poor quality of water will surely pose their own dangers (Umeoduagu *et al*., 2016). It is being noticed that the greatest consumers are the nursery, primary, secondary, and university students, so there is every need to ensure that it is produced under the most hygienic conditions such that it will not constitute a health hazard to these consumers. In view of this, it leads to stomach upset, diarrhea, vomiting, and body weakness.

In these areas, the product is readily available and close observation of the vendors and environment is of great public health importance. Furthermore, there is a need to enlighten the vendors and consumers about the importance of personal hygiene, since contamination is possible following an unhygienic preparation procedure. Soymilk consumption could threaten human health if harmful microorganisms are not adequately guided during production, storage, and distribution (Omale, 2021).

Medicinal plants and traditional medicine with antimicrobial activities have been used widely in the West African regions, and it is recognized in the primary healthcare system in many communities due to reasons such as affordability, low cost, and accessibility (Thonda *et al*., 2020). Plants of medicinal importance have been shown to be effective even where treatments with antibiotics have failed (Oshim *et al*., 2016). The World Health Organization (WHO) estimates that approximately 80% of the world's inhabitants rely on traditional or herbal medicines for their primary health care, and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments (Sofowora, 1993; Pravi, 2006; Akinjogunla *et al*., 2009). Herbal medicine is the oldest form of health care known to mankind, and over 50% of all modern clinical drugs are of natural product origin, and natural products play important roles in drug development in the pharmaceutical industry (Preeth *et al*., 2010). The rediscovery of the connection between plants and health is responsible for launching a new generation of multi-component botanical drugs, dietary supplements, and plant-produced recombinant proteins (Akinjogunla *et al.*, 2011). However, the increasing problem of food poisoning and stomach gastroenteritis after the consumption of soymilk is of great concern, hence, the use of affordable and available herbal medicine is important.

Vernonia amygdalina is a perennial shrub that belongs to the family Asteraceae (Gashe & Zeleke, 2017). *Vernonia amygdalina* is a shrub that grows to 10 m tall with a petiole leaf of about 6 mm in diameter and elliptic in shape and grows throughout tropical Africa and has been domesticated in various parts of West Africa, including Nigeria, where it is locally used as a vegetable in soups (Etim *et al*., 2012). It is used to treat many ailments, including diabetes, malaria, helminth infections, and fever (Magadula & Erasto, 2009), promote wound healing (Adetutu *et al*., 2011), and is used to treat microbial infections (Noumedem *et al.*, 2013). There is a need to implore the plant extract to treat common gastrointestinal infections, as it contains important bioactive constituents like: alkaloids, tannin, flavonoids, terpenoids, and phenolic compounds. This study aims to evaluate the antibacterial efficacy of *Vernonia amygdalina* (bitter leaf) on some bacteria isolated from soymilk.

2. Materials and Methods

2.1. Collection of Plant Materials

Fresh leaves of *Vernonia amygdalina* were collected from Eke-Agbani market in Nkanu-West Local Government Area Enugu State, Nigeria. The leaves were identified in the Department of Applied Biology and Biotechnology, ESUT. These leaves were then washed severally to remove dirt, dried in the shade at room temperature, ground to powder, and stored in a sterile air-tight container.

2.2. Preparation of Extracts

Fifty grams of *Vernonia amygdalina* leaf powder was soaked separately in 200 ml of methanol and ethanol, respectively, for 48 h and centrifuged at 3000 rpm(revolutions per minute) to enable proper diffusion of the active ingredients as described by Akinnibosun and Edionwe (2015). After that, the contents were filtered using muslin cloth and filtered again using Whatman's filter paper No 1. The filtrates were evaporated separately in a water bath and then used to check the antibacterial activities of some selected bacteria from soymilk.

2.3. Sample Collection

Twenty (20) locally produced samples of soymilk, which were packaged in plastic bottles, were bought from different vendors at different markets. The samples were labeled and taken to Department of Applied Microbiology and Brewing laboratory of Enugu State University of Science and Technology (ESUT), and it was analyzed immediately.

2.4. Sample Preparation and Inoculation of Sample

A total of one milliliter (1 ml) of each sample was added into a test tube containing 5 ml of sterile distilled water. This was swirled evenly in order to homogenize the mixture and this was labeled as the stock. A ten-fold serial dilution of the homogenates was made with sterile normal saline as diluents. This was carried out as described by (Cheesbrough, 2002). A total of 9 ml of the normal saline was measured into eight test tubes. One milliliter (1 ml) of the stock was collected using a pipette and serially diluted into the first tube and from the first tube to the second test tube up to the eighth test tube, respectively (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}). The 10^{-3} and 10^{-4} dilutions were used as the dilution factors and 0.1 ml was taken from each factor into a sterile agar petri dish in duplicates. Inoculation was by standard spread plate method. All plates were incubated at 37 ºC for 24 h. After incubation, the representative colonies on the plates were counted and subsequently purified by repeated sub-culturing. Pure colonies were transferred into nutrient agar slant for biochemical identification.

2.5. Calculations of Colony Forming Unit

The method described by Collins *et al*. (1989) for estimating bacteria counts was used to enumerate the total viable counts of the isolates. Countable plates were selected and counted. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was for 0.1 ml of the original sample and plating was done in duplicates for each dilution. The mean colony count on the plates was used to estimate the total count.

2.6. Characterization and Identification of the Isolates

All the isolated organisms were sub-cultured in nutrient agar plates to obtain a pure culture. The isolates were identified based on the method described by Cheesbrough (2002). Gram stain and biochemical tests were carried out to

identify the organisms. The biochemical tests include catalase, coagulase, oxidase, indole tests, and sugar fermentations like lactose, sucrose, mannitol, and glucose.

2.6.1. Indole Test

Sterile test tubes containing 5 ml of tryptophan broth were set on a test tube rack, the tubes were inoculated aseptically, and the bacteria growth was added into it. The tubes were incubated at 37 ºC for 24 h. After 24 h, 0.5 ml of Kovac's reagent was added to it and allowed to stand for 5 minutes. The formation of a pink or red colour ring in the reagent layer on the medium (within 10 seconds) indicates a positive result. The negative result shows no formation of any pink or red colour ring.

2.6.2. Methyl Red Test

The isolates were grown in 5 ml of MR broth (glucose-phosphate peptone water) and incubated for 24 h at 37 ºC. Thereafter, 3 drops of methyl red were added to each test tube. A reddish colour was observed on the addition of an indicator showing a positive result, while a yellowish colour showed a negative result.

2.6.3. Citrate Test

Simon citrate agar was prepared and sterilized into a test tube and slanted. It was allowed to solidify before the organism was inoculated on the surface of the solidified Simon citrate agar in the test tube. It was covered with cotton wool and incubated at room temperature for 24 hours. For a positive result, there will be visible growth, and the medium will be blue, while the negative result showed no visible growth and no change of colour.

2.6.4. Catalase Test

Catalase test was done using a test tube. A clean test tube was placed on the rack, and 1 ml of hydrogen peroxide solution was poured into the test tube. Using a sterile glass rod, bacteria growth was picked from an agar plate and immersed into the hydrogen peroxide solution. The presence of effervescence indicated a positive catalase reaction, whereas a negative reaction showed no effervescence.

2.7. Sugar Fermentations

10 ml peptone water was introduced into 5 sterile test tubes respectively. Three (3) drops of methyl red were added into each of the test tubes, then Durham's tubes were inserted in an inverted position into each of the tubes and sealed with foil before sterilization in an autoclave at 121 ºC for 10 minutes. One gram (1g) of respective carbohydrates: glucose, lactose, fructose, sucrose, and mannitol, were sterilized using a membrane filter and added into each of the sterilized test tubes that contained the peptone water. Thereafter, the cultured organisms were inoculated into each of the tubes respectively. They were then incubated at 37 ºC for 24 h. A positive result indicates the yellow colour, while gas production was seen in the Durham's tube.

2.8. Standardization of inoculum

A 24 h culture of the isolates on nutrient agar was used. Three colonies of each of the isolates were added into 3 ml sterile normal saline, and the turbidity was adjusted to match 0.5 McFarland standard, which is equivalent to $1.5x10^8$ bacterial cells.

2.9. Dilution of the Extracts

The stored extracts were reconstituted using dimethyl sulfoxide (DMSO) to obtain extracts of several concentrations 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.76 mg/ml and stored at 4 ºC prior to the determination of minimum inhibitory concentration.

2.10. Determination of Antibacterial Efficacy of Vernonia amygdalina

Antibacterial efficacy of *Vernonia amygdalina* was determined by agar well diffusion method as described by Ogata *et al*. (2000). The plates containing Mueller Hinton agar medium were spread with 0.1 ml of the bacterial inoculum. Wells (6 mm in diameter) were cut from the agar plates using a sterilized stainless steel borer, and wells were filled with 0.1 ml of each extract. The plates were incubated at 37 ºC for 24 h, and the diameter of the resultant zone of inhibition was measured. The least concentration that inhibited the growth of microorganisms was termed the minimum inhibitory concentration (MIC).

2.11. Antibiotic Sensitivity Test

A standard antibiotic, ciprofloxacin, was used as a control to test the susceptibility of the bacteria. This was employed to compare and contrast the antibacterial activities of the plant extract.

2.12. Phytochemical Evaluation of the Plant Extract

Phytochemical analysis was carried out using standard protocol to determine the following phytoconstituents: alkaloids, flavonoids, saponins, tannins, glycoside, reducing sugar, and steroids, as described by Odebiyi and Sofowora (1978).

The tests are as follows:

2.12.1. Test for Alkaloids

Five grams of the evaporated extract was boiled with 5 ml of dilute HCl in a water bath for 5 minutes. The mixture was cooled and filtered. Moreover, the filtrate was subjected to an alkaloids test using Dragendoff's reagent. The formation of a red precipitate indicates the presence of alkaloids.

2.12.2. Test for Flavonoids

Three grams of the extract were treated with ethyl acetate solution and heated in the water bath for 1 minute. The mixture was cooled and filtered. The filtrate was shaken with 1% aluminium chloride solution and left for 10 minutes. The formation of yellow colouration indicates the presence of flavonoids.

2.12.3. Test for Saponins

One gram of the extract was boiled with 5 ml of distilled water for 5 minutes. The content was filtered while hot, and the filtrate was treated with a few drops of olive oil and vigorously shaken. The formation of emulsion indicates the presence of saponins.

2.12.4. Test for Tannins

Two grams of the evaporated extract were boiled with 45% ethanol for 5 minutes. The mixture was cooled and filtered, and then the filtrates were treated with a few drops of lead acetate solution. The formation of a gelatinous precipitate indicates the presence of tannins.

2.12.5. Test for Glycosides

One gram of the evaporated extract was boiled with 15 ml of distilled water for 5 minutes in a water bath. The mixture was cooled and filtered. Then, 0.2 ml of Fehling's solutions A and B were added to 5 ml of the filtrate and further boiled for 2 minutes in a water bath. The formation of a brick-red colouration indicates the presence of glycosides.

2.12.6. Test for Reducing Sugars

The plant extract was treated with Fehling's solution (A and B) in a test tube. The colour change from deep blue to brick red indicates the presence of reducing sugar.

2.12.7. Test for Steroids

Two ml each of concentrated sulphuric acid (H_2SO_4) and acetic anhydride was poured into 5 ml of each of the aqueous extract samples. A colour changed to violet indicates the presence of steroids.

2.13. Statistical Analysis

All data collected were analysed using one-way ANOVA.

3. Results

3.1. Mean Bacterial Count (Cfu/Ml) of Soymilk Samples

The mean bacterial count of the soymilk samples indicates that the samples collected were heavily contaminated. The result is shown in table 1.

Sample Collection Market	Bacterial Count (Cfu/Ml)
Agbani Market	$7.6x10^5$
Mayor Market	$6.5x10^5$
Garriki Market	$8.6x10^5$
Eke-Otu Market	$4.9x10^5$

Table 1: Mean Bacterial Count (cfu/ml) of Soymilk Samples

3.2. Distribution of Contaminated Soymilk Samples Sold in Different Markets

It was discovered that the percentage of contaminated samples was high. The result is shown in table 2.

Market	Number of Samples	Number of Positive Samples	Percentage (%)
Agbani market	5	4	80
Mayor market	5	3	60
Garriki market	5	4	80
Eke-Out market	5	5	100
Total	20	16	80

Table 2: Percentage Distribution of Positive Soymilk Samples Sold in Different Markets

3.3. Distribution of Bacteria on Different Soymilk Samples Sold in Different Markets

Different bacteria were isolated from the various soymilk samples. The result is shown in table 3.

Market	Number of Samples	Number of Positive Samples	Suspected Bacteria
Agbani market	5	4	*E. coli, Staphylococcus aureus, Salmonella spp*
Mayor market	5	3	*E.coli, Staphylococcus aureus*
Garriki market	5	4	*Klebsiella spp, Escherichia coli*
Eke-Otu market	5	5	*Salmonella spp, Escherichia coli, Staphylococcus aureus*
Total	20	16	

Table 3: Distribution of Bacteria on Different Soymilk Samples Sold in Different Markets

3.4. Percentage Distribution of Bacteria Isolated from the Soymilk Samples

Different isolated bacteria were analyzed, and different percentages of bacteria were stated. This is shown in table 4.

Bacteria	Number of Bacteria	Percentage (%) Occurrence
E. coli	10	27.8
Staphylococcus aureus	11	30.6
Salmonella spp	9	25.0
Klebsiella spp	6	16.7
Total	36	100

Table 4: Percentage Distribution of Bacteria Isolated from the Soymilk Samples

3.5. Morphological Characteristics of the Isolates

Identification of the isolates was further confirmed by colony characteristics using Salmonella Shigella agar, MacConkey agar, and Mannitol salt agar. The result is presented in table 5.

Media Used	Choromo-genesis	Elevation	Shape	Suspected Organisms
Mannitol salt agar	Yellow	Convex	Round	S. aureus
MacConkey agar	Pink	Raised	Mucoid –Round	Klebsiella sp
MacConkey agar	Dark pink	Convex	Round	E. coli
Salmonella Shigella agar	Black	Convex	Round	Salmonella sp

Table 5: Morphological Characteristics of the Isolates

3.6. Biochemical Identification of the Bacterial Isolates

The isolates were further identified and characterized using Gram stain and other biochemical tests. The result is shown in table 6.

3.7. Antibacterial Efficacy of Methanolic and Ethanolic Extracts of Vernonia Amygdalina on Test Bacteria

The antibacterial efficacy of ethanolic and methanolic extracts of *Vernonia amygdalina* was tested on some bacteria. It was observed that both extracts had an appreciable effect on the test bacteria. It was also observed that both extracts had more effect on *S. aureus.* These results are shown in tables 7 and 8.

3.8. Minimum Inhibitory Concentration of Ethanolic and Methanolic Extracts of Vernonia amygdalina on Test Bacteria

The minimum inhibitory concentration of ethanolic and methanolic extracts of *Vernonia amygdalina* on test bacteria was determined. This is shown in table 9.

S/N	Isolates	Gram Reaction	Catalase Test	Methyl Red Test	Citrate Test	Coagulase Test	Oxidase Test	Indole Test	Sugar fermentation Lactose	Sucrose	Mannitol	Glucose	Fructose
1	*Staphylococcus aureus*	+ve cocci	+ve	+ve	+ve	+ve	-ve	-ve	A/G	A/G	A/G	A/G	A/G
2	*Escherichia coli*	-ve rod	+ve	+ve	-ve	-ve	-ve	+ve	A	A	A/G	A/G	-ve
3	*Klebsiella* spp	-ve rod	+ve	-ve	+ve	-ve	-ve	-ve	A	A/G	A/G	A	A/G
4	*Salmonella* spp	-ve rod	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	A/G	A/G	A

Table 6: Results of Gram Reaction and Biochemical Test

Keys: - A= Acid production, G= Gas Production, A/G= Acid and Gas production

Test Organisms	Different Concentrations (Mg/Ml)								
	200	100	50	25	12.5	6.25	3.125	1.56	0.76
Staphylococcus aureus	19	15	12	11	10	9	9	8	0.0
Escherichia coli	20	18	16	14	12	8	7	0.0	0.0
Salmonella spp	12	11	10	9	6	4	0.0	0.0	0.0
Klebsiella spp	18	16	12	10	9	7	4	0.0	0.0
E. coli NCTC 12241	17	16	15	13	10	8	6	0.0	0.0
Klebsiella pneumoniae NCTC 13368	18	15	14	12	11	9	7	0.0	0.0

Table 7: Antibacterial Efficacy of Ethanolic Extract of Vernonia Amygdalina on Test Bacteria Zones of Inhibition (Mm)

Test Organisms	Different Concentrations (Mg/Ml)								
	200	100	50	25	12.5	6.25	3.125	1.56	0.76
Staphylococcus aureus	13	12	11	10	10	9	9	8	0.0
Escherichia coli	16	14	12	11	9	8	0.0	0.0	0.0
Salmonella spp	12	10	9	8	0.0	0.0	0.0	0.0	0.0
Klebsiella spp	13	11	8	7	5	4	0.0	0.0	0.0
E. coli NCTC 12241	15	13	11	10	9	8	6	0.0	0.0
Klebsiella pneumoniae NCTC 13368	16	15	13	12	10	9	7	0.0	0.0

Table 8: Antibacterial Efficacy of Methanolic Extract of Vernonia Amygdalina on Test Organisms Zones of Inhibition (Mm)

Test Bacteria	Minimum Inhibitory Concentration (Mg/Ml)	
	Ethanol	Methanol
Staphylococcus aureus	1.56	1.56
Escherichia coli	3.125	6.25
Salmonella spp	6.25	25
Klebsiella spp	3.125	6.25
***E. coli* NCTC 12241**	3.125	3.125
***Klebsiella pneumoniae* NCTC 13368**	3.125	3.125

Table 9: Minimum Inhibitory Concentration of Ethanolic and Methanolic Extracts of Vernonia Amygdalina on Test Bacteria

Test Bacteria	Ciprofloxacin (50 Mg/Ml)	Ethanolic Extract (200mg/Ml)	Methanolic Extract (200mg/Ml)
Staphylococcus aureus	18	19	13
Escherichia coli	20	20	16
Salmonella spp	25	12	12
Klebsiella spp	19	18	13
E. coli NCTC 12241	13	17	15
Klebsiella pneumonia NCTC 13368	14	18	16

Table10: Antibacterial Activity (Mm) of Ciprofloxacin in Comparison with Plant Extract

Constituents	Ethanol Extract	Methanol Extract
Saponins	+	+
Flavonoids	+	+
Steroids	-	-
Alkaloids	+	+
Tannins	+	+
Glycosides	+	+
Reducing sugars	+	+

Table 11: Phytochemical Constituents of Vernonia Amygdalina Using Different Extracting Solvents

4. Discussion

The increased consumption rate of soymilk by most people due to its high protein content has led to its greater production without considering the possibility of contamination when produced under unhygienic conditions. In this study, the bacterial load of sold soymilk ranged from $4.9x10^5$-$8.6x10^5$cfu/ml (table 1). This is in agreement with the work of Umeoduagu *et al*. (2016), whose bacterial load ranged from $6.1x10^5$- $9.0x10^6$ cfu/ml, and with the work of Agboke *et al*. (2012), who reported a bacterial load of $1.4x10^8$ to $9.2x10^7$ cfu/ml. This is also in line with the work of Liamngee *et al*.

(2013), who reported a bacterial load of $3.8x10^7$ to $8.5x10^7$ cfu/ml. The high bacterial load could be attributed to poor hygienic practices during processing. The bottles used in packaging may not have been washed thoroughly and sterilized before re-using them for subsequent production. At times, these bottles are picked from the roadside without proper washing, thereby posing a risk to consumers' health. In the percentage distribution of positive soymilk samples, we discovered that the samples from Eke-Otu had all samples contaminated (100%), followed by Agbani and Garriki (80%) respectively, and then Mayor Market (60%) (table 2). These results could be attributed to the fact that some producers use unclean water and materials during production. The soymilk samples collected were contaminated with one or two bacteria species. The most dominant one was *Staphylococcus aureus* (30.6%). Other bacteria include *E.coli* (27.8%), *Salmonella* spp (25%), and *Klebsiella* spp (16.7%) (table 4). The presence of these bacteria in the soymilk samples poses a great health hazard to consumers. The presence of *E.coli* in the soymilk samples indicates fecal contamination. This result is in accordance with the work of Agboke *et al*. (2012) and Umeoduagu *et al*. (2016), which isolated similar organisms. Omale, 2013 had 6.1% of *Klebsiella* spp, which is lower than what we got in this study.

Vernonia amygdalina serves as a vegetable and is used for the preparation of food nutritive seasoning (Evbuomwan *et al*., 2018). It has been found to have the potential to inhibit the growth of microorganisms (Evbuomwan *et al*., 2018). Its low cost and easy availability encouraged us to assess its inhibitory potency on the test bacteria. The ethanolic extract of *Vernonia amygdalina* was effective against the isolated bacteria with a zone of inhibition ranging from 8-19 mm for *Staphylococcus aureus,* 7-20 mm for *E.coli,* 4-12 mm for *Salmonella* spp and 4-18 for *Klebsiella* spp (table 7). The methanolic extract had a zone of inhibition ranging from 8-13mm for *Staphylococcus aureus,* 8-16 mm for *E.coli,* 8-12 mm for *Salmonella* spp, and 4-13 mm for *Klebsiella* spp (table 8). These results agreed with the work of Evbuomwan *et al*. (2018), who had the zone of inhibition for both ethanol and methanol from 7-16.5 mm with varying concentrations. Likewise, Olamide *et al*. (2013) had a zone of inhibition ranging from 12-20 mm on *E. coli* at different concentrations and 10-20 mm on *Salmonella* spp. In this study, it was observed that ethanolic extract was more effective on the test bacteria than methanolic extract. The effectiveness of these extracts was due to their phytochemical contents. The finding in this study is in line with the work of Udochukwu *et al*. (2015), who reported the phytochemical and antibacterial activity of *Vernonia amygdalina*. Furthermore, the effectiveness of the extract depends on the nature of the extracting solvent and the concentration of the extract (Evbuomwan *et al*., 2018). The minimum inhibition concentration (MIC) of both ethanolic and methanolic plant extracts were 1.56 mg/ml for *Staphylococcus aureus* respectively, 3.125 mg/ml and 6.25 mg/ml for *E.coli*, 6.25 mg/ml and 25 mg/ml for *Salmonella* spp, 3.125 and 6.25 mg/ml for *Klebsiella* spp, and 3.125 mg/l for both typed strains: *E. coli* NCTC 12241 and *K. pneumoniae* NCTC 13368 (table 9). Ciprofloxacin used in this study inhibited the growth of all the test bacteria, showing a broad spectrum activity (table 10). In addition, ciprofloxacin showed a comparable zone of inhibition with the plant extracts at 200 mg/ml concentration (table 10). This finding indicates that *Vernonia amygdalina* can be used as an alternative to standard antibiotics in treating infections caused by these test bacterial species. The phytochemical analysis of *Vernonia amygdalina* revealed the presence of saponins, flavonoids, alkaloids, tannins, glycosides, and reducing sugar (table 11). This result is in line with Evbuomwan *et al*. (2018), who reported similar findings. The presence of these phytochemical constituents is important because of their antioxidant properties (Atangwho *et al*., 2013; Ashok & Ramaswamy, 2014), anti-diabetes, anti-inflammatory, anticancer, antibacterial and analgesic activities (Erasto *et al*., 2007; Nlandu *et al*., 2012; Alara *et al*., 2019).

5. Conclusion

The bacteriological quality of soymilk sold in the study areas revealed great contamination with different bacteria. This was deduced to be the result of the producer's poor hygiene and poor environmental condition. Therefore, it is recommended that adequate precaution and personal hygiene should be maintained for controlling the contamination of bacteria in soymilk. *Vernonia amygdalina* can be used as a potential antibacterial drug for these bacteria because of its low cost and availability. It is, therefore, recommended that there is an urgent need to discover a new drug that would be effective against these bacteria.

6. Conflict of Interest Statement

Authors declare that they have no conflict of interest.

7. Acknowledgement

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