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Enhanced Antibacterial Potential of *Xylopia aetiopica* Extracts in Consortium with Alum on Some Bacteria Isolated from *Achatina achatina* (Land Snail)

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A B S T R A C T

The study included the isolation, cultural characteristics, molecular identification of bacterial isolated from land snail *Achatina achatina* and the enhanced antibacterial potential of *Xylopia aetiopica* extracts in consortium with alum on some bacteria isolated from *Achatina achatina* (land snail). Isolates associated with the intestine of the snails were isolated using standard microbiological methods and subjected to standard microbiological procedures such as, culturing isolation, identification; the cultural characteristics of all isolated bacterial strains were elucidated on International Streptomyces Project media (ISP2-ISP-7). 16S rRNA marker gene was used for molecular identification using 27F and 1492R universal primers and sensitivity testing using alum and plant extracts (Agar well Diffusion method). Tube dilution method was used to determine the Minimum Inhibitory Concentration (MIC) using double-fold serial dilutions at concentrations 62.5mg/ml to 400 mg/ml. The bacterial isolates identified were; *Escherichia coliMW46885*, *Bacillus cereus AP007209*, *S. aureus CP051191* and *Salmonella typhimurium AE006468*. The result of phytochemical component present in *X. aethiopica* were; Tannin Alkaloid, Flavonoid, and Saponin with values, 2.22±0.08, 5.55±0.78, 6.55±0.21, and 10.55±0.07mg/kg respectively. Results of the inhibitory activity of the extracts and alum were dose-dependent. Methanolic extract of *X. aethiopica* were more active on *E. coli*24.50±0.71 at 250mg/ml, Aqueous extract of *X. aethiopica* were more active on *E. coli* and *Salmonella typhi* (21.0±0.00mm), at 250mg/ml and *Staphylococcus aureus* (20.0±0.00mm) at 125mg/ml and 62.5mg/ml concentrations respectively. Alum were more active on *Staphylococcus aureus* (25.0±0.00mm) at 250mg/ml concentrations. The result of the combination methanol extract of *X. aethiopica* and alum showed it more effect on *Bacillus cereus* (22.0±0.00) at 250mg/ml concentrations. The combination effect of aqueous extract of *X. aethiopica* and alum revealed the inhibitory effect were more on *Bacillus cereus* (21.0±0.00) at 250mg/ml concentrations while it was more active on *Staphylococcus aureus* (15.5±0.71mm) at 62.5mg/ml conc. The result of the combination treatments of methanolic, aqueous extracts of *X. aethiopica,* and alum showed that the inhibitory effect was more on *E, coli* and *Staphylococcus aureus* (18.0±0.00) at 250mg/ml conc., *E. coli* and *Salmonella typhi* (15.0±0.00) at 125mg/ml conc. and *Salmonella typhi* (14.0±0.00mm) at 62.5mg/ml conc. and ciprofloxacin which serves as control has higher inhibition potentials. *Xylopia aetiopica* has been reported to possess anticancer, antidiabetic, antimalarial, antioxidant, enzyme inhibitory and antibacterial properties. Tube dilution method was used to determine the Minimum Inhibitory Concentration (MIC) using double-fold serial dilutions at concentrations 62.5mg/ml to 400 mg/ml. statistically, there was a significant difference ($p \leq 0.05$) in the antibacterial activity of the alum, methanol, and aqueous extracts of *X. aethiopica*. The minimum inhibitory concentrations at which the extracts were effective against the bacterial isolates was at the 400mg/ml to 300mg/ml concentrations. The MICs values of the extracts and their combinations revealed significantly the inhibitory activities. The study has revealed some level of antibacterial activity and antibacterial activity of the extracts on these bacterial isolates is promising as the extracts could be used as a cheap antibacterial for the treatment of infections caused by these bacteria.

Introduction

Snail meat has been documented to be a good and high source of protein, iron and calcium and it is known to contain almost all the aminoacids needed by humans (Ademola *et al.,* 2004; Cobbinah *et al.,* 2008). The invasive land snail *Achatina achatina*, is a species known to carry parasites and harbours a dense and metabolically active bacterial community, the diversity and composition of which is however unknown (Cardoso *et al.,* 2012). A study by Bukola *et al.,* (2011), on the microbiological composition of snails also indicated the presence of *Proteus* sp, *Streptococcus* sp, *Shigella flexneri, Staphylococcus aureus, E. coli, Klebsiella aerogenes, Citrobacter* sp, *Bacillus subtilis, Bacillus cereus, Aeromonas* sp., *Micrococcus liteus, Salmonella typhi, Vibrio parahaemolyticus* and *Vibrio cholerae*.

The food spoilage potential of bacteria is tied to the ability of bacteria to produce metabolites that are linked with spoilage (Bukola *et al.,* 2011). Naturally, there are a lot of bacteria present in foods as contaminants and bacteria are the most predominant pathogens and spoilage bacteria. For instance, *Pseudomonas* species and other gram-negative psychrotrophic bacteria have been reported to be responsible for the reduction in the shelf life of high-protein, chilled foods such as meat and dairy products stored under aerobic conditions (Bukola *et al.,* 2011)

In Nigeria, for an instance, it has been reported that over 40% of known plant species serve as food whereas about 30% serve as spices and medicinal plants. The plant spices are used to give aroma and flavor to food and at the same time they act as food preservatives as a result of the fact that they possess active ingredients which are either bacteriostatic or bactericidal and prevalent among these medicinal plants is *Xylopia aethiopica* commonly called Negro pepper (Ogbonna *et al.,* 2013).

The plant *Xylopia aethiopica* is medicinal containing a variety of complex chemical compounds and has great repute in ethnobotany. The active ingredients in this plant are usually extracted in various forms such as crude aqueous or organic extracts or in the form of essential oils and used for various purposes (Ogbonna *et al.,* 2013).

The medical importance of *X. aethiopica* has been extensively reported with its fresh and dried fruits, leaf, stem bark, and root bark essential oils reported to have various degrees of activity against some gram-positive and negative bacteria.

The plant is widely distributed in the West African rainforest and its range is from Senegal to Sudan in Eastern Africa, and down to Angola in Southern Africa, and virtually every part of the plant is used in traditional medicine for managing various ailments like skin infections, candidiasis, dyspepsia, cough, and fever although the literature on its antibacterial activities and potentials as a food preservative is scarce (Ogbonna *et al.,* 2013).

Studies have revealed that the fresh and dried fruits, leaf, stem bark, and root bark essential oils in *Xylopia aethiopica* produced varying degrees of activity against the gram-positive bacteria, *Bacillus subtilis,* and *Staphylococcus aureus*, the gram-negative bacteria *Pseudomonas aeruginosa* (Erhirhie and Moke, 2014).

Alum has also been shown to have a bactericidal effect; however, the mechanism is unknown. However, it was speculated that the impact was due to a decrease in acidity or harmful effects on the bacterial cell wall. Alum's antibacterial activity against four malodour-producing axillary bacterial flora (*Micrococcus luteus* (ATCC 49732), *Staphylococcus epidermidis* (ATCC 14990), *Corynebacterium xerosis* (ATCC BAA-1293), and *Bacillus subtilis* (ATCC 19659) was determined in another study as an alternative natural product for reducing axillary malodour.

Hence, this study was carried to investigate the antibacterial efficacy of *Xylopia aethiopica* extracts in combination with alum on some bacteria isolated from *Achatina achatina* (Land Snail)

Materials and Methods

Samples Collection

The snail samples were purchased from Rumuokoro market under hygienic condition and put in a sterile polyene bags. The samples were labelled properly, put into an ice-chest container and transported to the Department of Animal and Environmental Biology, Rivers State University and was identified by Prof. G. C Akani. While the fruits of *Xylopia aethiopica* (Uda) were purchased from Abua Central Markets in Abua/Odual Local Government Area of Rivers State, Nigeria. The fruit was taken to the Department of Plant Science and Biotechnology, Rivers State University and was identified by Dr. M. J. Ajuru. The both samples was taken to Microbiology Laboratory Rivers State University for analyses

Bacteria Isolation and Preparations of Extracts

Ten-fold serial dilution method was carried out on the snail sample by weighing 25g of the snail intestine and dispensed into 225ml of normal saline and continuing pipetting 1 mL of the sample into 9 mL of sterile normal saline up to 6 dilutions (dilution from 10^{-1} to 10^{-6}) onto nutrient agar and *Salmonella-shigella* agar. Representative colonies were described and sub-cultured onto nutrient agar plates and incubated for 24 h at 37^{o}C to obtain pure cultures. Pure cultures were stored in sterile 10% v/v glycerol for preservation and subsequently used for identification (Taylor, 2008). The fruits of *Xylopia aethiopica* were prepared according to the method of (Ogbonna *et al.,* 2013). The fruits were dried for five days at room temperature before being crushed into powder. Then, 100g each of the powder was placed in separate 500 mL Erlenmeyer flask, containing 200 mL of methanol and sterile distilled water. For three days, the flasks were maintained at room temperature with periodic shaking. Each sample was filtered with Whatman No. 1 filter paper into a 500ml beaker which was later dried at 40°C using an oven drier (Kenkpamp). The extracts after drying, were stored in the fridge for further use. Stock solution of the Methanol and aqueous extract was used in preparing the concentrations of the extract and alum. One gram (1g) of extract was transferred into 2ml of sterilized distilled water and 2ml of DMSO to achieve the concentrations of 250mg/ml, 125mg/ml, and 62.5mg/ml. while the concentrations of the extract used in combination was constituted by combining 0.33g of methanol extract of *X. aethiopica,* aqueous extract of *X. aethiopica* and alum respectively. Ciprofloxacin 500mg concentrations were used as control.

Bacterial Identification

The following tests were performed on each of the isolates to confirm their identity: Gram staining, sugar fermentation tests, oxidase test, catalase test, indole test, methyl red test, vogues proskauer test, citrate utilization test, motility test, (Srinivasan *et al.,* 2015)

Antimicrobial Susceptibility of Extracts and Alum

The antimicrobial susceptibility of the extract on the test isolates was carried out using the well in agar method as described by (Amadi *et al.,* 2016). The test bacterial isolates which had been standardized using the 0.5 McFarland standard were swabbed on the surface of freshly prepared labelled Mueller-Hinton agar plates and allowed to dry. Wells of 6mm diameter were made on the agar plates using sterile cork-borer at equidistant positions in the medium and labelled based on the concentrations. The extract concentrations were aseptically transferred into the wells and incubated. Zone diameters were obtained by measuring the observed zones (CLSI, 2017).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) were determined for alum and plant extracts as described by Manaphraim (2014). Each bacterium was pre-cultured before being diluted with medium to a

concentration of around 10^6 CFU/ml. The presence or absence of colonies was determined after 10 µL of diluted cell culture was injected into the medium using a micro-planter and incubated at 30°C for 72 hours. The minimal inhibitory concentration was determined to be the lowest extract concentration that inhibited the bacteria's growth.

Phytochemical Analysis

The coarse dried powder of leaves of the plant (200g) was subjected to extraction with 2000 ml ethanol for 48 hours. The ethanol extract was collected, filtered and concentrated in vacuum under reduced pressure and dried in desiccator and stored for further analysis. The concentrated ethanol extract was further subjected to phytochemical screening and the test substance was shaken with few drops of 2N HCL.

An aqueous layer formed, decanted and to which one or two drops of Mayer's reagent was added. Formation of white precipitate indicates the presence of alkaloids. To the substance in alcohol, a few magnesium turnings and few drops of concentrated HCl were added and boiled for five minutes. Red coloration shows the presence of flavonoids. The substance mixed with basic lead acetate solution the formation of white precipitate indicates the presence of Tannins. 5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins (Harborne, 1973).

Data Analysis

Statistical Package for Social Sciences (SPSS) version 25 and Microsoft Office Excel 2010 was used to analyze the data obtained from the measurement of the zones of inhibition of the extracts. Descriptive statistics was used to summarize all data obtained. ANOVA was carried out to test for significant difference in the minimum inhibitory concentration (MIC) obtained for the different herbs

Results and Discussion

The result of the morphological and biochemical reaction of the bacterial isolates revealed the following probable bacteria; *Staphylococcus* spp, *E. coli*, *Bacillus* spp, *Salmonella* spp as show in Table 1.The phytochemical composition of the above plant is presented in Fig 1. Results showed that the concentrations of Alkaloid, Flavonoid, Saponin, and Tannin were 5.55±0.78, 6.55±0.21, 10.55±0.07, and 2.22±0.08mg/kg respectively.

Results of the inhibitory activity of the extracts and alum were dose-dependent. For the bacterial isolates the methanolic extract of *X. aethiopica* were more active on *E. coli* (24.50±0.71; 18.00±0.00 and 16.00±0.00mm), at 250mg, 125mg and 62.5mg concentrations respectively. Aqueous extract of *X. aethiopica* were more active on *E. coli* and *Salmonella typhi* (21.0±0.00; 21.0±0.00mm), at 250mg and *Staphylococcus aureus* (20.0±0.00mm) at 125mg and 62.5mg concentrations (conc.) respectively. Alum were more active on *Staphylococcus aureus* (25.0±0.00mm; 20.0±0.00 and 11.0±0.00mm), at 250mg, 125mg and 62.5mg concentrations respectively as showed in table 2a to 2d.

The result of the combination effect of methanol extract of *X. aethiopica* and alum showed it more effect on *Bacillus cereus* (22.0±0.00; 20.0±0.00 and 18.0±0.00mm) at 250mg, 125mg and 62.5mg concentrations respectively as showed in table 3.

The combination effect of aqueous extract of *X. aethiopica* and alum revealed the inhibitory effect were more on *Bacillus cereus* (21.0±0.00; 19.0±0.00mm) at 250mg and 125mg concentrations while it was more active on *Staphylococcus aureus* (15.5±0.71mm) at 62.5mg conc. as shown in table 4.

The result of the combination effect of methanol extract, aqueous extract of *X. aethiopica,* and alum showed that the inhibitory effect was more on E, coli and *Staphylococcus aureus* (18.0±0.00, 18.0±0.00mm) at 250mg conc., *E. coli* and

Salmonella typhi (15.0±0.00, 15.0±0.00mm) at 125mg conc. and *Salmonella typhi* (14.0±0.00mm) at 62.5mg conc. as showed in table 5 and ciprofloxacin which serves as control has higher inhibition potentials. Statistically, there was a significant difference ($p \leq 0.05$) in the antimicrobial activity of the alum, methanol, and aqueous extracts of *X. aethiopica*. Bacteria of interest were isolated from land snail and the presence of these bacteria in the intestines of snail could be associated to different factors not limited only to their life style and environmental factors but also from handling, storage and processing methods. During the purchase of these snails, the farmers/ sellers kept the snails in containers and mixed those crawling on the ground from the storage to those already in the storage. More so, these handling processes and their interaction with the environment could predispose these snails to different types of microorganisms. It is well documented that one of the routes of microbial contamination of food is through exposure to the environment (CDC, 2012; Manaphraim, 2014). Isolated *Enterobacter* spp, *Escherichia coli, Salmonell*a spp, *Bacillus cereus,* and *Staphylococcus aureus* from the land snail agreed with the present study. Most of the bacteria isolated has been implicated to cause several illnesses; *E. coli* for urinary tract infections, particularly in older people.

The proximate composition of the plant showed that saponins had the highest concentration amongst other phytochemicals and at such was the most abundant in the leaf followed by flavonoids and alkaloids while tannins was the least phytochemical present. Plant secondary metabolites such as phytochemicals are known to provide plants with enhanced antibacterial activity. According to Ilusanya *et al.,* (2012), the extract of *X. aethiopica* fruits contain tannins, phlobatannins, flavonoids and steroids which are pharmaceutical constituents, and these bioactive components are known to be bactericidal in nature. These substances are primarily secondary metabolites capable of causing defined physiological activities on the body, and they are the most significant bioactive elements of natural goods (Ilusanya *et al.,* 2012). Tannins in plants are astringents that aid wound healing and are anti-parasitic. They are used to treat intestinal illnesses including diarrhea and dysentery and have been shown to have therapeutic effect against a variety of pathogens (Ilusanya *et al.,* 2012).

Howbeit, (Kanife *et al.,* 2012) has reported the presence of tannins, terpenoids, flavonoids, alkaloids and steroids and that tannin and flavonoids have antimicrobial properties that can coagulate protoplasm of microorganisms

The antibacterial activity of alum, aqueous and ethanolic extracts of *X. aethiopica* indicates that the plant extract, as well as the alum, were very potent against *Bacillus* cereus, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*. The potency of the extracts on the bacterial isolates was influenced by the concentration. Higher inhibition property is observed when the concentration is high and vice visa and this correlates with findings of previous studies (Ikeyi *et al.,* 2013). The result showed that the aqueous extract of *X. aethiopica* exhibited a higher zone diameter on *Bacillus cereus, Salmonella typhi*, and *Staphylococcus aureus* at all concentrations than the methanol extract while the methanol extract on the other hand showed a larger zone of inhibition on *E. coli* at all concentrations than the aqueous extract. Aqueous extracts of plants have been reported to show better antibacterial activity than ethanol extracts and this observation could either be attributed to incomplete extraction of the secondary metabolites responsible for antimicrobial action or destruction of secondary metabolites required. This agreed with Ikeyi *et al.,* (2013) who also reported similar findings and attributed their findings to the loss of active components of these extracts by the acid components. The antibacterial activity of the extracts showed that the extracts of *X. aethiopica* caused higher inhibition on *Staphylococcus, Bacillus* sp and *E. coil* while the inhibition rate of the extracts on *Salmonella* sp was the least. This observation could be attributed to either the upsurge in antibacterial resistance of *Salmonella* sp or due to the nature of their cell wall.

Table.1 Morphology and Biochemical Characteristics of Bacterial Isolates

Isolate Code	Morphology	Gram Reaction	Shape	Catalase	Oxidase	Citrate	Motility	Indole	MR	VP	Glucose	Lactose	Sucrose	Mannitol	Suspected Organisms
An1	Small round metallic sheen, flat	-Ve	Rods	+	+	+	+	+	+	+	Ag	Ag	Ag	Ag	*E. coli*
An2	Cream round flat dry	+Ve	Rods	+	+	-	+	-	-	+	-	Ag	A	-	*Bacillus* sp
An3	Cream round flat dry	+Ve	Rods	+	-	-	+	-	+	+	A	-	A	-	*Bacillus* sp
An4	Cream round flat dry, cerrated edge	+Ve	Rods	+	+	-	+	-	+	+	A	-	A	-	*Bacillus cereus*
As1	Large pink raised	-Ve	Rods	+	-	+	+	-	+	+	Ag	Ag	Ag	Ag	*Enterobacter*sp
As2	Pale small round black centre	-Ve	Rods	+	-	+	+	-	+	+	A	-	Ag	Ag	*Salmonella* sp
As3	Pale small round raised	-Ve	Rods	+	+	+	-	-	+	-	Ag	Ag	Ag	Ag	*Shigella*sp
As4	Small round metallic sheen, flat	-Ve	Rods	+	+	-	+	+	+	+	Ag	Ag	Ag	Ag	*E. coli*
As5	Golden yellow, small round	+ve	Cocci	+	-	+	-	+	+	+	Ag	Ag	Ag	Ag	*Staphylococcus* sp

Keys: A = acid; G = gas, MR = Methyl Red; VP: VogesProskauer, -ve = negative; +ve = positive

Table.2a Effect of Methanol, Aqueous and Alum Extract on *Bacillus cereus*

Isolates	Type of Extract	62.5mg	125mg	250mg	Ciprofloxacin
Bacillus cereus	ME	15.0 ± 0.00^{c}	16.0 ± 0.00^{b}	18.0 ± 0.00^{a}	30.0±0.00
Bacillus cereus	AQ	12.0 ± 0.00^{b}	18.0 ± 0.00^{c}	20.0 ± 0.00^{b}	30.0±0.00
Bacillus cereus	AL	8.0 ± 0.00^{a}	13.0 ± 0.00^{a}	22.0 ± 0.00^{c}	32.0±0.00

*Means with similar alphabet (^{abc}) down the group show no significant difference (P<0.05)
Keys: ME = methanol extract, AQ = aqueous extract, AL = alum.

Table.2b Effect of Methanol, Aqueous and Alum Extract on *E. coli*

Isolates	Type of Extract	62.5mg	125mg	250mg	Ciprofloxacin
E. coli	ME	16.5 ± 0.71^{c}	18.00 ± 0.00^{c}	24.50 ± 0.71^{c}	35.0±0.00
E. coli	AQ	13.0 ± 0.00^{b}	17.0 ± 0.00^{b}	21.0 ± 0.00^{b}	40.0±0.00
E. coli	AL	10.0 ± 0.00^{a}	12.0 ± 0.00^{a}	17.0 ± 0.00^{a}	40.0±0.00

*Means with similar alphabet (^{abc}) down the group show no significant difference (P<0.05)
Keys: ME = methanol extract, AQ = aqueous extract, AL = alum.

Table.2c Effect of Methanol, Aqueous and Alum Extract on *Salmonella typhimurium*

Isolates	Type of Extract	62.5mg	125mg	250mg	Ciprofloxacin
Salmonella typhi	ME	13.5±0.71[b]	14.5±0.71[a]	16.5±0.71[a]	31.5±0.71
Salmonella typhi	AQ	15.0±0.00[b]	18.0±0.00[b]	20.0±0.00[c]	37.0±0.00
Salmonella typhi	AL	8.0±0.00[a]	14.0±0.00[a]	18.0±0.00[b]	30.0±0.00

*Means with similar alphabet ([abc]) down the group show no significant difference (P<0.05)
Keys: ME = methanol extract, AQ = aqueous extract, AL = alum.

Table.2d Effect of Methanol, Aqueous and Alum Extract on *S. aureus*

Isolates	Type of Extract	62.5mg	125mg	250mg	Ciprofloxacin
Staphylococcus sp	ME	15.0±0.00[b]	15.0±0.00[a]	17.5±0.71[a]	39.5±0.71
Staphylococcus sp	AQ	18.0±0.00[c]	20.0±0.00[b]	21.0±0.00[b]	35.0±0.00
Staphylococcus sp	AL	11.0±0.00[a]	16.0±0.00[c]	25.0±0.00[c]	35.0±0.00

*Means with similar alphabet ([abc]) down the group show no significant difference (P<0.05)
Keys: ME = methanol extract, AQ = aqueous extract, AL = alum

Table.3 Combination of Methanol Extract of *X. aethiopica* and Alum on Bacterial Isolates

Isolates	Ascension No.	62.5mg	125mg	250mg	Ciprofloxacin
Bacillus cereus	AP007209	18.0±0.00	20.0±0.00	22.0±0.00	30.0±0.00
E. coli	MW46885	15.0±0.00	18.0±0.00	20.0±0.00	40.0±0.00
Salmonella typhi	AE006468	12.0±0.00	15.0±0.00	17.0±0.00	35.0±0.00
S. aureus	CP051191	14.0±1.41	15.0±0.00	17.0±0.00	35.0±0.00

Table.4 Combination of Aqueous Extract of *X. aethiopica* and Alum on Bacterial Isolates

Isolates	Ascension No.	62.5mg	125mg	250mg	Ciprofloxacin
Bacillus cereus	AP007209	11.0±0.00	19.0±0.00	21.0±0.00	45.0±0.00
E. coli	MW46885	11.50±0.71	13.0±0.00	15.0±0.00	42.0±0.00
Salmonella typhi	AE006468	10.0±0.00	12.0±0.00	15.0±0.00	36.0±0.00
S. aureus	CP051191	15.5±0.71	16.5±0.71	19.5±0.71	36.0±0.00

Table.5 Combination of Methanol, Aqueous Extract of *X. aethiopica* and Alum on Bacterial Isolates

Isolates	Ascension No.	62.5mg	125mg	250mg	Ciprofloxacin
Bacillus cereus	AP007209	10.0±0.00	13.0±0.00	15.0±0.00	30.0±0.00
E. coli	MW46885	6.50±0.71	15.0±0.00	18.0±0.00	40.0±0.00
Salmonella typhi	AE006468	14.0±0.00	15.0±0.00	16.0±0.00	32.0±0.00
S. aureus	CP051191	10.0±0.00	12.0±0.00	18.0±0.00	35.0±0.00

The minimum concentrations at which the extracts were effective against the bacterial isolates was at the 400mg/ml to 300mg/ml concentrations.

Table.6a Minimum Inhibitory Concentration of the Extracts on Bacterial Isolates

Isolate	Concentration (mg/ml)																	
	ME						AQ						AL					
	400	350	300	250	125	62.5	400	350	300	250	125	62.5	400	350	300	250	125	62.5
S. aureus	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Bacillus cereus	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
E. coli	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Salmonella typhi	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+

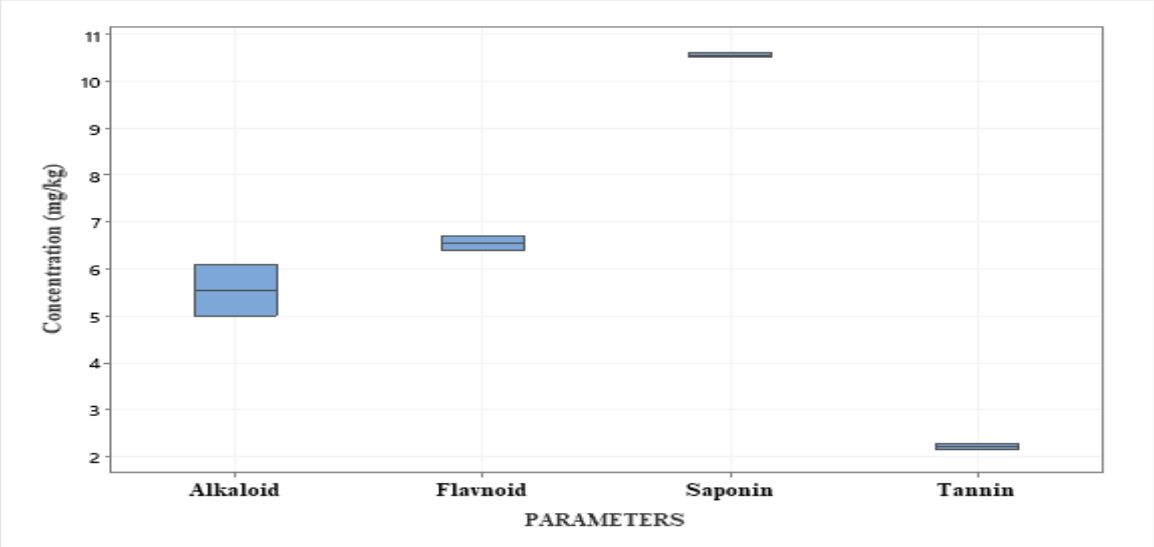
Keys: + Presence of microbial growth; - no microbial growth; ME = methanol extract, AQ = aqueous extract; AL = alum

Table.6b Minimum Inhibitory Concentration of the Combinations on Bacterial Isolates

Isolate	Concentration (mg/ml)																	
	ME+AL						AQ+AL						ME+AQ+AL					
	400	350	300	250	125	62.5	400	350	300	250	125	62.5	400	350	300	250	125	62.5
S. aureus	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Bacillus cereus	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
E. coli	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Salmonella typhi	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+

Keys: + Presence of microbial growth; - no microbial growth; ME = methanol extract, AQ = aqueous extract; AL = alum

Fig.1 Phytochemical Composition of *X. aethiopica*



This agreed with (Nweze and Onyishi, 2010) who also reported that Gram negative bacterium has an extra outer membrane that may be impermeable to the plant extract, thus, their ability to resist antimicrobial agent. More so, the present study showed that the plant extract of *X. aethiopica* were very potent against *E. coli* which contradicts (Nweze and Onyishi, 2010) who reported no antibacterial activity of the plant on *E. coli* in their study. This could be due to variation in strains or environment since they could influence the effect of antimicrobial actions since environment with more antimicrobial use tend to produce resistant isolates (Douglas and Robinson, 2019; Nweze and Onyishi, 2010). The antibacterial activity of *X. aethiopica* has been well studied and have shown great potency in inhibiting the growth of both gram positive and gram negative bacterial isolates (John-Dewole *et al.*, 2012; Nweze and Onyishi, 2010; Ogbonna *et al.*, 2013).

The alum was more potent as it showed a higher zone of inhibition to all the bacterial isolates than the methanol and aqueous extract of *X. aethiopica*. Results also showed that the combination of alum with aqueous extract of *X. aethiopica* showed increased antimicrobial activity on the bacterial isolates. This also agreed with a previous study that had earlier reported enhanced antibacterial activity of extracts of *Gongronema latifolium* combined with alum (Amadi *et al.*, 2016). The potency of alum as an antibacterial agent had been visibly demonstrated over the years through the myriads of its beneficial activities and relevance in a broad spectrum of human research and development (Amadi, 2020).

This study has shown that land snails harbor pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi* which could be pathogenic or act as opportunistic pathogens if not properly processed. Flavonoids, tannins, alkaloids and saponins revealed to be present in the plant in the present study could no doubt be a great tool in the antibacterial activity of the plant This research has vehemently revealed that *X. aethiopica* is a promising plant, with the profiles of its extracts with and without alum indicating excellent bioactivity and broad-spectrum effectiveness both in inhibiting the growth *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*.

The vast inhibitory zones suggest that this plant might be a valuable source of low-cost, safe antibacterial. The use of alum to enhance plant extracts in the treatment of bacterial infections especially for the bacteria highlighted in the present study is a valuable resource. Aqueous extract of *X. aethiopica* is should for use in the treatment of *Staphylococcus*, *Bacillus*, *Salmonella* and *E. coli*.

The combination of alum and aqueous extract of *X. aethiopica* should be practiced to achieve high potency especially in the treatment of bacterial mediated infections from consumption of infected land snails.

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