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Antimicrobial Activity Of Sourplum (*Ximenia Caffra*) Leaf Extracts Against *Staphylococcus Aureus* And *Escherichia Coli*

BY

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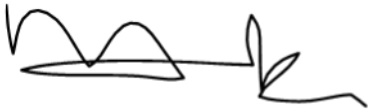
APPROVAL FORM

The undersigned certify that they have and recommended to Bindura University of Science Education for acceptance of research project titled "Antimicrobial activity of sourplum (*Ximenia caffra*) leaf against *Staphylococcus aureus* and *Escherichia coli*" submitted by Munashe Mudariki(B192103B)



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(Signature of Student) Date

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DEDICATION

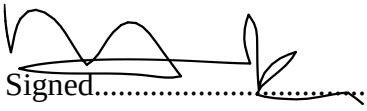
This research is dedicated to friends and family**.**

ACKNOWLEDGEMENT

The success of this study was a result of the efforts made by many people who helped me along the way. I owe a lot to my supervisor, Mr J. Ndava, who helped me understand what needed to be done. I also want to thank Mr D. Katsande and his team from the biology department's lab, as well as Mr Shumba from the chemistry department's lab, for helping me with the technical aspects of the laboratory.

DECLARATION

I, Munashe Mudariki (192103B), declare that this research project herein is my original work and affirm that it has not been copied or extracted from previous sources that have been submitted to this or other University in support of any application for a degree or any other qualifications without due acknowledgement of the source.



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ABSTRACT

Traditional medicine has become one of the latest innovations in dealing with drug resistant bacteria. *Ximenia caffra* is being used in rural areas to treat stomach pains, fever and also other skin conditions, by using different parts of the tree. This study serves to exploit the antimicrobial activity *Ximenia caffra* leaf extracts in ethanol and hexane thereby determining the best solvent for extraction and also to observe the different phytochemicals present. The disc diffusion assay was adopted to determine the antibacterial activity and evaluating the minimum inhibitory concentration against *Escherichia coli* and *Staphylococcus aureus*. Phytochemical screening was done by adding chemicals such as ferric chloride, acid, Benedict's reagent, alkaline solutions and potassium iodide, and observing the colour changes. Hexane was the best extraction solvent with mean extraction yield percentages of 12.6, 9.6 and 8 whilst ethanol had 8.5, 1.16 and 6 and water had 5.Tannins,reducing sugars, alkaloids, flavonoids and phenols were present in the aqueous solution ;ethanol extract contained tannins, reducing sugars, alkaloids and phenols ;hexane extract had alkaloids and reducing sugars. Ethanolic extracts had greater antibacterial activity with mean zones of inhibition of 22.7±8.9 and 13±4.9 mm whilst hexane had 16.7±6.4 and 2.2±0.4 mm of *E. coli* and *S. aureus* respectively. Serial dilutions of 10 mg/ml, 5 mg/ml and 2.5 mg/ml were prepared. *Staphylococcus aureus* growth was supported in hexane at all concentrations whilst in ethanol the mean diameters were 11±1.5mm, 4±0.9mm and 0 ±0.4 respectively. For *E. coli* in ethanol the mean zones were 20±5.4, 15±3.1 and 9±2.2 mm respectively whilst in hexane antimicrobial activity was only shown at 10 mg/ml with a diameter of 10 mm. When the data was analysed, the p value was 0.00 which is below the alpha level 0.05 therefore Ximenia caffra has antimicrobial activity against *E. coli* and *S. aureus*. Ethanolic extracts have greater antimicrobial activity than hexane and aqueous extracts and also *Ximenia caffra* is highly active against *Escherichia coli* than S*taphylococcus aureus*.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF STUDY

According to Eldeen and Van Staden (2007), there is an increase in the prevalence of infectious disorders, particularly bacterial infections with varying degrees of medication resistance. Infectious infections cause over 50,000 deaths every day worldwide (Ahmad and Beg, 2001). In addition to offering food, shelter, and aid in the treatment of various diseases, plants have been man's first companions (Jaleel et al., 2009). As a result, people are using natural products more frequently and are looking for novel antimicrobial medications (Tshikalange et al., 2005).

The primary component of most drugs now comes from medicinal plants. Approximately 80% of Africans rely on herbal therapy to treat and manage their illnesses. Due to their variety, availability for collecting, widespread use across cultures, ability to be used for self-medication, low cost, and few negative health consequences, medicinal herbs are preferred to synthetic medications. Medicinal plants contain a wide variety of phytochemicals, such as polyphenols, which have antioxidant qualities and can disarm potentially harmful free radicals, thereby preventing oxidative stress on tissues.

Medicinal plants are those that have antibacterial qualities. In folkloric medicine, diseases like parasite infections, malaria, typhoid fever, and diarrhoea have all been treated with plants and plant products (Nasri et al., 2013). Spices and herbs have a significant role in medicine and are effective treatments for many pathogenic infections. Finding new substances to treat infectious diseases is becoming more and more difficult due to organisms' rising multidrug resistance (Nasri et al., 2013).

The large sour plum, *Ximenia caffra*, is a member of the Olacaceae family, which is indigenous to tropical areas. According to Orwa et al. (2009), the sour plum is a native of South East Africa, specifically Botswana, Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe. Each year, the sour plum tree yields a number of fruits. After being planted, the seeds will begin to grow after 14 to 30 days and will reach a height of 0.5 meters each year (Baloyi et al., 2004). These often taste sour

and have a dry aftertaste, but they also contain significant amounts of potassium (Ndhlala et al., 2008).

Escherichia coli also referred to as *E. coli* is a rod-shaped bacterium which is gram negative. The bacteria can either undergo respiration in the presence of oxygen or fermentation in the absence of oxygen making it a facultative aerobe (Idalia and Bernado, 2017). It is mainly located in the intestines of healthy humans and animals. However, some of the strains such as STEC result in diarrhoea and stomach pains. *S.aureus* known as *Staphylococcus aureus* is the one of the important bacteria that is disease causing in humans (Horvath and Barrangou, 2010). It causes diseases such as boils, furuncles and cellatiis.*S.aureus* can also lead to serious infections such pneumonia and joint infections. As time proceeds and also some genetic changes due to intake or contact with genetically modified organisms it has resulted in the resistance of antibiotics by some of the bacteria. A resistant bacterial infection increases the risk of death compared to a drug-sensitive infection. Methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* resistant to third generation cephalosporins (3GC) were found to have about 12.11% and 36.0% resistance, respectively, according to an antimicrobial resistance indicator used in 2019 (Brown and Macgowan, 2010).. This resistance has resulted in the extraction of plants for medical purposes in order to reduce serious infections.

1.2 PROBLEM STATEMENT

Staphylococcus aureus and *Escherichia coli* are some of the bacteria that cause disease in humans, plants and animals such as food poisoning, abscesses and cellulitis. Over the counter medication and antibiotics have become less effective since some of the strains have become resistant. So, medication leaves resistant strains which then multiply and become more difficult to treat resulting in outbreaks.

1.3 AIM

The aim of the study was to in vitro determine the antimicrobial activity of Sour plum (*Ximenia Caffra*) leaf extracts on *Staphylococcus aureus* and *Escherichia coli.*

1.4 OBJECTIVES

- To assess the antibacterial efficacy of *Ximenia caffra* leaf extracts, against *E. coli* and *S. aureus.*
- To compare antimicrobial effects of different leaf extracts of *Ximenia caffra* against *E. coli and S. aureus.*
- To determine Minimum inhibitory concentration (MIC) of leaf extracts.

1.5 RESEARCH QUESTIONS

- Which leaf extract of *Ximenia caffra* is most effective on *E.coli and S.aureus?*
- What bioactive compounds are found in *Ximenia caffra* leaves?
- Which concentration has the minimum inhibitory activity?

1.6 HYPOTHESIS

Ximenia caffra leaf extracts have antimicrobial activity on *S.aureus* and *E.coli.*

1.7 SIGNIFICANCE OF STUDY

Since medication has proved to be expensive in developing countries it is better to exploit the plants and herbs we already have to treat various infections. . There is a continuous need of new antimicrobial components due to rapid emergence of multi-drug resistant pathogens and dreadful infections .Hence assessing plants will assure more strategic treatment. There is a continuous need of new antimicrobial components due to rapid emergence of multi-drug resistant pathogens and dreadful infections. Hence this study serves to exploit medical effects of *Ximenia caffra* on *E.coli* and *S.aureus* so as to avoid outbreak and growth of resistant strains.

1.8 LIMITATIONS OF THE STUDY

There was absence of other reagents to further test for other phytochemicals .The Astra laboratory cannot contain other bacteria such as *helicobacter pylori* therefore only used *E.coli* and *S.aureus*. The solvents were only limited to 2 per person and also for dilution we used solvents due to absence of DMSO.

1.9 DELIMITATIONS

This study is delimited to investigation the antimicrobial properties of only two bacterial species, Escherichia *coli* and *Staphylococcus aureus*, excluding other bacterial strains that may also exhibit relevant characteristics. In this study extracts were solely from the leaves of the plant, excluding extracts from other parts such as roots which may contain different bioactive compounds.

1.10 DEFINITION OF TERMS

Drug resistance it is when the intended use of a drug such as an antimicrobial is no longer very effective.

Antimicrobial activity is the ability of a certain substance to inhibit growth of microbes.

Zone of inhibition is the area around in which the growth of bacteria was not supported (Banu and Cathrine, 2015).

CHAPTER 2: LITERATURE REVIEW

2.1 DESCRIPTION OF *XIMENIA CAFFRA*

The *Ximenia caffra* commonly known as sour plum is a drought resistant wild fruit tree species found in Africa from Tanzania in the North to South Africa. It grows to a height of 6 meters and has some stout axillary spines. Around August to October the tree produces sour plums which are 3.5 long and 2.5 cm wide and are green when immature but are orange or red when ripe and they have a red seed on the centre (Corrigan et al., 2011). On younger branches, the bark is pale green or brown rather than dark grey and rough. It has dark green clustered leaves. The fruits are rich in vitamins though they are bitter. The most effective method to grow this tree is using fresh seeds however, some use stem cuttings. Their growth require them to make contact with other roots hence they are hemi parasitic. The trees are mostly allocated on river banks, woodlands and grasslands and on rocky outcrops (De Wet et al., 2012).

2.2 USES OF *XIMENIA CAFFRA* AS A MEDICINAL PLANT

Traditionally it is used in treating abdominal pain, cough and wounds and in some cases sexually transmitted infections and tuberculosis. The powdered roots when put in soup and beer they stimulate sexual desires .The porridge made from the roots treats nausea in pregnancy .The fruits promote clear skin, healthy hair and relief from constipation. The *Ximenia caffra* seed can be used to produce oil which moisturises aging skin and to treat acne because it contains ximenynic acid which stimulates the circulation .It is also ideal for skin serums ,hair care products especially damaged or dry hair and lotion bases(Semenya and Maroyi,2012).

2.3 ESCHERICHIA COLI

E.coli also referred to as *Escherichia coli* is a rod shaped bacteria which is gram negative .The bacteria can either undergo respiration in the presence of oxygen or fermentation in the absence of oxygen making it a facultative aerobe (Idalia and Bernado, 2017) .It is

mainly located in the intestines of healthy humans and animals. However, some of the strains such as STEC result in diarrhoea and stomach pains. Exposure to contaminated water or food especially raw vegetables and undercooked ground beef .However, it can also be acquired through contact with another who has it and also cleaning up after an infected person and not washing hands. Symptoms of *E.coli* include fatigue, loss of appetite or nausea low fever and stomach pains and cramps (Lee and Choi, 2006).

2.4 STAPHYLOCOCCUS AUREUS

S.aureus known as *Staphylococcus aureus* is the one of the important bacteria that is disease causing in humans (Kannappan, 2017).S.aureus is spread to others by contaminated hands. Barriers to infection such as skin and mucous membranes when they are breached through incidents such as skin damage in an accident, it gives *S.aureus* access to underlying tissues and cause infection. *S.aureus* causes diseases such as boils, furuncles and cellatiis.*S.aureus* can also lead to serious infections such pneumonia and joint infections (Rowe et al., 2021).

2.5 ANTIMICROBIAL RESISTANCE

Since ancient times, the use of plants or their products as a source of traditional remedies has remained significant in the treatment of numerous illnesses, including diarrhoea. Notably, the exorbitant expense of contemporary medications and their associated negative effects, particularly antibiotic resistance, have reignited public interest in using traditional treatments. According to reports, traditional medicines are used by 80% of the people in African civilizations for both cultural and financial reasons. Due to this, numerous African nations are making an effort to establish effective regulation and oversight of traditional medicines (Kostakioti, 2013).

Due to their abundance of beneficial bioactive chemical components, plants have maintained their status in conventional disease treatment (Crozier et al., 2006). In plant tissues, bioactive substances build up as secondary metabolites. Extraction methods are frequently used to obtain these metabolites. Some plant components must, however, be completely consumed in order to provide the appropriate treatment. The chemical make-

up of an extract, the solvent employed, and the extraction procedure followed all affect its composition.

With significant morbidity and mortality, diarrheal illnesses continue to be one of the biggest dangers to global public health, particularly in children below the age of 5.Scientists from all around the world have continued to describe or confirm the anti-diarrhoeal capabilities of medicinal plants (Maroyi, 2016). Because they have more health-promoting qualities and less negative effects than conventional treatment, medicinal plants are regarded as the preferred choice.

In order to attain medical satisfaction from plants, long ago they would chew the raw plant roots, leaves and bark. They also inhale the vapour generated from burning some of the medical plant species. In this manner it would result in taking more medication than required and also adverse reactions due to an allergy to a certain tree species (Loizzo, 2009).

As time proceeds and also some genetic changes due to intake or contact with genetically modified organisms it has resulted in the resistance of antibiotics by some of the bacteria. A resistant bacterial infection increases the risk of death compared to a drug-sensitive infection. Methicillin-resistant Staphylococcus aureus (MRSA) and E. coli resistant to third generation cephalosporins (3GC) were found to have about 12.11% and 36.0% resistance, respectively, according to an antimicrobial resistance indicator used in 2019 (Brown and Macgowan, 2010). This resistance has resulted in the extraction of plants for medical purposes in order to reduce serious infections.

Modern day medication is very precise since the efficacy is laboratory approved and it is produced based on tests on sick patients .However, for example in Zimbabwe which is a developing country with low resources ,poor sanitation and inadequate infection prevention and control has given rise to multidrug-resistant microorganisms. Due to continuous reoccurrence of bacterial infections, there is over prescription of antibiotics and the patients are not finishing their courses resulting in antibiotic resistance (Srinivasan, 2021).

CHAPTER 3: METHODS AND MATERIALS

3.1 Study Area

Fresh samples of Ximenia caffra leaves were collected at Greenhill forests (around 17˚18' 06" S, 31˚ 19' 50" E) in Bindura town, Mashonaland Central Province of Zimbabwe.

3.2 Collection and processing of plant materials

During the months from February to March 2023 the leaves were collected. The collected leaves were washed and placed in the sun to dry. After a week of drying, pestle and mortar were used to grind up the dried leaves. The powder of the leaf extract were measured using a balanced and 200g were collected.

3.3 Extraction

Maceration method was used following the method by Tripathi and Pandey (2014). Then 20 g, 15 g and 10 g of powdered leaves were measured and put into conical flask respectively. They were then cold extracted using hexane, ethanol and water separately. The flasks were closed and placed on an orbital shaker for 72 hours, to ensure complete extraction. Whatman filter paper number 1 was used to filter the plant extracts. After filtration, the filtrates were evaporated by means of a water bath until solid precipitates were formed .The extracts were stored under 4˚C until antimicrobial screening. The percentage yield were calculated by the formula:

Percentage yield = mass of extract/original mass x 100

3.4 Bacterial strains

In this antimicrobial activity assessment the bacterial strains were *E.coli* and *S.aureus.* They came from Astra Biology Laboratory's internal pool of strains that were isolated from numerous environmental sources .They were maintained on nutritional agar. Subcultures were created by moving bacteria colonies into test tubes with 10 ml of nutrient broth apiece, where they were allowed to grow for roughly 12 hours at 37 °C..

3.5 Antibacterial screening

For antibacterial test the disc diffusion assay was used. The discs were made by punching holes on a bond paper, the disc were about 2mm in diameter. In a laminar flow cabinet, the extracts were allowed to diffuse for one hour while being kept at room temperature. Mueller Hinton agar was prepared and sterilised for *E.coli* and *S.aureus* agar plates. Using the spread plate the bacterial colonies in the nutrient broth were spread on the agar plates using sterile swabs sticks. Aqueous extract was the negative control whilst alamycin was the positive control. The disc were then put on the prepared plates and incubated at 37˚C for about 24 hours. To measure the antibacterial activity the diameter of zones of inhibition of each treatment was measured using a ruler in millimeters following a method by Das (2014).

3.6 Determination of Minimum Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of the plant extracts, the agar disc diffusion assay was used. Sterile Mueller Hinton agar was prepared following manufacturer's instructions. Concentrations of (0.1; 0.05 and 0.025 g/ml) were prepared for each plant extract .Dilutions were done by dissolving the solid extracts with the respective solvents for each concentration. The Mueller Hinton agar was poured into sterile petri dishes and *E.coli* and *S.aureus* colonies were spread into the plates using a sterile swab stick. Discs (3 mm in diameter) were made for each plate with a puncher and were dipped into the differently concentrated extracts. The diffusion of the extracts was allowed at room temperature for 1 h in a sterile laminar flow cabinet. The discs were later placed on the medias using sterile forceps. The tests were done in triplets. Incubation was done at 37˚C for 24 hours and antimicrobial activity was observed by measuring the zones of inhibition in millimetres using a ruler.

3.7 Identification of phytochemicals in *Ximenia caffra*

Screening of phytochemicals are tests which are carried out in order to determine if there are any primary and secondary metabolites in the extract. The phytochemicals test included testing for flavonoids, reducing sugars, alkaloids, phenols and tannins.

3.7.1 Test for reducing sugars

Benedict's reagent, an alkaline solution comprising cupric citrate solution, was added to 1 ml of the plant extract in a test tube. The mixture was then heated in a water bath. A reddish-brown precipitate is a sign of reducing sugars (Ingle et al., 2017).

3.7.2 Test for phenols

The Ferric chloride test was used. In a test tube 1ml of extract were put followed by 1% gelatin solution containing sodium chloride were added and shaken. Bluish-black color indicates presence of phenols (Doughari, 2019).

3.7.3 Test for alkaloids

The Wagner's test was used.1ml of extract were put in test-tube then 1ml 0f Wagner's reagents (potassium iodide) were also added. Presence of alkaloids is indicated by a reddish brown precipitate (Rungsung et al., 2015).

3.7.4 Test for flavonoids

The Alkaline reagent test was used. In a test tube 1ml extract was added followed by a few drops of sodium hydroxide solution then it was shaken. Immediately after adding dilute acid color change from yellow to colorless implies presence of flavonoids (Sasidharan et al., 2011).

3.7.5 Test for tannins

2ml of aqueous extract solution were placed in a test tube and then a few drops of 0.1 ferric chloride were added. Blue coloration shows presence of tannins (Altemimi et al., 2017).

3.8 Data analysis

The data was subjected to a Statistics Package for the Social Sciences (SPSS) IBM 20, a statistical software for analysis .The data obtained was subjected to a normality test and One Way ANOVA was used to determine the levels of difference among hexane, ethanol and aqueous extracts as antimicrobials on *E.coli* and *S.aureus*.

CHAPTER 4: RESULTS

4.1 Phytochemical screening

This current study shows that in aqueous solution *Ximenia caffra* showed presence of all phytochemicals which are alkaloids, phenols, tannins, reducing sugars and flavonoids; hexane contained alkaloids and reducing sugars whilst ethanolic extracts showed presence of alkaloids, phenols, tannins and reducing sugars (Table 4.1).

Table 4.1 Phytochemical screening for *Ximenia caffra*

Extracts	Aqueous	Hexane	Ethanol
Phytochemical			
Alkaloid	+	+	+
Phenols	+	-	+
Tannins	+	-	+
Reducing sugars	+	+	+
Flavonoids	+	-	-

Key

\+ = Presence

\- = Absence

4.2 Extraction yield

The yields of extraction were expressed as the percentages of initial mass of the sample macerated. Extraction using hexane produced the most yields with the highest percentages, compared to ethanol and water. Ethanol had greater yields than water (Table 4.2).

Table 4.2 Yields of extraction

Solvent	Original mass (g)	Mean mass of extract (g)	Percentage yield (%)
Hexane	20	2.503 ±0.6	12.5
	15	1.440±0.2	9.6
	10	0.801±0.08	8
Ethanol	20	1.708±0.3	8.5
	15	0.905±0.04	6
	10	0.116±0.4	1.16
Distilled water	20	1.008±0.003	5

4.3 Antibacterial screening

The ethanol extracts had a greater inhibition than hexane and aqueous solutions against *E.coli. S.aureus* was greatly inhibited by ethanol than hexane. Alamycin as the positive control had a greater diameter whilst the aqueous solution as the negative control had no inhibition activity. It was measured in millimetres (Figure 4.1).

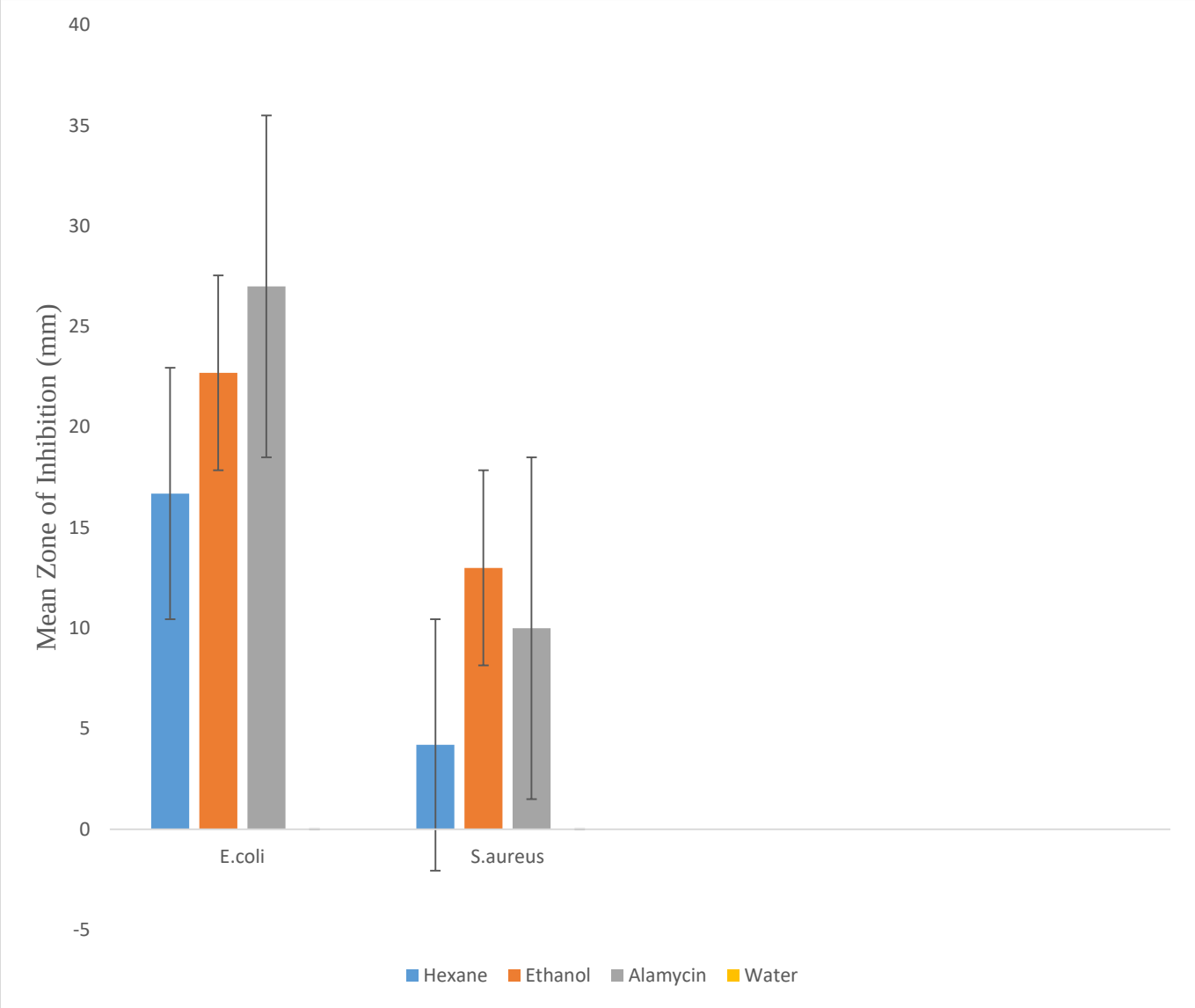

Figure 4.1: Mean zones of inhibition of the ethanol and hexane extracts against *E.coli* and *S.aureus*

4.4 Determination of MIC

Ethanolic extracts at 10mg /ml they showed a greater inhibition for both the *E.coli* and S.aureus than at 5mg/ml .The lowest concentration of 2.5mg/ml inhibited growth of E.coli whilst *S.aureus* growth was supported. For *S.aureus* in all the concentration of hexane extracts there was no inhibitory action whilst at only 10mg/ml zones of inhibition were recorded for *E.coli*. Alamycin for both bacteria they were high zones of inhibition and in the water no zones were observed(Figure 4.2).

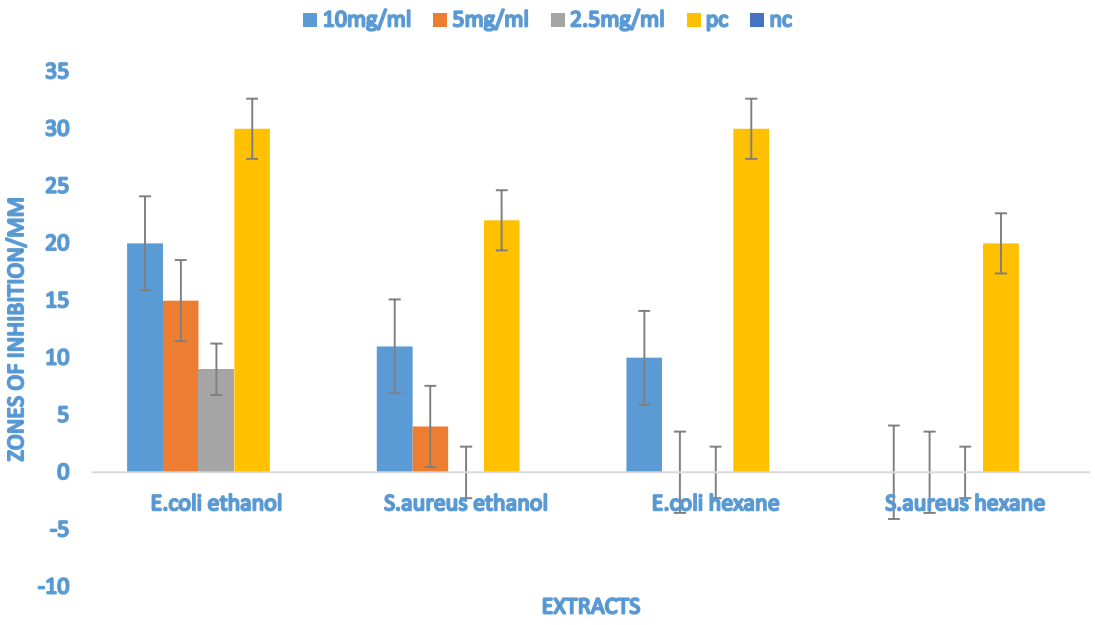

Figure 4.2: Minimum Inhibitory Concentration (MIC) of the ethanol and hexane extracts against *E.coli* and *S.aureus*

Key

pc = positive control

nc = negative control

4.4 Data Analyses

The zones of inhibition were roughly normally distributed for all extracts, according to a Shapiro-Wilk test (p0.05) and visual examination of their histograms, normal Q-Q plots, and box plots showed …….. The F value is 362.098 which reaches significance with a p value of 0.000, which is less than the 0.05 alpha level (Appendix6). This means that there is a statistically significant difference between the means of the different levels of the zone of inhibition variables. A high standard deviation was obtained of 10.498 with 14 as the degrees of freedom.

CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The increase in drug resistance by different microorganisms has led to scientists exploiting different trees for medical purposes. There are many published reports which prove the efficacy of traditional against bacteria. In this study *Ximenia caffra* extracts were analysed for their antimicrobial activity against *E.coli* and *S.aureus (*Ngari et al., 2014).

5.1.1 Phytochemical screening

The results of this study revealed the presence of phenols, reducing sugars, tannins, alkaloids and flavonoids in *Ximenia caffra*. Among the solvents, in distilled water all the phytochemicals mentioned above were present, in ethanol all were present except for flavonoids whilst in hexane alkaloids and reducing sugars were present. These results on the ethanol extract are in agreement with previous studies done by Dzoyem et al.,(2014) ,on the presence of tannins and reducing sugars .These phytochemicals display physiological and medical traits (Richardson, 2015). For instance, alkaloids when it comes to herbivores and pathogens they produce a chemical defence mechanism and most of them are used in production of drugs which treat pain, fever and infections and others such as caffeine and nicotine, have stimulant effects on the nervous system, while others, such as morphine and codeine, have analgesic properties .Polyphenol compounds such as tannins, they bind to proline rich protein which interfere with protein synthesis and they also provide protection against ultra violet radiation (Majiwa et al., 2018). Phenols exhibit antioxidant properties and are also responsible for aroma. Reducing sugars are mostly responsible for energy supply whilst flavonoids help in the reduction of inflammation and also block the growth of tumours. Extracts of *Ximenia caffra* have been shown to possess anti-inflammatory, analgesic, and antidiarrheal activities, which may be due to the presence of tannins, flavonoids, and alkaloids in the plant. It has revealed the presence of various secondary metabolites that may be responsible for the plant's pharmacological properties. Further studies are needed to fully elucidate the pharmacological activities of *Ximenia caffra* and to determine its potential as a source of new drugs. However, the difference in the results with other previous researches may be due to the different locations and conditions in which the plants grew and also the extraction methods differing from researcher to researcher (Revutska, 2015).

5.1.2 Extraction yields

Hexane yielded more extracts than ethanol and water. Hexane had percentage yields of 12.5%, 9.6% and 8%; ethanol had 8.5%, 6% and 1.16% and water had 5%. For this particular plant, hexane tends to be a better solvent than ethanol and water. *Ximenia caffra* leaves compounds may be less polar hence they were able to dissolve in hexane with polarity of about 0.009 other than ethanol with 0.654 and water with 1.000 (Majekodunmi, 2015). However, when it comes to phytochemicals water was able to extract all the phytochemicals other than ethanol and hexane.

5.1.3 Antibacterial activity

The ethanol and hexane extracts of *Ximenia caffra* inhibited the growth of bacteria as shown by this current study on the graph. The growth of *E. coli* was most inhibited as it has mean zones of inhibition of 17mm and 22.2 mm whilst *S. aureus* had 4.2mm and 13mm.For the distilled water, growth of both bacteria was supported whilst in alamycin 27mm was the zone of inhibition for *E. coli* and 10mm was for *S.aureus*. Despite the gram-negative bacteria containing a lipopolysaccharide outer membrane the polarity of the ethanol enabled it to penetrate and block the growth of *E. coli*. According to a journal by Idris et al. on a related study on the assessment of *Ximenia caffra* the results are aligning on the greater inhibition for gram negative than gram positive bacteria which may be due to the compounds in the leaves that highly active against *E. coli*. However, on the related study done by Ejim (2021), for hexane extracts the mean zones of inhibition recorded were 6.60mm and 9.45 mm for *S. aureus* and *E. coli* respectively.

5.1.4 Minimum Inhibitory Concentrations (MIC)

The different extracts were diluted using serial dilutions, in the hexane extract in all concentrations *S.aureus* growth was supported whilst *E.coli* growth was inhibited in the highest concentration. According to Akinpelu and Kolawole (2004), the bacteriostatic quality of hexane extract was exhibited on *E.coli* only and also that it is more effective in higher concentrations. The positive control showed greater inhibition for *E.coli* than *S.aureus*, this might be because alamycin is more effective on gram negative than positive bacteria. However, in the ethanol extracts as concentration increases so as the zones of inhibition increased for both bacteria but *E.coli* having the greatest zones on inhibitions. The active ingredients in the leaves were more effective on *E.coli*.

5.2 CONCLUSIONS

The antimicrobial ability of *Ximenia caffra* leaves was demonstrated by the study above using different solvents. Greater yields of extracts were produced by hexane, this proves that hexane is best for dissolving less polar substances than ethanol and water. The *Ximenia caffra* leaves contains phytochemicals such as tannins, alkaloids ,phenols and reducing sugars which provided defence mechanisms which aided to the inhibition of growth of bacteria .The phytochemical screening of *Ximenia caffra* has showed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, reducing sugars , and phenolic compounds. These compounds are known to have various pharmacological properties, such as antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. The presence of these compounds in *Ximenia caffra* suggests that the plant may have potential as a source of natural products for the development of new drugs or nutraceuticals. However, further studies are necessary to isolate and identify the specific compounds responsible for the observed activities and to determine their mechanisms of action. Overall, the phytochemical screening of *Ximenia caffra* has provided valuable information on the plant's chemical composition and potential bioactivity, highlighting its importance as a medicinal plant and potential source of new natural products for various applications.

The antimicrobial activity property of extracts of *Ximenia caffra* leaves were shown in this study and therefore it is acceptable for it to be considered as an antimicrobial agent. For the best results in bioactive compounds test, water and ethanol are more reliable since they are more polar. It is clear from the above analysis that ethanolic extracts have the greatest inhibitory effect. However, *Ximenia caffra* has proved to be more effective on *E.coli*. An examination with other related studies done before, the differences in methods used contributes to the variances in the outcomes. The assessment of antimicrobial activity for *Ximenia caffra* has shown promising results, indicating that the plant may have potential as a source of natural antimicrobial agents. Several studies have reported that various extracts and compounds derived from *Ximenia caffra* have exhibited significant antimicrobial activity against a range of pathogenic microorganisms, including bacteria, fungi, and viruses. However, it is important to note that further research is needed to fully evaluate the antimicrobial potential of *Ximenia caffra* and to identify the active compounds responsible

for the observed activity. Additionally, studies on the toxicity and safety of *Ximenia caffra* extracts and compounds are necessary to ensure their safe use in humans and animals. Overall, the available evidence suggests that *Ximenia caffra* has potential as a source of natural antimicrobial agents, but further research is necessary to fully evaluate its efficacy and safety.

5.3 RECOMMENDATIONS

Medicinal plants should undergo further tests before use in order to reduce adverse reactions and effects caused by toxins. Since *Ximenia caffra* showed antimicrobial activity, they should undergo clinical tests to observe if they can treat serious related infections. Other extraction methods can also be used to produce more efficient results. Evaluation of the phytochemical properties should be conducted to evaluate its safety as a possible antimicrobial agent. A panel of microorganisms that are relevant to the intended use of the plant extract or compound should be chosen during selection of microorganisms. This may include both Gram-positive and Gram-negative bacteria, fungi, and viruses. During extraction method, several extraction methods have been reported in the literature for Ximenia caffra. Choosing an appropriate extraction method that is suitable for the intended use and that has been shown to yield high yields of bioactive compounds is recommended. Fractionate the crude extract into different fractions using appropriate solvents or chromatography techniques to isolate the active compounds responsible for the observed antimicrobial activity. Using appropriate bioassays to evaluate the antimicrobial activity of the crude extract and the isolated fractions. This may include methods such as the disk diffusion assay, broth micro dilution assay, or time-kill assay. Positive and negative controls: including appropriate positive and negative controls in the bioassay to validate the results and ensure the accuracy of the assay. Overall, a comprehensive approach that includes the selection of appropriate microorganisms, extraction and fractionation methods, bioassays, and determination of MIC and MBC/MFC, as well as investigation of the mechanism of action, can provide valuable information on the antimicrobial activity of *Ximenia caffra* and its potential as a source of natural antimicrobial agents.

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APPENDICES

Appendix 1:Crushing using pestle and mortar

Appendix 2: Extraction using solvents

Appendix 3:Filtration of the extracts

Appendix 4:Zones of inhibition

Appendix 5: Data Analysis

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Maximum	3	20.0000	.20000	.11547	19.5032	20.4968	19.80	20.20
Moderate	3	15.0000	.50000	.28868	13.7579	16.2421	14.50	15.50
Minimum	3	9.0000	1.00000	.57735	6.5159	11.4841	8.00	10.00
PC	3	30.0000	2.00000	1.15470	25.0317	34.9683	28.00	32.00
NC	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	15	14.8000	10.49823	2.71063	8.9863	20.6137	.00	32.00

Appendix 6

Data analysis

The data was subjected to the SPSS software to test for normality .The following were observed,

ANOVA

Diameter in mm

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1532.400	4	383.100	362.098	.000
Within Groups	10.580	10	1.058		
Total	1542.980	14