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Assessment of the Antimicrobial Activities of *Ximenia caffra* (Sour Plum) Leaf

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ABSTRACT

The antimicrobial activities of sour plum (*Ximenia caffra*) leaf extracts was investigated against *Staphylococcus Aureus*, *Bacillus Subtilis*, *Escherichia Coli* and *Pseudomonas Aurignosa*, using Methanol, n-Hexane and Chloroform as solvents for extraction. The agar wall diffusion method as described by Irobi *et al.*, (1994) was used for the determination of antimicrobial activity. The results showed that the extracts were all bacterial static with the methanol and chloroform extract, having the highest and least activities, respectively. *Ximenia caffra* leaves are therefore recommended for use as antimicrobial agents.

Keywords: antimicrobial activities, extracts, solvents, methanol, n-hexane, chloroform

1.0 INTRODUCTION

The use of plants as medicine is a worldwide phenomenon; plants not only provide safe and cost effective remedies, they are also available and accessible at affordable prices. the use of resources already available, forms the basic core of any public health practices and what is better than plant as medicine as they associated with fewer side effect and no known resistance to microorganisms (Phillipson, 2001).

Many plants have been used by tribal and folklore in different countries for the treatment of various diseases. The medicinal value of plants lies in their bioactive phytochemical constituents. The rise in interest on medicinal properties of herbs is consequent on the failing efficacy and toxicity associated with conventional drugs and their inaccessibility to poor rural dwellers or low-income earners. For a long time, there has been a resurgence of interest in the investigation of natural materials as a source of potential drug substance (Kumor *et al.*, 2015).

Plants that possess antimicrobial properties are known as medicinal plant. Plants and plant products have been used in folkloric treatment of ailments such as diarrhea, malaria, typhoid fever and parasitic infections (Nasri *et al.*, 2013). Herbs and spices are very important and useful

as therapeutic agent against many pathological infections. Increasing multidrug resistance of pathogenens forces to find alternative compounds for treatment of infectious diseases (Nasri *et al.*, 2013).

Ximenia caffra, the large sour plum, is part of the Olacaceae family which is native throughout tropical regions. In particular, the sour plum is native to regions in South East Africa, mainly Botswana, Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe (Orwa *et al.*, 2009). The sour plum tree produces several fruits on an annual basis. Once planted the seeds will germinate after 14–30 days, and have a moderate growth rate of 0.5 m per year (Baloyi *et al.*, 2004).). These are generally sour with a dry aftertaste, however contains significant amounts of potassium (Ndhlala *et al.*, 2008).

The paper is a study that evaluates the antimicrobial activities of sour plum (*Ximenia caffra*) leaf extracts in three different solvents (methanol, n-hexane and chloroform).

2.0 MATERIALS AND METHODS

2.1 Sample collection and Treatment

The plant *Ximenia caffra* (PT-001) was collected from Anyigba town in Dekina Local Government Area of Kogi State, and was identified at the Department of Plant Science

and Biotechnology, Kogi State University, Anyigba, Kogi State. The leaves were separated from the plant, washed to remove altering matter, air-dried and pounded using mortar and pestle. The extraction was done using cold maceration method. The powdered sour plum leaves were subjected to maceration for 72 hours at room temperature using methanol (300 mL). The extract was filtered and the solvent was evaporated using water bath at 40°C. A brownish pasty residue was obtained. The same procedure was followed for n-hexane and chloroform respectively.

2.2 Antimicrobial screening methods

In the determination of the antimicrobial activity of the extract from Methanol, N-Hexane and Chloroform, the agar wall diffusion method as described by Irobi *et al.*, (1994) was used for each of the respective Mueller Hinton agar plates, a sterile cork borer (6 mm) in diameter was used to create wells on its solidified agar. Each of the wells were filled with the extract (0.1 mL) concentration of 100mg/mL, 150 mg/mL, 200 mg/mL and 250 mg/mL. and labeled appropriately. Distilled sterile water was used to fill one of the wells to serve as control. These were allowed to diffuse in to the agar at room temperature for one hour, then incubated

at 37°C for 18-24hrs. The plates were observed for zones of inhibition or clearance after 18-24 hrs incubation.

2.3 Collection of Test Organism

The test organisms were gotten from the Microbiology Laboratory of Kogi State University, Anyigba, Nigeria. The organisms include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

2.4 Zone of Inhibition

The zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The estimation of MIC in the extract was carried out using the method of Akinpelu and Kolawale (2004). The extracts (0.5mL) of varying concentration in mg/ml (100, 150, 200, 250) were placed into each test tubes containing nutrient broth, inoculated with loopful of each test organism, 0.5 McFarland turbidity standard was adopted. A test tube containing nutrient broth with test organism, but no extract served as control. The MIC was taken as the tube with the least concentration with no visible turbidity after 24hrs incubation at 37°C for 24hrs and then examined for growth by observing for turbidity.

3.0 RESULTS

Table 1a: Antimicrobial Activities of Various Concentrations of Methanol Extracts On the Test Organisms Using Zone of Inhibition Method (mm)

Concentration (mm)	100	150	200	250	+ve control	-ve control
<i>Staphylococcus aureus</i>	12.00	19.00	20.00	21.00	37.00	0.00
<i>Escherichia coli</i>	17.00	18.00	20.00	21.00	31.00	0.00
<i>Pseudomonas Aeruginosa</i>	16.60	6.60	12.00	18.00	33.00	0.00
<i>Bacillus subtilis</i>	10.00	14.00	17.00	20.00	30.00	0.00

Table 1b: Minimum Inhibitory Concentrations (MIC) of The Methanol Extracts Against Test Organisms (mg/mL)

Concentration (mm)	100	150	200	250	+ve control	-ve control
<i>Staphylococcus Aureus</i>	G	G	NG	NG	NG	G
<i>Escherichia Coli</i>	G	G	NG	NG	NG	G
<i>Pseudomonas Aeruginosa</i>	G	G	G	NG	NG	G
<i>Bacillus Subtilis</i>	G	G	G	NG	NG	G

key: NG= no growth, G = growth

Table 2a: Antimicrobial Activities of Various Concentrations of n-Hexane Extracts in The Test Organisms, Using Zone of Inhibition Method (mm)

Concentration (mm) Test organism	100	150	200	250	+ve control	-ve control
<i>Staphylococcus Aureus</i>	6.60	6.60	6.60	6.60	29.00	0.00
<i>Escherichia Coli</i>	6.60	6.60	6.60	18.00	32.00	0.00
<i>Pseudomonas Aeruginosa</i>	6.60	8.00	11.00	13.00	35.00	0.00
<i>Bacillus Subtilis</i>	6.60	9.00	10.00	12.00	37.00	0.00

Table 2b: Minimum Inhibitory Concentration (MIC) of the n-Hexane Extract Against Test Organisms (mg/mL)

Concentration (mm) Test organism	100	150	200	250	+ve control	-ve control
<i>Staphylococcus Aureus</i>	G	G	G	G	NG	G
<i>Escherichia Coli</i>	G	G	G	NG	NG	G
<i>Pseudomonas Aeruginosa</i>	G	G	G	G	NG	G
<i>Bacillus Subtilis</i>	G	G	G	G	NG	G

Key: NG = No growth, G = growth

Table 3a: Antimicrobial Activities of Various Concentration of Chloroform Extract on the Testy Organisms, Using Zones of Inhibition Method (mm)

Concentration (mm) Test organism	100	150	200	250	+ve control	-ve control
<i>Staphylococcus Aureus</i>	6.60	6.60	14.00	16.00	31.00	0.00
<i>Escherichia Coli</i>	8.00	10.00	12.00	12.00	30.00	0.00
<i>Pseudomonas Aeruginosa</i>	6.60	6.60	6.60	6.60	34.00	0.00
<i>Bacillus Subtilis</i>	6.60	6.60	6.60	6.60	34.00	0.00

Table 3b: Minimum Inhibitory Concentration (MIC) of The Chloroform Extracts Against Test Organisms (mg/mL)

Concentration (mm) Test organism	100	150	200	250	+ve control	-ve control
<i>Staphylococcus Aureus</i>	G	G	NG	NG	NG	G
<i>Escherichia Coli</i>	G	G	G	NG	NG	G
<i>Pseudomonas Aeruginosa</i>	G	G	G	G	NG	G
<i>Bacillus Subtilis</i>	G	G	G	G	NG	G

Key: NG = no growth, G = growth

Figure 4: Comparison of The Antimicrobial Activities of the Extracts from The Different Solvents at 250 mg/mL

Extract		<i>Staphylococcus Aureus</i>	<i>Escherichia-coli</i>	<i>Pseudomonas Aeruginosa</i>	<i>Bacillus Subtilis</i>
METHANOL	Zone of Inhibition (mm)	21	21	18	20
	Positive Control	37	31	33	30
	Minimum Inhibitory Control	NG	NG	NG	NG
N-HEXANE	Zone of Inhibition (mm)	6;6	18	13	12
	Positive Control	29	32	35	37
	Minimum Inhibitory	G	NG	G	G

Control					
CHLOROFORM	Zone of Inhibition (mm)	16	12	6.6	6.6
	Positive Control	31	30	34	34
	Minimum Inhibitory Control	NG	NG	G	G

4.0 DISCUSSION

Results from Table 1a and Figure 1 show that the methanolic extract inhibits the growth of the test organisms at 250 mg/mL ranging from 18 mm to 21 mm. Results from Table 1b shows that at a concentration of 250 mg/mL, there were no growth of all the test organisms, indicating that the methanolic extract is bacteriostatic towards *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Results from Table 2a and Figure 2 show that the n-hexane extract inhibits the growth of the test organisms at 250 mg/mL ranging from 6.6 mm to 18 mm. Results from Table 2b shows that at a concentration of 250 mg/mL, there were growth of all the test organisms, except *Escherichia coli* indicating that the n-hexane extract is bacteriostatic towards only *Escherichia coli*.

Results from Table 3a and Figure 3, show that the chloroform extract inhibits the growth of the test organisms at 250 mg/mL ranging from 6.6

mm to 16 mm. Results from Table 3b shows that at a concentration of 250 mg/mL, there were no growth of *staphylococcus Aureus* and *Escherichia coli*, while there was growth of *Pseudomonas aeruginosa* and *Bacillus subtilis*, indicating that the chloroform extract is bacteriostatic towards *Staphylococcus aureus*, *Escherichia coli* only.

Results from Table 4 shows Comparison of the Antimicrobial Activities of the extracts from the different Solvents at 250 mg/mL, and it shows that all the extracts from the three different solvents show varying antimicrobial activities, as seen in their respective zones of inhibitions, with the methanolic extract having (18 mm to 21 mm), n-hexane extract (from 6.6 mm to 18 mm) and chloroform extract (6.6 mm to 16 mm). this result shows that the methanolic extract showed more antimicrobial activity at a concentration of 250 mg/mL.

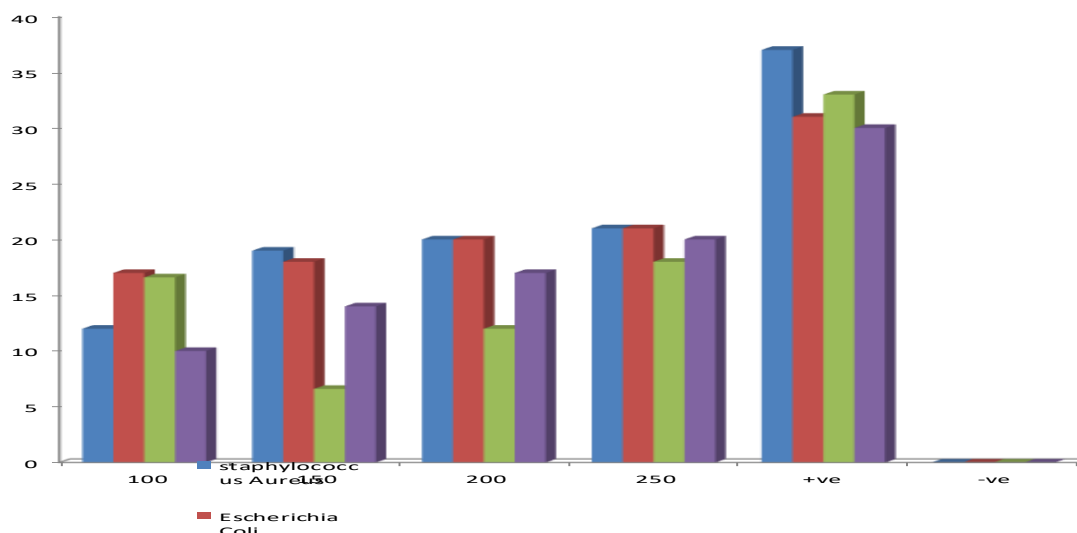


Figure 1: Graphical Representation of Various Concentrations of Methanol Cold Extracts.

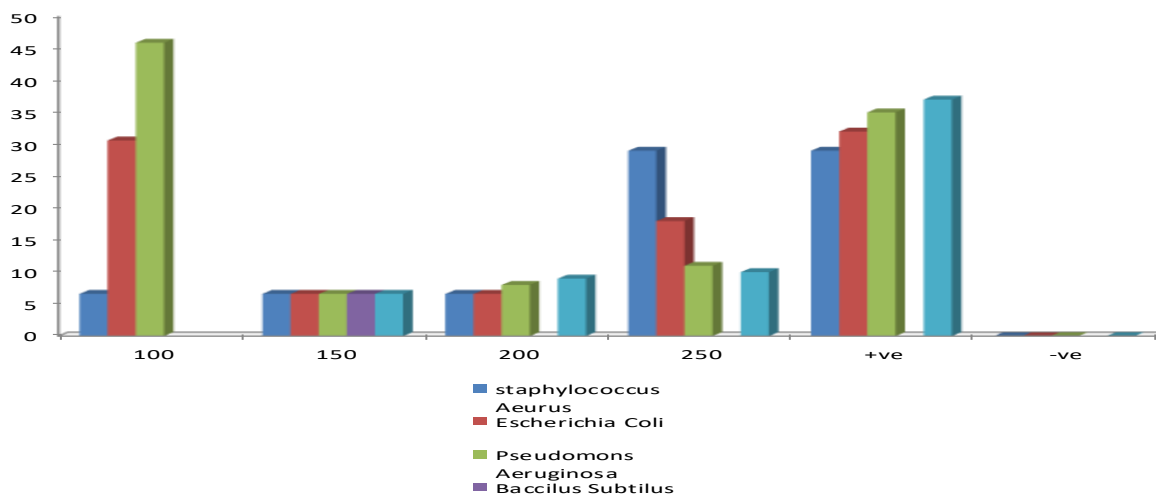


Figure 2: Graphical Representation of Various Concentrations of N-Hexane Cold Extracts

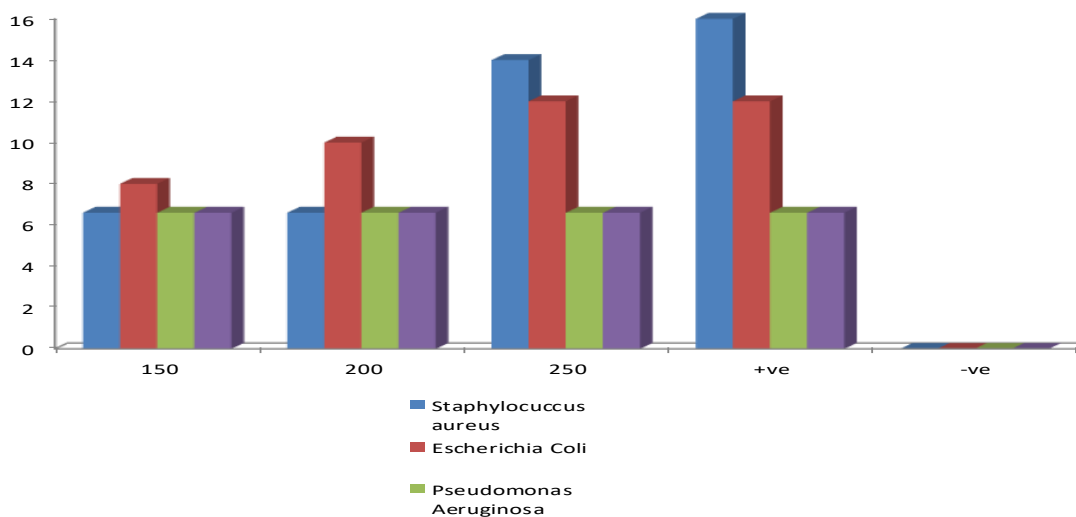


Figure 3: Graphical Representation of Various Concentrations of Chloroform Cold Extracts

5.0 CONCLUSION

The results of the present study, shows that, extracts from *Ximenia caffra* (sour plum) leave shows antimicrobial activities (bacterial static), and is therefore recommended that it be considered as an antimicrobial agent.

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