

Phytochemical analysis of *Xylopi aethiopica* (Dun), *Citrus limon* (L.) and *Allium sativum* (L.) extracts and their effect on selected human pathogens

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ABSTRACT

A study was carried out to evaluate the effects of phytochemical properties of plant extracts of *Xylopi aethiopica*, *Citrus limon* and *Allium sativum* on selected human pathogens. Phytochemical analysis of ethanol and chloroform of *Xylopi aethiopica*, *Citrus limon* and *Allium sativum* extracts showed presence of flavonoid, alkaloid, HCN, saponin, tannin and sterol. Quantitative phytochemical analysis of *C. limon*, *X. aethiopica* and *A. sativum* revealed *C. limon* has highest flavonoid (2.635 ± 0.021), sterol (0.160 ± 0.000), terpenoid (0.520 ± 0.000) and anthraquinone (2.760 ± 0.057). *X. aethiopica* has highest composition of hydrogen cyanide (0.835 ± 0.021) while *A. sativum* has highest composition of alkaloid (2.080 ± 0.000), saponin (2.080 ± 0.000) and tannin (1.040 ± 0.000). The three plants; *Allium sativum*, *C. limon* and *X. aethiopica* showed a significant difference in their composition of all assayed phytochemicals ($P < 0.05$). The inhibitory effect of plant extracts against *S. aureus*, *E. coli* and *Pseudomonas* spp. in all concentrations had *C. limon* and *A. sativum* as the highest while *X. aethiopica* showed the least inhibitory activity against *Aspergillus* spp. and *Rhizopus* spp. The study proves the effectiveness of the plant extracts in the control of human pathogens.

Keywords: Phytochemical, *Xylopi aethiopica*, *Citrus limon*, *Allium cepa*, extracts, human, pathogens.

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INTRODUCTION

Plant medicine has played a key role in the world health care (Calixto, 2000). This plant medicine is any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or basis for herbal products (WHO, 2001). These herbal medicines were first selected for treatment based on simple, botanical evidence carried out by local practitioners. With the improvement in standardization, efficacy, stability and safety of phytomedicine, plant medicine formulations are based on well controlled double blind clinical trials

(Akerle, 1993; Petrovick et al., 1999).

Staphylococcus aureus constitutes a nuisance in post infection and post-operative wound infection causing formation and production of wound diseases in both cases (Okigbo and Omadamiro, 2006). One of the avenues for such a research is to screen local medicinal plants for likely antimicrobial activities. With recent advances made in using plant extracts in inhibiting microbial growth, it was observed that Phytomedicine have antimicrobial effect against some human pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and so on.

People solicit for phytomedicine to their synthetic

counterpart because they prefer natural therapies. Three plants were selected for this work based on ethno botanical evidence of the plant in the community.

Xylopia aethiopica, Ethiopian pepper (Annonaceae): *X. aethiopica* is a tree of 20 m high or more with clear straight bore to 75cm girth (Burkill, 1985). The fruits are rather small and look like twisted bean-pods. When dry, the fruit turn dark brown, cylindrical, 2.5 to 5 cm long and 4 to 6 mm thick (Acquaye et al., 1982). The plant, *X. aethiopica* is used as carmitive, cough remedy, post-partum tonic and lactation aid, stomach remedy for bronchitis, biliousness and dysentery (Smith et al., 1996). The plant is used externally as poultice for headache and neuralgia, used with lemon grass for female hygiene and rich in copper, manganese and zinc (Smith et al., 1996). Adewuyi et al. (2008) reported that the fruit oil of *X. aethiopica* in liquid paraffin repels *Aedes* mosquito known to be carriers of deadly diseases such as yellow fever, dengue fever filariasis and encephalitis.

Citrus limon, lemon (Rutaceae): *Citrus limon* is a plant whose tree reaches 10 to 20 ft (3 to 6 m) in height and usually has sharp thorns on the twigs. The alternate leaves, reddish when young, become dark-green above, light green below are oblong, elliptic or long ovate, 2 1/2 to 4 1/2 in (6.25 to 11.25 cm) long, finely toothed with slender wings on petioles. The fruit is oval with nipple-like protuberance at the apex; the peel is usually light yellow though some lemons are varied with longitudinal stripes of green and yellow or white. It is aromatic, dotted with oil glands (Julia, 1987).

Allium sativum, Garlic (Liliaceae): *A. sativum* has shallow adventitious root system at the bottom of the bulb (Sterling, 2000). The leaves are similar to that of the other alliums and might be confusing but it has flat leaves whereas chives and onions have round leaves. The leaves are long and grass-like. The fruit contains many seeds. Each of its flowers has four white petals. Garlic blooms in spring (Catwell, 2001).

The aims of this research were to determine the inhibitory properties of these plants, *X. aethiopica*, *C. lemon* and *A. sativum* on disease causing organisms in human body and to measure the zone of inhibition of these plants on the organisms and also to ascertain the phytochemical constituents of the plants used.

MATERIALS AND METHODS

Collection of plant samples

The medicinal plants used, *X. aethiopica* (Ethiopian pepper), *C. limon* (lemon), *A. sativum* (Garlic) were collected on the basis of traditional medicinal history from Eke Awka Market in Anambra State, Nigeria. The plant identities were verified and authenticated by the Horticultural Unit of National Root Crops Research Institute, Umudike. The plants were dried at 50°C for four days and stored in

containers for use.

Extraction of plant materials

The plants; *X. aethiopica*, *C. limon* and *A. sativum* were washed and dried, placed in sterile mortar and pestled and ground with mechanised grinder. The plants in aqueous form were used for the analysis.

Quantitative test

Determination of anthraquinone

About 5 g of fine powder was soaked in 50 ml of distilled water for 16 h. This suspension was heated in water bath at 70°C for one hour. After the suspension was cooled, 50 ml of 50% methanol was added and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450 nm and compared with standard solution containing 1 mg/100 ml alizarin and 1 mg/100 ml purpurin with the absorption, maximum 450 nm.

Determination of terpenoids

About 5 ml of the extract was mixed with 2 ml of chloroform and carefully added to 3 ml of H₂SO₄ to form a layer which determines its presence.

Determination of alkaloid

The determination was carried out using alkaline precipitation gravimetric method described by Harborne (1973). Five gram of the powder samples were soaked in 20 ml of 10% ethanolic acetic acid. The mixture was stood for 4hours at room temperature. Thereafter, the mixture was filtered using filter paper. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60°C for 30minutes, cooled in dessicator and reweighed.

Determination of flavonoid

Determined by gravimetric method as described by Harborne (1973). Five gram of the powdered sample was placed into a conical flask and 50 ml of water and 2 ml of HCl solution were added and allowed to boil for 30 min. The boiled mixture was allowed to cool before it was filtered. 10ml of ethyl acetate extract which contained flavonoid was recovered while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filter the second (ethyl acetate layer), the residue was then placed in an oven to dry at 60°C. When cooled, it was reweighed.

Determination of phenol

Powdered sample of 0.2 g was added into a test tube and 10 ml of methanol was added to it and shaken thoroughly, the mixture was

left to stand for 15 min before being filtered. 1 ml of the extract was placed in a test tube and 1 ml of folin-cio caltean reagent in 5 ml of distilled water was added and colour was allowed to develop for about 1 to 2 h at room temperature. The absorbance of the developed colour was measured at 760 nm wavelength. The process was repeated.

Determination of saponin

Saponin is determined by double extraction gravimetric method Harborne (1973). Five gram of powdered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 min at 55°C, it was then filtered. The residue and extract were poured together and the combined extract was reduced to about 40 ml at 90°C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re extraction by partitioning was done repeatedly until the aqueous layer becomes clear in colour. The saponins were extracted with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a reweighed evaporation dish.

Determination of sterol

Five gram of powdered sample was hydrolysed by boiling in 50 ml of hydrochloride acid solution for about 30 min. It was filtered; the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) recovered while the aqueous layer was discarded. The extract was dried at 100°C for 5 min in a steam bath. It was then heated with concentrated ethyl alcohol to extract the sterol. The mixtures became turbid and a Whatman filter paper was used to filter the mixture properly. The dry extract was then cooled in a desiccator and reweighed.

Qualitative test

Methanol extract of the samples were used for the test. The dried samples were soaked in the solvent overnight and filtered before heating to one quarter volume of flask.

Test for alkaloid

The extract (1.0 ml) was shaken with 5.0 ml of 2% HCl on a steam bath and filtered. To 1 ml of the filtrate, Wagner reagent (iodine in potassium iodine solution) was added. A reddish brown precipitate confirms its presence.

Test for saponin

One millimetre of the filtrate was diluted in 1ml of water and shaken vigorously. A strong frothing confirms presence of saponin.

Test for tannin

Five ml of the extract was added to 2.0ml of 1% HCl. Deposition of

a red precipitate shows the presence of tannin.

Test for hydrogen cyanide

One millimetre of the extract was added with 2 to 3 drops of toluene solution. A change from yellow colour of the spot paper test used to brick red is a positive result for hydrogen cyanide.

Test for sterol

The extract (1 ml) was dissolved in 2.0 ml of chloroform in a test tube and then 1ml of concentrated H₂SO₄ was added. Formation of reddish brown colour at the interphase confirms the presence of sterol.

Test for phenol

The extract of 1.0 ml was added with 1.0ml of 10% ferride chloride. The formation of greenish brown or black precipitate or colour is taken as positive for phenolic test.

Test for flavonoid

The extract of 1.0 ml was diluted in 1.0 ml of diluted NaOH. Formation of precipitate shows presence of flavonoid.

Antimicrobial activities

Sample preparation and extraction procedures

The fresh leaves were oven dried at 65 to 70°C and ground into fine powder using a mechanised grinder. Soxhlex extraction was employed for the extraction of plant's active principles. About 100 g of the ground plant material were wrapped in Whatman filter paper, each containing 5 g. They were put in the thimble of a Soxhlet apparatus. Then 300ml of the 75% ethanol was put in a round bottom flask and this was used to mount the Soxhlet apparatus. The round bottom flask was heated and extraction of the plant material was stopped after seven refluxes. The solution was then evaporated under reduced pressure at 50°C using a rotary evaporator.

Preparation of stock solution of extract

The method of Akujobi (2004) was adopted for the preparation of stock solutions. The crude extracts obtained were diluted with 20% dimethyl sulphoxide (DMSO) solution to obtain 50, 25, 12.5 and 3.2 mg/ml concentrations. They were stored at 10°C in amber coloured bottle until required.

Test microorganisms and sources

The bacterial isolates used for the study were clinical isolates of food importance. These were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp. The fungi isolates used were *Aspergillus* spp and *Rhizopus* spp. They were obtained from Pathology Laboratory of National Root Crops Research Institute,

Umudike. They were re-identified; sub cultured on nutrient agar slants for *Escherichia coli*.

Evaluation of antimicrobial activity

The agar diffusion method as described by Esimone et al. (1998) and (Osadebe and Ukwueze, 2004) were adopted for the study. Standardised nutrient broth culture isolates containing approximately 10^7 cells/ml organisms were used. 6 ml of the broth cultures were introduced into sterile Petri dishes and 15 ml of molten nutrient agar was poured into the Petri dishes. The content was thoroughly mixed and allowed to solidify. Three holes, each measuring 5.0 mm in diameter were made in each of the solid agar plates using a sterile cork borer (6 mm). Different concentration of 0.04 ml of plant extracts were transferred into the holes using a Pasteur pipette. Positive control (amikacin 30 mcg and nitrofurantoin 300 mcg) for bacteria and 1 mg/ml of cyclohexylamine for fungal isolates and negative/solvent control (DMSO) respectively. Two plates were used for each concentration of the extracts. The plant extracts were thereafter allowed to stand for one hour for pre-diffusion of the extracts (Esimone et al., 1998) and were subsequently incubated at 37°C for 24 h. After incubation, plates were observed for formation of a clear zone around the hole which corresponds to the antimicrobial activity of the tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Organic solvent extraction

About 12 g of the plant was soaked in 100 ml of 99.91/100% ethanol for a day at room temperature with occasional stirring. The content was filtered and evaporated to dryness in a water bath at 78°C. The extract was collected and stored in the refrigerator at 4°C until required for use.

Sterility test

The extract disc was tested in sterile nutrients agar at 37°C for a day. The disc sample sterility was confirmed because there was no growth.

Culturing and sensitivity test

Test organisms *Staphylococcus aureus* and *E. coli* were cultured on nutrient agar media prepared by dissolving 28 g of nutrient agar in one litre of water. The media was autoclaved at 121°C for 15 min. The media was poured into plates and left to gel. The extract disc was placed in the cultured plates and incubated for a day at 37°C. Then, the zone of inhibition of plant extract was observed and measured in mm.

Determination of minimum inhibitory concentration

The broth microdilution method was used to determine the minimum inhibitory concentration according to Clinical and Laboratory Standard Institute (CLSI). Twofold serial dilutions of extracts were prepared directly in a microtiter plate containing Mueller Hinton broth to obtain various concentrations. The bacterial inoculum was added to give a final concentration of 5×10^5 CFU/ml

in each well. The positive control was used containing amikacin as a standard drug. The plate was covered with a sterile sealer and incubated for 24 h at 37°C. Resazurin was added in each well of the microtiter plate and was incubated at 37°C for 30 min. The wells containing the bacteria growth turned into pink colour whereas the well without bacterial growth remained blue. The MIC was considered as the lowest concentration of the extract that completely inhibits the bacterial growth.

Statistical analysis

Test for significance in the zone of inhibition was done with analysis of variance (ANOVA) via statistical analysis system (SPSS) of Version 21 and means were separated with Duncan's Multiple Range Test (DMRT) to know the effectiveness of plant extract and the susceptibility of the test organism.

RESULTS

Qualitative phytochemical composition of *Citrus limon*, *Xylopia aethiopica* and *Allium sativum*

Result of the qualitative phytochemical analysis of ethanol and chloroform extract of *Citrus limon*, *Xylopia aethiopica* and *Allium sativum* showed the presence of flavonoid, alkaloid, HCN, saponin, tannin and sterol. Flavonoid was very deeply present in the methanol and chloroform extract of *Citrus limon* and deeply present in methanol extract of *Xylopia aethiopica* and *Allium sativum*. Alkaloid was deeply present in chloroform extract of *Allium sativum* and methanol extract of *Citrus limon* (Table 1).

Quantitative phytochemical composition of *Citrus limon*, *Xylopia aethiopica* and *Allium sativum*

Quantitative phytochemical analysis of *Citrus limon*, *Allium sativum* and *Xylopia aethiopica* revealed that *C. limon* had highest composition of flavonoid (2.635 ± 0.021), sterol (0.160 ± 0.000), phenol (0.182 ± 0.028), terpenoid (0.520 ± 0.000) and anthraquinone (2.760 ± 0.057). *Xylopia aethiopica* had highest composition of hydrogen cyanide (0.835 ± 0.021) while *Allium sativum* had highest composition of alkaloid (2.080 ± 0.000), saponin (2.080 ± 0.000), and tannin (1.040 ± 0.000). *Citrus limon*, *Xylopia aethiopica* and *Allium sativum* showed a significant difference in the composition of all assayed phytochemicals ($P < 0.05$) (Table 2).

Inhibitory activity of plant extract against human pathogens

The inhibitory activity of extract of *Citrus limon*, *Xylopia*

Table 1. Qualitative phytochemical composition of *Citrus limon*, *Allium sativum* and *Xylopiya aethiopica*.

Plant extract	Solvent	Flavonoid	Alkaloid	HCN	Saponin	Tannin	Sterol
<i>C. limon</i>	Methanol	+++	++	+	+	+	+
	Chloroform	+++	+	+	+	+	+
<i>X. aethiopica</i>	Methanol	++	+	+	+	+	+
	Chloroform	+	+	+	+	+	+
<i>A. sativum</i>	Methanol	++	+	+	+	+	+
	Chloroform	+	++	+	+	+	+

NB: + -Present; ++ - Deeply Present; +++-Very deeply present.

Table 2. Quantitative phytochemical composition of *Citrus limon*, *Allium sativum* and *Xylopiya aethiopica* extract.

Treatment	% Phytochemical composition								
	Flavonoid	Alkaloid	HCN	Saponin	Tannin	Sterol	Phenol	Terpenoid	Anthraquinone
<i>C. limon</i>	2.635 ± 0.021 ^c	1.780 ± 0.000 ^b	0.750 ± 0.014 ^b	0.475 ± 0.021 ^a	0.670 ± 0.042 ^a	0.160 ± 0.000 ^b	0.182 ± 0.028 ^b	0.520 ± 0.000 ^c	2.760 ± 0.057 ^c
<i>X. aethiopica</i>	1.830 ± 0.000 ^a	1.660 ± 0.057 ^a	0.835 ± 0.021 ^b	0.690 ± 0.014 ^b	0.850 ± 0.000 ^b	0.074 ± 0.000 ^a	0.085 ± 0.002 ^a	0.350 ± 0.014 ^a	1.450 ± 0.000 ^a
<i>A. sativum</i>	2.450 ± 0.000 ^b	2.080 ± 0.000 ^c	0.380 ± 0.028 ^a	2.080 ± 0.000 ^c	1.040 ± 0.000 ^c	0.135 ± 0.021 ^b	0.180 ± 0.000 ^b	0.480 ± 0.000 ^b	1.760 ± 0.057 ^b
P-value	**	**	**	**	**	**	**	**	**

Means with the same letters in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT).

aethiopica and *Allium sativum* against human pathogens at 50 mg/ml revealed that *C. limon* extract gave higher inhibition against *Staphylococcus aureus* (9.200 ± 0.000), *E. coli* (8.525 ± 0.106), *Pseudomonas* spp (10.475 ± 0.035), *Aspergillus* spp (12.380 ± 0.113), and *Rhizopus* (11.450 ± 0.07) than the plant extracts of *Allium sativum* and *Xylopiya aethiopica*. The control however gave highest inhibitory activity against the human pathogens than the plant extracts. The plant extracts and control showed significant difference in their inhibitory activity

against the test pathogens at 50 mg/ml concentration (P < 0.05) (Table 3).

The inhibitory activity of extracts of *C. limon*, *X. aethiopica* and *A. sativum* against human pathogens at 75 mg/ml revealed that *C. limon* extract gave higher inhibition against *Staphylococcus aureus* (10.775 ± 0.035), *E. coli* (9.610 ± 0.014), *Pseudomonas* spp (12.725 ± 0.035), *Aspergillus* spp (14.820±0.028), and *Rhizopus* spp (13.800±0.000) than the extracts of *A. sativum* and *X. aethiopica*. The control however showed highest inhibitory activity against the test

pathogens than the plant extracts. The plant extracts and control showed significant difference in their inhibitory activity against the test pathogens at 75 mg/ml concentration (P < 0.05) (Table 4).

The inhibitory activity of extracts of *A. sativum*, *C. limon* and *X. aethiopica* against human pathogens at 100 mg/ml revealed that *C. limon* extract gave higher inhibition against *S. aureus* (12.770 ± 0.042), *E. coli* (11.490 ± 0.014), *Pseudomonas* spp (14.675 ± 0.035), *Aspergillus* spp (16.770 ± 0.042), and *Rhizopus* spp (15.800 ±

Table 3. Diameter of zones of inhibition (mm) of plant extracts against microorganisms at 50 mg/ml concentration.

Treatment	Human pathogens				
	<i>Staphylococcus</i> *	<i>E. coli</i> *	<i>Pseudomonas</i> *	<i>Aspergillus</i> *	<i>Rhizopus</i> *
<i>A. sativum</i>	7.200 ± 0.000 ^b	5.780 ± 0.028 ^b	8.910 ± 0.014 ^b	9.760 ± 0.057 ^a	9.475 ± 0.035 ^a
<i>X. aethiopica</i>	6.425 ± 0.035 ^a	4.500 ± 0.000 ^a	7.510 ± 0.014 ^a	10.525 ± 0.106 ^b	9.760 ± 0.05 ^a
<i>C. limon</i>	9.200 ± 0.000 ^c	8.525 ± 0.106 ^c	10.475 ± 0.035 ^c	12.380 ± 0.113 ^c	11.450 ± 0.07 ^b
Control	13.475 ± 0.035 ^d	11.770 ± 0.042 ^d	13.790 ± 0.014 ^d	15.600 ± 0.000 ^d	15.800 ± 0.000 ^c
p-value	**	**	**	**	**

Means with the same letters in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT).

Table 4. Diameter of zones of inhibition (mm) of plant extracts against microorganisms at 75 mg/ml concentration.

Treatment	Human Pathogens				
	<i>Staphylococcus</i> *	<i>E. coli</i> *	<i>Pseudomonas</i> *	<i>Aspergillus</i> *	<i>Rhizopus</i> *
<i>A. sativum</i>	8.410 ± 0.014 ^b	6.725 ± 0.035 ^b	11.500 ± 0.000 ^b	11.675 ± 0.106 ^a	11.865 ± 0.120 ^a
<i>X. aethiopica</i>	7.500 ± 0.000 ^a	5.820 ± 0.028 ^a	9.200 ± 0.000 ^a	12.625 ± 0.035 ^b	11.380 ± 0.113 ^a
<i>C. limon</i>	10.775 ± 0.035 ^c	9.610 ± 0.014 ^c	12.725 ± 0.035 ^c	14.820 ± 0.028 ^d	13.800 ± 0.000 ^b
Control	16.310 ± 0.014 ^d	14.820 ± 0.028 ^d	16.550 ± 0.071 ^d	18.500 ± 0.141 ^c	18.550 ± 0.071 ^c
P-value	**	**	**	**	**

Means with the same letters in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT).

0.000) than the extracts of *A. sativum* and *X. aethiopica*. The control however showed highest inhibitory activity against the test pathogens than the plant extracts. The plant extracts and control showed significant difference in their inhibitory activity against the test pathogens at 100 mg/ml concentration ($p < 0.05$) (Table 5).

The inhibitory activity of extracts of *C. limon*, *X. aethiopica* and *A. sativum* against the human pathogens at 150 mg/ml revealed that the *C. limon* extract gave higher inhibition against *Staphylococcus aureus* (14.540 ± 0.085), *E. coli* (13.825 ± 0.035), *Pseudomonas* spp (17.525 ± 0.106), *Aspergillus* spp (19.525 ± 0.106) while *X. aethiopica* gave higher inhibition of *Rhizopus* spp (18.700 ± 0.141) than that of *A. sativum* and *C. limon*. The plant extracts and the control showed a significant difference in their inhibitory activity against the test pathogens at 150 mg/ml concentration of extract ($P < 0.05$) (Table 6).

DISCUSSION

Phytochemicals have antimicrobial properties. Plant extracts play vital role in human health. *X. aethiopica* had effect on *S. aureus*. *A. sativum* and *C. limon* showed inhibition on *E. coli* and *Pseudomonas* spp. *X. aethiopica* and *C. limon* showed inhibition on *Aspergillus* spp. *A. sativum* showed great antimicrobial properties (Ginseng,

2011). The difference in antimicrobial properties of plant extracts is attributed to the age of the plants used, freshness of plant materials, physical factors (temperature, water, light) contamination by field microbes, adulteration and substitution of plants incorrect preparation and dosage (Calixto, 2000; Okigbo and Omodamiro, 2006; Okigbo and Mmeka, 2006). As reported by Iwu (1993), the antimicrobial properties of *A. sativum* are attributed to allitridium and micro dose of selenium that prevent gastric cancer (Lin et al., 1996). *X. aethiopica* contains high amount of manganese, copper and zinc. Diterpenic and xylopic acids have antimicrobial properties against gram positive and negative bacteria (Iwu, 1993). *C. limon* composition; potassium and vitamins especially biflavonoid and volatile oil of the peel promotes clarity of thought (Aslin, 2014). It aids in the treatment of asthma, cough, biliousness, cold, heart burn, liver, fever and rheumatism (Michael, 1998). *C. limon* is anti-carcinogenic, it lowers the rates of colon, prostate and breast cancers (Aslin, 2014). *A. sativum* on the other hand, is a spice for seasoning and condiment; which enhances taste of food and drugs (Parry et al., 1995; Dziezak, 1989; Iwu, 1993; Manandhar, 1995). *A. sativum* is used for treatment of fever, cough, nervous disorders, asthma, skin diseases and rheumatism pains (Manandhar, 1995). *A. sativum* has been recognised as valuable folk medicine with favourable effect against large number of pathogenic conditions, as anti-

Table 5. Diameter of zones of inhibition (mm) of plant extracts against microorganisms at 100 mg/ml concentration.

Treatment	Human pathogens				
	<i>Staphylococcus</i> *	<i>E. coli</i> *	<i>Pseudomonas</i> *	<i>Aspergillus</i> *	<i>Rhizopus</i> *
<i>A. sativum</i>	10.490 ± 0.014 ^b	9.710 ± 0.014 ^b	12.525 ± 0.106 ^b	13.800 ± 0.000 ^a	13.775 ± 0.035 ^a
<i>X. aethiopica</i>	9.490 ± 0.099 ^a	7.635 ± 0.021 ^a	11.825 ± 0.035 ^a	14.525 ± 0.389 ^b	13.875 ± 0.106 ^a
<i>C. limon</i>	12.770 ± 0.042 ^c	11.490 ± 0.014 ^c	14.675 ± 0.035 ^c	16.770 ± 0.042 ^c	15.800 ± 0.000 ^b
Control	19.525 ± 0.106 ^d	17.710 ± 0.127 ^d	20.550 ± 0.071 ^d	21.775 ± 0.035 ^d	22.250 ± 0.354 ^c
p-value	**	**	**	**	**

Means with the same letters in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT).

Table 6. Diameter of zones of inhibition (mm) of plant extracts against microorganisms at 150 mg/ml concentration.

Treatment	Human pathogens				
	<i>Staphylococcus</i>	<i>E. coli</i>	<i>Pseudomonas</i>	<i>Aspergillus</i>	<i>Rhizopus</i>
<i>A. sativum</i>	12.375 ± 0.035 ^b	11.500 ± 0.000 ^b	15.800 ± 0.000 ^b	17.870 ± 0.042 ^a	17.460 ± 0.057 ^a
<i>X. aethiopica</i>	11.500 ± 0.00 ^a	9.610 ± 0.014 ^a	14.775 ± 0.035 ^a	18.400 ± 0.141 ^b	18.700 ± 0.141 ^b
<i>C. limon</i>	14.540 ± 0.085 ^c	13.825 ± 0.035 ^c	17.525 ± 0.106 ^c	19.525 ± 0.106 ^c	18.525 ± 0.177 ^b
Control	22.500 ± 0.141 ^d	20.500 ± 0.141 ^d	23.720 ± 0.170 ^d	25.820 ± 0.028 ^d	25.780 ± 0.028 ^c
p-value	**	**	**	**	**

Means with the same letters in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT).

inflammatory used as cell influx (Dorsch, 1999).

X. aethiopica show activity against gram positive organisms (Iwu, 1993). Chemical components of *X. aethiopica* have been helpful in the treatment of cancerous tumours (Graham et al., 2000). It also has anti-inflammatory and anti-cancer actions (Shoji, 2011).

CONCLUSION

The study revealed that *C. limon*, *X. aethiopica* and *A. sativum* used possessed antimicrobial properties. The ability of the three plants to inhibit the pathogens (*Aspergillus* spp, *S. aureus*, *E. coli*, *Pseudomonas* spp and *Rhizopus* spp) justifies the use of these plants by Physiotherapist in Nigeria in combating different human ailments arising from effect of these human pathogens. Also, other forms of extraction and solvents can be conducted to know the best form of use of each plant extract by future researchers.

REFERENCES

- Acquaye** CTA, Young JD, Ellory JC, Goreck M, Wilcher M, **1982**. Mode of transport and possible mechanism of action of L-Phenylalanine benzylester as an anti-sickling agent. *Biochem Biophys Acta*, 693: 407-416.
- Adewuyi** G, Obi-Egbedi N, Babayemi J, **2008**. Evaluation of ten different African wood species for potash production. *Int J Phys Sci*, 3(3): 63-65.
- Akerele** O, **1993**. Nature's medicinal bounty: don't throw it away. *World Health Forum*, 14: 390-395.
- Akujobi** C, Anyanwu BN, Onyeze C, Ibekwe VI, **2004**. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. *J Appl Sci*, 7(3): 4328-4338.
- Aslin** CU, **2014**. Medicinal Plant of Nigeria. Offset-Happen, Netherlands. 86p.
- Burkill** TU, **1985**. Medical Plants and Traditional Medicine in Africa. The Pitman Press, Bath, Avon. Pp. 314-318.
- Calixto** JB, **2000**. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (Phytotherapeutic agents). *Braz J Med Biol Res*, 33(2): 179-189.
- Catwell** SM, **2001**. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998. Champaign, Illinois. Pp. 62-78.
- Dorsch** W, **1999**. Anti-inflammatory activity in human skin. It prevents edema formation in vitro. *INTAALIM*, 118(2-4): 236-239.
- Dziezak** JD, **1989**. Innovative food trends: Species. *Food Technol*, 43: 102-116.
- Esimone** CO, Adiukwu MU, Okenta JM, **1998**. Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usnea subfloridans* (L). *J Pharmaceut Res Dev*, 3(2): 99-102.
- Ginseng** CD, Lawson LD, Block E, Chatterjee LM, Kiazand A, Balise RR, Kraemer HC, **2011**. The effect of raw garlic vs. garlic supplements on plasma lipids concentrations in adults with moderate hypercholesterolemia: A clinical trial. *Arch Internal Med*, 167: 346-353.
- Graham** S, Graham JG, Quinn DS, Fabricant DS, Farnsworth NR, **2000**. Effect of *Xylopiia aethiopica* on some biochemical parameters following ethanol-induced toxicity. *Brit J Med Med Res*, 73(3): 347-377.
- Harborne** JB, **1973**. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd, London. 279p.
- Iwu** M, **1993**. Handbook of Africa Medicinal Plant. CRC Press, Ann Arbor, U.S.A. 435p.
- Iwu** M, **1993**. Pharmacological Properties and Chemical Composition,

- Advances in the Development, Production and Utilization of Medicinal and Aromatic Plants in India: A survey of some plants used in Native Medicine of West Africa of interest to India; 35p.
- Iwu M, 1993.** Handbook of African Medicinal Plants. CRC Press, Boca Raton, FL. 367p.
- Julia FF, 1987.** Roselle. In: Fruits of warm climates. Edited by Morton J., Miami. Pp. 281-286.
- Lin CC, Chang CH, Yang JJ, Namba T, Hattori M, 1996.** Hepatoprotective effects of emodin from *Ventilago leiocarpa*. J Ethnopharmacol, 52(2): 107-111.
- Manandhar NP, 1995.** A survey of medicinal plants of Jajarkot district, Nepal. J Ethnopharmacol, 48: 1-6.
- Okigbo RN, Mmeko EC, 2006.** An appraisal of phytomedicine in Africa. KMITL Sci Technol J, 6(2): 83-94.
- Okigbo RN, Omodamiro OD, 2006.** Antimicrobial effect of leaf extracts of pigeon pea (*Cajanus cajan* (L) Millsp) on human pathogens. J Herbs Spices Med Plants, 12(1/2): 117-127.
- Osadebe PO, Ukwueze SE, 2004.** A comparative study of the phytochemical and antimicrobial properties of the Eastern Nigeria species of African mistletoe (*Loranthus micranthus*) sourced from different host trees. J Biol Res Biotechnol, 2(1): 18-23.
- Parry DW, Jenkinson P, MCleod L, 1995.** Fusarium ear blight (scab) in small grain cereals: A review, Plant Pathol. 44: 207-238.
- Petrovick PR, Marques LC, Paula IC, 1999.** New rules for phytopharmaceutical drug registration in Brazil. J Ethnopharmacol, 66: 51-55.
- Shoji S, 2011.** Chemistry and cancer presenting activities of Ginseng saponins and some related terpenoid compounds. J Korean Med Sci, 16: 28-37.
- Smith G, Clegg M, Keen C, Grivetti L, 1996.** Mineral values of selected plant foods common in Southern Burkina Faso and to Niamey, Niger, West Africa. Int J Food Sci Nutr, 47: 41-53.
- Sterling S, 2000.** Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois. Pp. 62-78.
- World Health Organization (**WHO**), **2001.** Legal Status of Traditional Medicines and Complementary/Alternative Medicine: A Worldwide Review. WHO publishing. 1092p.

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