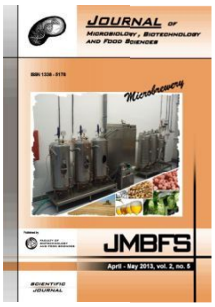

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ASSESSMENT OF BITTER LEAF (*Vernonia amygdalina*) ON SOME SELECTED PATHOGENIC MICROORGANISMS FROM UNIVERSITY OF ILORIN TEACHING HOSPITAL

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ABSTRACT

Vernonia amygdalina is a medicinal plant which is employed to cure various infections in traditional medicine. The aqueous and ethanolic extracts of this plant were analyzed phytochemically and screened against different microorganisms responsible for various human infections. Phytochemical analysis of the extracts showed the presence of many secondary metabolites including tannins, saponins, alkaloids, flavonoids, phylobatannins, steroids and phenolics. The result shows that the ethanolic extracts showed more antibacterial activity than the aqueous extracts. The extracts displayed potent antimicrobial activity against the test organisms which were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*, the minimum inhibitory concentration ranges from 2.5-15mg/ml in all the plant extracts. The results confirmed the potency of this plant in treating human infections.

Keywords: Aqueous, bitter leaf, chemotherapy, extracts, incubation, phytochemicals

INTRODUCTION

Medicinal plants contain certain physiologically active principles, which over the years have been exploited in traditional medical practice for the treatment of various ailments (**Adebanjo *et al.*, 1983**). Plants of both lower and higher groups are known to produce chemical substance with which they defend themselves against invading microorganisms. The chemical materials or substances which are commonly referred to as antimicrobial agents are developed from the noticeable conditions such as viral disease attack, mammalian predation attack and the development of extra-ordinary array of defenses against chemicals and the struggles to survive under intense competition for resources and nutrients (**Tempester and King, 1994**).

A study carried out by **Tajhr *et al.* (1998)** revealed the effectiveness of an antibacterial substance obtained from the crude, ethanolic and chloroform extracts of the medicinal plant (*Myricineafricana)* against *Pseudomonas aeruginosa, Streptococcus* sp. and *Staphylococcus aureus.* **Mann *et al.* (1997)** studied the antimicrobial activity of the leaf extracts of *Calotropis procera* and reported that the *in vitro* (agar streak dilution) bioassay had strong activity at a 250g/ml concentration of the water soluble extract against *Clostridium perfringens* and *Streptococcus faecalis* The interface layer extract has strong activity on *Klebsiella ozaena*, the petroleum soluble extract against *Pseudomonas aeruginosa* and the methanolic extract showed activity against *Salmonella typhi.*
The Yoruba name is 'Ewurojije' 'Olugbu' in Igbo and 'Shiwaka' in Hausa. The shrub is usually about 5m high. The leaves are simple and entire (5×15cm), finely glandular below and displaying few lateral nerves. The flowers occur in a panicle, white and fragments. It is differentiated from its counterpart *V. colorata*, which grows widely with hairy leaves of the latter (**Iwu, 1999**).

Vernonia amygdalina have been used in many homes in the eastern parts of Nigeria as food especially in the preparation of soups. The characteristic bitter taste is believed to have after taste of sweetness. The peeled stem is often used in as chewing stick for cleaning the teeth and is very effective as anticaries (**Adekunle, 2002**). The bitterness of the leaves is also exploited by nursing mothers to assist in weaning their babies by rubbing the juice on their breast (**Iwu, 1999**). It is also suggested to be useful to nursing mother as it improves lactation. The plants leaves and other parts have been used solely or mixed with other plants for treatment of various suspected illness. Interaction with some traditional medicine healers in Ilorin metropolis revealed that the leaves have been extracted raw in aqueous solution and taken as anti-dysentery decoction. It is also said to be useful for sugar level control in diabetes patients; a cup full is prescribed to patients in serious cases.

Dysentery refers to an acute inflammatory reaction of the intestinal tract characterized by watery stool, blood and pus. It is caused by any one of several members of *Enterobacteriaceae*. The *Enterobacteriaceae* or enteric bacteria are a large heterogeneous group of gram negative rods whose natural habitat is the intestinal tracts of human and some other animals (**Jawatz *et al.*, 2004**). They are facultative anaerobes or aerobes that ferment a wide range of carbohydrates, possess a complex antigenic structure and produce variety of toxin and other virulent factors. The family includes many genera*: Escherichia, Salmonella, Shigella, Proteus, Serratia* and *Enterobacter* etc. historically enteric bacteria have been divided into opportunistic pathogens and the intestinal pathogens. The intestinal pathogens are traditionally members of the genera *Salmonella, Shigella* and *Yersina*, while the opportunistic pathogen which infect any body site when given an altered host include *Escherichia coli,* and *Proteus* (**Joklik *et al.*, 1992**). Some are normal flora of the gastrointestinal tract and incidentally cause disease e.g. *Escherichia coli* while others regularly pathogenic for humans e.g. *Salmonella* and *Shigella*. The routes of transmission and avenues of infection are many and varied but the final common route is ingestion of a large number of the bacteria or a dose of their toxic product.

The objective of our study was to investigate the effectiveness of *Vernonia amygdalina* against some selected microorganisms which are implicated in human infections.

MATERIAL AND METHODS

Collection of plant materials

Leaves, barks and roots of *Vernonia amygdalina* plant were collected in sterile polythene bags at Jalala quarters University of Ilorin, Kwara State, Nigeria. The leaves, the backs and the roots were taken to Department of Plant Biology, Faculty of Science, University of Ilorin, Ilorin, Kwara State for proper identification and authentication. The roots were washed with distilled water sliced/cut into pieces and air-dried.

Preparation of extracts

Aqueous and ethanolic extracts of the plant were prepared. The plant leaves, barks and roots were grounded with mortar and pestle.

Aqueous extract

Twelve grams each of the grounded leaves, barks and roots were measured into different sterile conical flasks and 100 ml of hot and sterile distilled water was added, covered with a cork, mixed together properly and left on the shaker at 100 revolution per minute for 24 hours after which the extracts were filtered and squeezed through four layers each of muslin cloth. The filtrates were then centrifuged at 2,000 revolutions per minute (r.p.m.) 5 minutes after which they were decanted. The pellets were discarded and the supernatants were sterilized by using the membrane filtration unit with type HC filters. The filtrates obtained were stored in sterile McCartney bottles and kept in the refrigerator at 4˚C and later used for antimicrobial tests.

Ethanolic extract

Twelve grams each of the grounded leaves, barks and roots were measured into different conical flasks and 100 ml of 95% ethanol was added to each sample, covered with a cork, mixed together and left on the shaker at 100 r.p.m. for 24 hours after which the extracts were filtered and squeezed through four different layers of muslin cloth. The filtrates were then centrifuged at 2,000 r.p.m. for 5 minutes after which they were decanted. The pellets were discarded and the supernatants were sterilized by using the membrane filtration unit with type HC filters. The filtrates obtained were stored in sterile McCartney bottles and kept in the refrigerator at 4˚C and later used for antimicrobial tests.

Sterility test of the Plant extracts

Each of the above extracts (aqueous extract and ethanolic extract) was tested for growth or contaminants. This was carried out by inoculating 1ml of each of them on nutrient agar and incubated at 37˚C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicated that the extracts were sterile. The extracts were then assayed for antimicrobial activity.

Sterilization of materials

All glassware used were washed with detergent, rinsed properly with tap water and dried. They were then sterilized in the oven at 160˚C 2 hours. Cork borer was sterilized by dipping into 70% alcohol followed by flaming over Bunsen burner. Inoculating loop was heated to redness in an open flame. All the media such as Nutrient agar, Potato dextrose agar, Nutrient broth and Muller Hinton agar, distilled water, and McCartney bottles used were sterilized in the autoclave at 121˚C for 15 minutes at 1.8kg/m2. Finally, the laboratory bench was swabbed with 70% alcohol before and after each round of experiment.

Collection and maintenance of test organisms

The test organisms were selected based on their availability, although the organisms of choice were medically important pathogenic bacteria and fungi. Care was taken to cover morphologically broad range of microorganisms. Hence five bacteria and two fungi (yeast and mould) were used for the test. The organisms were collected from University of Ilorin Teaching Hospital Ilorin, Kwara State, Nigeria. The organisms were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Candida albicans*, and *Aspergillus flavus*. The bacteria were maintained on Nutrient agar slant and stored in the refrigerator at a temperature of 4˚C. *Candida albicans* was maintained on Potato dextrose agar (PDA) and stored in the refrigerator at a temperature of 4˚C. Both bacteria and *Candida albicans* were subcultured onto fresh media at regular intervals while *Aspergillus flavus* was cultured on Potato dextrose agar (PDA) and subcultured at regular intervals until it was used for the test.

Preparation and standardization of each bacterial inoculum

Preparation and standardization of each bacterial inoculum was done using the method described by **(Barry *et al*., 1980)**. This was carried out by picking test organism growing as a pure culture on solid media and then transferred into sterile nutrient broth and incubated for 18 -24 hours to produce a growth of the same turbidity. Each standardized inoculum was used for antimicrobial test.

Antibiotic and antifungal used in this study

The following antibiotics which were available as powder, capsule and caplet were used in this study: Amoxicillin, Erythromycin, Streptomycin, Ciprofloxacin and Ketoconazole.

Preparation of Antibiotic dilution

The antibiotic used were purchased at a pharmaceutical store at Tanke, Ilorin, Kwara State, Nigeria and was reconstituted by dissolving 500 mg of powder/granules in a 500ml of distilled water so as to get a concentration of 1.0 mg/ml while 500mg of powder in 250ml of sterile distilled water gave a concentration of 2mg/ml. The prepared dilution of the antibiotic was used to compare for the antimicrobial effect of the extract at concentration of 30mg/ml. Reconstitution of antifungal was carried out in the same manner as antibiotic

Phytochemical Screening of the extracts of the leaves, bark and the roots of *Vernonia amygdalina*

Phytochemical screening was done in order to detect the presence of plant constituents such as alkaloids, saponins, tannins, phenolics, glycosides, flavonoids, and phylobatannins in the plant's extract.
The methods used by **Odebiyi and Sofowora (1978)** were used to test for the presence of alkaloids, saponins, tannins, phenolics, flavonoids, Lieberman Burchad reaction as described by **Herbune (1973)** was used to test for steroids, phylobatannins, while Salkowski test was used to test for the presence of glycosides.

Antibacterial test

The antibacterial properties of the leaves, barks, and the roots were determined using the agar diffusion method of **Bookye- Yiadam (1979)**.

Antifungal test

Agar diffusion method previous described for antibacterial was used in determining antifungal action of the leaves, barks, and roots extract against *Candida albicans*, 10mg/ml, 20mg/ml, and 30mg/ml of the extracts were incorporated in the wells in the Potato dextrose agar plates containing the organisms.

Determination of Minimum Inhibitory Concentration (MIC) of the leaves, bark and the root extracts

Nutrient broth medium was used to determine the MIC. Varying concentrations of the extracts were used which, ranged from 10 mg/ml, 20 mg/ml and 30 mg/ml each concentration contain 0.1ml was added to each 9 ml Nutrient broth containing zero point, 1ml of standardized test organism of bacterial cells. The tubes were incubated aerobically for 24 hours at 37 ˚C. Controls were equally set up using the Nutrient broth and the extract without the test organisms.

RESULTS AND DISCUSSION

Physical characteristic of different parts of *Vernonia amygdalina*

The physical characteristics of ethanol, aqueous cold, aqueous hot of leaf, bark and root extracts of *V. amygdalina* is shown in Table 1.

Phytochemical screening of different parts of *Vernonia amygdalina*

All the plant extracts were rich in alkaloids, saponins, flavonoids, tannins, phenols and cardiac glycosides, phlobatannins and steroids were not present in the extracts as shown in Table 2. Some of these bioactive substances have been found to be present in bitter kola (*G. kola*), honey and bitter leaf (*V. amygdalina*) (**Almagoul *et al.*, 1985**). The phytochemical compounds found in this study were in agreement with **Adegboye *et al.*, (2008)** using *G. kola* they found similar compounds. Other researchers reported the occurrence of such compounds in plants based extracts. (**Almagoul *et al.*, 1985; Odiongenyi *et al.*, 2009**).cts.

Table 1 Physical characteristic of different parts of *Vernonia amygdalina*

Plant Parts	Samples	Colour of the extract
Leaves	Hot water extract	Deep green
	Cold water extract	Yellow
	Ethanolic extract	Light green
Bark	Hot water	Light yellow
	Cold water extract	Yellow
	Ethanolic extract	Green
Root	Hot water extract	Deep green
	Cold water extract	Light yellows
	Ethanolic extract	Green

Table 2 Phytochemical screening of different parts of *Vernonia amygdalina*

Chemical compounds	Part of *Vernonia amygdalina* assayed		
	Leaf	Bark	Root
Alkaloids	+	+	+
Steroids	_	_	_
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Phlobatannins	_	_	_
Phenols	+	+	+

Legend:+ - Present, ─ - Absent

Sensitivity patterns of some pathogenic organisms to ethanol extract, aqueous cold and aqueous hot extracts as measured by zone of inhibition

All the plant extracts have antimicrobial properties. *V. amygdalina* ethanol and aqueous extracts had inhibition on *S. arureus* (gram +ve) and *E. coli* (gram -ve) and against *C. albicans*as shown in (Tables 3 and 4). These concurs with the findings of **Al-Magboul *et al* (1985)**, but contrary to **Ashebir and Ashenafi (2007)** observation of the plants' ability to inhibit *E. coli. Klebsiella pneumoniae* was not inhibited in the aqueous cold extracts. However, it was inhibited by the ethanolic extracts. This might be due to the fact that this organism produces capsule which could not be readily dissolved in water. Investigators in the past had clearly shown that ethanolic extract were more effective than water extract (**Dutta 1993**; **Ibekwe *et al.*, 2001**). They attributed this to the high volatility of ethanol which tends to extract more active compounds from the sample than water.

Pseudomonas aeruginosa showed inhibition on both the ethanolic and cold extracts but not as much as in the case of *E. coli, Staphylococcus aureus* and *Candida albicans*. However\ *Bacillus subtilis* and *Aspergillus niger* are less sensitive to ethanolic, cold and aqueous hot extract of *V. amygdalina* as shown in table 3, 4 and 5.

Table 3 Sensitivity patterns of some pathogenic organisms to ethanol extract as measured by zone of inhibition

Organisms	Ethanolic extract									
	Leaf			Bark			Root			Control
	Concentration of extract in mg/ml									
	10	20	30	10	20	30	10	20	30	2 (ml)
	* Zone of inhibition									
B. subtilis	15.3	17.2	18.5	14.0	14.4	15.2	_	_	14.8	_
E. coli	18.6	20.0	20.5	17.0	17.6	19.5	14.2	16.0	17.0	_
P. aeruginosa	8.5	8.8	10.5	13.0	17.0	17.8	_	12.4	14.5	_
K. pneumoniae	13.0	15.4	16.8	10.7	15.0	15.6	12.0	12.5	13.8	_
S. aureus	18.4	20.5	22.2	15.3	17.1	17.5	16.2	18.4	18.5	_
A. niger	_	12.0	14.0	_	_	_	_	_	14.5	_
C. albicans	14.3	15.0	17.6	12.4	14.5	15.1	16.2	17.5	18.8	_

Legend: * Zone of inhibition in milliliter in triplicate expressed as means, ─ - No zone of inhibition.

On the other hand sensitivity patterns shown by the hot aqueous extracts of *V. amygdalina* plants have relative inhibition on *Escherichia coli, Staphylococcus aureus*, and on *Candida albicans. Klebsiella pneumoniae* had small inhibition on the extracts and this was because there was relatively small zone of inhibition on all the extracts, while *Bacillus subtilis, Pseudomonas aeruginosa* and *Aspergillus niger* had no zone of inhibition on the test organisms as presented in Table 5.

The difference in antimicrobial properties of a plant extract is attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage **(Calixto, 2000; Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007**). It was found out that the higher the concentration of the extract, the higher the diameter of zones of the inhibition.

Table 4 Sensitivity patterns of some pathogenic organisms to Aqueous cold extract as measured by zone of inhibition

Organisms	Aqueous cold									
	Leaf			Bark			Root			Control
	Concentration of extract in mg/ml									
	10	20	30	10	20	30	10	20	30	2(ml)
	* Zone of inhibition									
B. subtilis	_	_	10.2	12.4	10.5	8.4	_	_	_	_
E. coli	16.2	16.2	17.1	18.1	22.2	24.5	16.5	18.1	18.5	_
P. aeruginosa	_	15.4	17.5	16.2	16.4	18.2	_	14.2	15.2	_
K. pneumoniae	_	_	12.4	_	_	10.5	_	_	12.4	_
S. aureus	16.4	18.1	19.0	18.2	18.1	20.3	16.4	20.3	22.1	_
A. niger	12.3	13.2	16.2	_	_	14.1	_	_	12.5	_
C. albicans	22.1	22.5	24.4	18.3	20.5	22.3	16.0	18.2	18.3	_

Legend: * Zone of inhibition in milliliter (mm) in triplicate expressed as means, ─ - No zone of inhibition.

Table 5 Sensitivity patterns of some pathogenic organisms to Aqueous hot extract as measured by zone of inhibition

Organisms	Aqueous hot									Control
	Leaf			Bark			Root			
	Concentration of extract in mg/ml									
	10	20	30	10	20	30	10	20	30	2(ml)
	*Zone of inhibition									
B. subtilis	_	_	_	_	_	_	_	_	_	_
E. coli	12.5	14.2	14.4	_	10.4	11.2	_	8.4	10.2	_
P. aeruginosa	_	_	_	_	_	_	_	_	_	_
K. pneumoniae	8.2	10.2	12.5	_	10.3	10.5	_	7.3	9.4	_
S. aureus	8.4	10.4	12.1	_	10.5	12.1	_	9.2	10.5	_
A. niger	_	_	_	_	_	_	_	_	_	_
C. albicans	10.5	12.1	12.4	_	8.3	12.5	_	8.4	10.2	_

Legend: * Zone of inhibition in milliliter in triplicate expressed as means, ─ - No zone of inhibition, Con – Control

The minimum inhibitory concentration (MIC) exhibited by *Vernonia amygdalina* extracts (from leaf, bark and root) against some selected bacterial isolates

The MIC were determined to be 15mg/ml for ethanolic extracts of the leaves plant on *Bacillus subtilis* and *Pseudomonas aeruginosa*, 12.5mg/ml for *E. coli, Staphylococcus aureus* and *Klebsiella pneumoniae* while it was also found out to be 7.5mg/ml for *E. coli* and *S. aureus,* 15mg/ml for *Bacillus subtilis* and *Pseudomonas aeruginosa* and 12.5mg/ml for *Klebsiella pneumoniae* for the cold aqueous extract of leaves of *V. amygdalina* as presented in Table 6.
In Tables 7 and 8, the bark and root extracts of *V. amygdalina* for both ethanolic and aqueous extract had the same minimum inhibitory concentration for the microorganisms but variesd among them. *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* had 7.5mg/ml concentration of ethanolic extract of the barks and roots, 15mg/ml for *Bacillus subtilis* and 5.0mg/ml for *Pseudomonas aeruginosa* for both the barks and roots of ethanolic extracts. On the other hand, 5.0mg/ml for *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*, 10mg/ml for *Bacillus subtilis* and 2.5mg/ml for *Pseudomonas aeruginosa* of cold aqueous extract of the barks and leaves.

In the broth dilution tubes for the MIC, the hot aqueous extract of *V. amygdalina* was not inhibitory on all the test organisms. This might be due to the fact that the crude hot aqueous extracts of the plants was not suitable at (2.5 - 15mg/ml) in tackling diseases caused by these test organisms as it is used by the local populace if hot aqueous must be used for extraction.

Table 6 The minimum inhibitory concentration (MIC) exhibited by *Vernonia amygdalina* extracts against some selected bacterial isolates

S/No	Bacterial Isolate	Concentration of *Vernonia amygdalina* extract (mg/ml)			Control
		Medium of leaf extract			
		Ethanolic	Hot aqueous	Cold aqueous	
		Concentration in mg/ml			
1	*B.* subtilis	15.0	_	15.0	_
2	*E. coli*	12.5	_	7.5	_
3	*P. aeruginosa*	15.0	_	15.0	_
4	*K. pneumoniae*	12.5	_	12.5	_
5	*S. aureus*	12.5	_	7.5	_

Legend: ─ - No inhibition at concentration used

Table 7 The minimum inhibitory concentration (MIC) exhibited by *Vernonia amygdalina* extracts against some selected bacterial isolates

S/No	Bacterial Isolate	Concentration of *Vernonia amygdalina* extract (mg/ml)			Control
		Medium of bark extract			
		Ethanolic	Hot aqueous	Cold aqueous	
		Concentration in mg/ml			
1	*B. subtilis*	15.0	_	10.0	_
2	*E. coli*	7.5	_	5.0	_
3	*P. aeruginosa*	5.0	_	2.5	_
4	*K. pneumoniae*	7.5	_	5.0	_
5	*S. aureus*	7.5	_	5.0	_

Legend: ─ - No inhibition at concentration used

Table 8 The minimum inhibitory concentration (MIC) exhibited by *Vernonia amygdalina* extracts against some selected bacterial isolates

S/No	Bacterial Isolate	Concentration of *Vernonia amygdalina* extract (mg/ml)			Control
		Medium of root extract			
		Ethanolic	Hot aqueous	Cold aqueous	
		Concentration in mg/ml			
1	*B. subtilis*	15.0	_	10.0	_
2	*E. coli*	7.5	_	5.0	_
3	*P. aeruginosa*	5.0	_	2.5	_
4	*K. pneumoniae*	7.5	_	5.0	_
5	*S. aureus*	7.5	_	5.0	_

Legend: ─ -No inhibition at concentration used

Sensitivity patterns of zone of inhibition by standard antibiotics and antifungal

The plant extracts were more susceptible to *S. aureus* (gram +ve) followed by *E. coli* (gram -ve) and *C. albicans*. Plant extracts (leaf, bark and root) showed stronger retardation effect on the gram positive test organisms than on the gram-negative ones (**Desta, 1993; Okigbo *et al*., 2006; Ashebir and Ashenafi, 2007**). In standard antibiotics used, Amoxycillin and Ciproflaxin had more effect on *S. aureus* than on *E. coli* and other bacterial used in the study even at concentration of 2mg/ml as presented in Table 9. Amoxycillin, Tetracycline, Ciproflaxin and Streptomycin were used as for the antibacterial test, while, Ketoconazole and Streptomycin were used as for antifungal test. Streptomycin had effect on *Candida albicans* than *Aspergillus niger* as presented in Table 10. The observation made on the minimum inhibitory concentration (MIC) of the plant extracts seemed to correlate with the report that bacteria varied widely in the degree of their susceptibility (**Emeruwa, 1982; El-feraly *et al*., 1983; and Prescott *et al*., 2008**). High minimum inhibitory concentration was observed for bacteria whose growths could not be inhibited at lower concentration.

It is worth nothing that this plant showed activity against microorganisms causing diseases not traditionally used for and since these test organisms are also implicated in a wide variety of infections, it therefore means that the constituents of the leaves, barks and roots of this plant could be very useful in chemotherapy.

Table 9 Sensitivity patterns of zone of inhibition by standard antibiotics

Microorganisms	Zone of inhibition (mm)*							
	Antibiotics concentration (mg/ml)							
	1 (mg/ml)				2 (mg/ml)			
	Amoxy	Cipro	TCN	Strep	Amoxy	Cipro	TCN	Strep
B. subtilis	-	-	-	-	-	-	-	-
E. coli	12.2	28.4	-	-	22.3	30.5	-	25.2
P. aeruginosa	13.1	-	18.2	12.4	16.2	-	21.1	13.3
K. pneumoniae	29.4	20.1	-	30.5	23.2	20.2	-	-
S. aureus	23.2	28.0	-	22.4	23.0	-	-	-

Legend: ─- Resistant, *mm - Mean of three replicates in mm, Amoxy- Amoxycillin, Cipro – Ciproflaxin, TCN – Tetracycline, Strep – Streptomycin.

CONCLUSION

This present study has shown that the leaves, bark and roots of *Vernonia amygdalina* hav antimicrobial effects against some pathogenic microorganisms . There is need for further research on the active principles ,to purify them and to carry out toxicological studies before they can be formulated into dosage forms for use against potential disease causing microbes.

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