Journal of Science Research and Reviews
PRINT ISSN: 1595-9074
E-ISSN: 1595-8329
DOI: https://doi.org/10.70882/josrar.2025.v2i5.110
Homepage: https://josrar.esrgngr.org
Original Research Article

Antimicrobial Potential of the Yellow Plum (*Ximenia americana)*: A Qualitative Evaluation of Ethyl Acetate Extracts from the Various Plants' Parts

***[1]Bitrus, Solomon, [2]Shoge, Mansurat O., [1]Agbo, Boniface O., [1]Moses, Simon and [1]Shehu, Genesis M.**

[1]Department of Biological sciences, Faculty of Life Sciences, College of Computing, Engineering & Sciences, Kaduna State University (KASU) PMB 2339 Tafawa Balewa way, Kaduna Northwest, Nigeria.
[2]Department of Chemistry, Air force Institute of Technology, Kaduna Northwest Nigeria.
*Corresponding Author's email: bitrus.solomon@kasu.edu.ng

KEYWORDS
Extracts,
Harness,
Inhibition,
Potential,
Susceptibility,
Bioactive.

CITATION
Bitrus, S., Shoge, M. O., & Agbo, B. O. (2025). Antimicrobial Potential of the Yellow Plum (Ximenia americana): A Qualitative Evaluation of Ethyl Acetate Extracts from the Various Plants' Parts. *Journal of Science Research and Reviews*, *2*(5), 117-126. https://doi.org/10.70882/josrar.2025.v2i5.110

ABSTRACT
Medicinal plants have long been traditionally used as remedies for various ailments which include antimicrobials. *Ximenia americana* which belongs to the Olacaceae family is one of the plants used in traditional medicine in Africa. This study was targeted at assessing qualitatively, the antimicrobial potential of the ethyl acetate extracts from the roots, stem bark and leaves of the plant at different concentrations on selected environmental and clinical samples of bacterial and fungal pathogens using standard susceptibility test procedures. Extracts from the roots, stem bark and leaves of the plant revealed the presence of some phytochemicals ranging from glycosides (saponins and flavonoids), polyphenolics (tannins and steroids) to alkaloids. Alkaloids, flavonoids and steroids were found to be present in the same intensity in all parts while the leaves contained alkaloids, tannins, flavonoids steroids and steroids at varying intensities. The highest susceptibility was recorded from the leaf extracts where three of the test bacterial pathogens (*Salmonella* sp, *Shigella* sp and *Staphylococcus aureus*) showed varying degrees of susceptibilities at 20mg/ml, 25mg/ml, 30mg/ml, 35mg/ml and 40mg/ml. Furthermore, *Staphylococcus aureus* within this category exhibited the highest zone of inhibition (ZI) of 12mm indicating an increase in the disc size of 8mm conforming with Gentamycin (10µg) and Erythromycin (10µg) used as controls from commercially available antibiotic multi disks with ZIs of S (12mm). This study further reveals that the root extracts of the plant had no inhibitory effects on *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* at concentrations of 20mg/ml. 25mg/ml, 30mg/ml, 35mg/ml and 40mg/m. This finding potentially reveals the suitability of X. *americana* plant to be harnessed for its antibacterial potential through a further quantitative, safe and efficient evaluation of the specific bioactive components of the plant parts responsible for each antimicrobial activity as indicated in this study.

INTRODUCTION

It is a common sight especially around the northern part of Nigeria to see local herbal drug sellers hawking extracts of certain plants claiming the products have healing potentials against series of infectious diseases ranging from bacterial, fungal and/or viral infections. There is no doubt that plants have served as the source of constituting different drugs and have being the major source of raw materials in pharmaceutical industries (Ngeiywa *et al.,* 2024). Plants have played and still play a vital role in pharmaceutical sciences, contributing significantly to the development of new medicines and therapies such as providing a reliable source of medicinal compounds providing a rich source of bioactive molecules, such as alkaloids, glycosides, and terpenoids, which are used to develop new drugs; drug discoveries, Pharmacognosy and synthetic biology production of recombinant proteins, vaccines, and other pharmaceuticals (Suresh & Abraham, 2020). Traditionally, plants have been used for centuries in traditional medicine, providing a foundation for modern pharmaceutical research. Plants continue to be a vital source of inspiration and material for pharmaceutical sciences, driving innovation and improving human health (Ngeiywa *et al.,* 2024)

The potentiality of plants cuts across a very wide range of effects as a result of the numerous bioactive compounds present in different variety of plants such as antioxidants, anti-inflammatory, and antimicrobial effects. Plants exhibit vast genetic diversity, providing a rich source of novel compounds and therapeutic leads. The advantages of harnessing plants for their drug potentials can be explained in a number of ways such as; accessibility (Plants are widely available, making them a valuable resource for healthcare, especially in underserved communities); cost-effectiveness (Plant-based treatments can be more cost-effective than conventional therapies) and low toxicity (Plant-based treatments which often have lower toxicity and fewer side effects compared to synthetic drugs and Holistic approach). Plant-based medicine often addresses the whole body, promoting overall wellness and prevention (Sauvêtre, Eichhorn & Pérez, 2021).

In addition to providing raw materials for drug formulations and therapeutics, some plants also serve as sources of nutrients where human beings and animals use to maintain healthy living.

Plants provide essential vitamins such as those in fruits and vegetables which are rich in vitamins A, C, D, E, K, and B vitamins; minerals like calcium, iron, magnesium, potassium, and zinc can be found in many plants; proteins can be found in legumes, beans, and lentils which are excellent plant-based protein sources. Whole grains, fruits, and vegetables are rich in dietary fiber while plants like nuts, seeds, and avocados provide healthy fats, carbohydrates can be found in grains, fruits, and vegetables rich in complex carbohydrates. Most plants have high water content, contributing to hydration and many provide a high amount of nutrients per calorie /nutrient-dense calories (Ounyambila *et al.,* 2018; Bazezew *et al.,* 2021)

Bacterial diseases are illnesses caused by bacterial pathogens, which are single-celled microorganisms that can multiply quickly and cause a range of symptoms. These pathogens are said to be ubiquitous (present everywhere) in the soil, water, air, faecal materials, urine, skin and numerous other discharges and exudates from both animals and plants respectively. The diseases or infection they cause can be described based on the area they localize such as; lungs (e.g., tuberculosis, pneumonia); gastrointestinal tract (e.g., salmonellosis, cholera); skin (e.g., impetigo, cellulitis); urinary tract (e.g., cystitis, pyelonephritis); genital parts (e.g., chlamydia, gonorrhea); bloodstream (e.g., sepsis, bacteremia) and foodborne illnesses (e.g., food poisoning, botulism). The treatment, prevention and control of these diseases require deeper knowledge of the respective causative agent(s), their pathogenicity, virulence and epidemiology (Foning *et al.,* 2022)

Some of these pathogens and their respective diseases are; *Staphylococcus aureus* (causing skin infections/boils, abscesses, respiratory tract infections (pneumonia), food poisoning and toxic shock syndrome); *Streptococcus pneumoniae* (causing pneumonia, meningitis, sinusitis and otitis media/ear infections); *Escherichia coli* commonly known as *E. coli* (causing urinary tract infections, diarrhea, gastroenteritis and pneumonia); *Salmonella sp* (causing food poisoning, gastroenteritis, typhoid fever and paratyphoid fever); *Clostridioides difficile* (causing diarrhea, colitis and pseudomembranous colitis); *Mycobacterium tuberculosis* (causing tuberculosis –TB);

Bordetella pertussis (causing pertussis - whooping cough); *Haemophilus influenzae* (causing meningitis, pneumonia, otitis media-ear infections and sinusitis); *Neisseria gonorrhoeae* (causing gonorrhea); *Neisseria meningitides* (causing meningitis and septicemia); *Chlamydia trachomatis* (causing chlamydia, conjunctivitis and pneumonia); *Legionella pneumophila* (causing legionnaires' disease); *Yersinia pestis* (causing Plague); *Vibrio cholerae* (causing Cholera) and *Campylobacter* sp causing gastroenteritis and diarrhea Sethi & Murphy 2001; MacInnes *et al.,* 2022).

Ximenia americana also known as Dumbi or tsada in Hausa is a plant species commonly found in the woodland savannah regions of Nigeria including Kaduna. It thrives in the woodland savannah ecosystem characterized by tropical climate with high temperatures and distinct wet and dry seasons with well drained soils with a mix of sand, silt and clay. Its vegetation is dominated by grasses, shrubs and scattered trees. In Kaduna, Dumbi has been

used in traditional medicine for various purposes including treatment of fever, rheumatism and indigestion also used in certain skin conditions or infection. These conditions could be caused by different microorganisms such as bacteria, fungi, protozoans and viruses (Fatope *et al.,* 2000).

This study intends to evaluate the antibacterial potential of the different parts of *X. americana* (leaves, stem and roots) which is a plant species native to Africa, commonly known as the "yellow plum". Its fruit, bark, and leaves have been used in traditional medicine for various purposes for the claim on its antimicrobial, antioxidant and anti-dermal infections (skin and hair care) properties amongst many others. Findings from this work will also add and/or contribute to existing knowledge by validating or revalidating such knowledge (MacInnes *et al.,* 2022).

MATERIALS AND METHODS

Plant collection, authentication and extraction

Ximenia americana plant was obtained by digging the roots and pulling them carefully in order to have the whole plant intact. Each plant was cut into different sizes and packaged in polyethene bags and then transported to the Botanical laboratory, Biological Sciences Department where it was identified and authenticated as *Ximenia americana*, family olacacaceae with voucher number 1631 by the Botanist in charge (Adomat and Thomas 2021)

After identification and authentication, the plant was carefully sorted where the leaves, stem, bark and roots were separated and dried under the shade, this was followed by the pulverization with a pestle and mortar. A very fine powder of the leaves, stem, bark and roots was obtained and weighed using the weighing balance (Adomat and Thomas 2021). The soxhlet extraction method was used for the extraction of the phytocompounds of *Ximenia americana* where a ratio of 1:50 (plant powder to ethyl acetate was used). The round bottom flask was connected to the soxhlet apparatus with the condenser attached to the top of the apparatus. The round bottom flask was then filled with 500ml ethyl acetate (solvent) leaving 2cm at the top. 50g of each powdered root, stem and leaf of *X. americana* was placed into the thimble and attached to the apparatus. The inlet and outlets of water of the condenser were securely connected appropriately. The heating mantle was then turned on and set to a temperature 35°C which allowed the solvent to vaporize and rise into the condenser and condensed back into liquid form. The liquid slowly dripped into the thimble extracting the crude phytocomponents of the plant. The rotary evaporator was finally used to remove the solvent from the extract which remained at the bottom of the conical flask (Lopez and Castro 2020).

Phytochemical tests

Test for Alkaloids

Five drops of dragendorff's reagent was added to a portion of each of the crude extracts of *X. americana*. The formation of a reddish-brown precipitate indicated a positive outcome while the absence of a reddish precipitate indicated a negative result (Shubham *et al.*, 2019; Shaikh and Patil 2020)

Test for Tannins

Six drops of 1% gelatin containing sodium chloride was added to each of the of the crude extracts of *X. americana*. A formation of yellowish precipitate indicated the presence of tannins while absence of yellowish precipitate indicated absence of tannins (Shubham *et al.,* 2019)

Test for Flavonoids

Five drops of 10% NaOH was added to a portion of each crude extract of *X. americana*. A yellowish coloration which appeared but cleared away by adding 10% HCl indicated the presence of flavonoids while the absence of a yellowish coloration indicated an absence of flavonoids (Shubham *et al.,* 2019)

Test for Saponins

Five milliliter (5ml) of H_2O was added to a portion of each extract in a test tube and was vigorously shaken. A formation of stable form indicated the presence of saponins while the absence of stable form indicated an absence of saponins (Shubham *et al.,* 2019).

Test for Steroids

A portion of each extract was placed in a test tube followed by an addition of 3ml of chloroform which dissolves the extracts. Three milliliter (3ml) of concentrated H_2SO_4 was added gently through the side of the test tube. The formation of a ring as an interphase between the organic layer and the acid indicated the presence of steroids while the absence of the ring was reported as a negative result. All positive results of the phytochemical screenings in this study were reported in varying intensities of presence using the plus sign (+, ++, +++, ++++) while the negative sign (-) was used to indicate absence of the phytochemical screened (Shubham *et al.,* 2019).

Susceptibility tests

Media Preparation

Nutrient agar (NA) and potato dextrose agar (PDA) were used for the antimicrobial testing of the extracts of *X. americana* plant extracts. The media were prepared according to the manufacturer's instructions where 28g and 39g of NA and PDA respectively were each weighed and dissolved in 1000ml of sterile distilled water in a conical flask. This was loosely sealed with cotton wool wrapped with aluminium foil and autoclaved at 121°C for

15 minutes. This was allowed cool down to 40°C after which they were aseptically poured onto sterile plastic petri dishes and allowed to cool and solidify (Patra, 2020).

Preparation of test organisms

The test organisms (*E. coli, Salmonella typhii, Shigella Staphylococcus aureus, Staphylococcus epidermidis, Aspergillus niger, Aspergillus flavus and Candida sp*) were obtained from the microbiology laboratory and Zoology laboratories of the Departments of Microbiology and Biology respectively. These organisms were sub-cultured to confirm and obtain pure cultures on Eosin methylene blue agar (EMB), *Salmonella Shigella* agar (SSA), Mannitol salt agar (MSA) and potato dextrose agar (PDA). *E. coli* was confirmed by its characteristic green metallic sheen colonies on EMB and gram negative rod stain; oxidase, Voges-Proskauer (VP) negative, while catalase, methyl red (MR) and Indole positive. *Salmonella* sp and *Shigella* sp were confirmed by their characteristic blackish and transparent colonies with Gram negative rods respectively; oxidase, Indole, VP negative and Catalase, MR positive. *Aspergillus niger* was microscopically confirmed following a stain with lacto-phenol cotton blue (LPCB) by their blackish and greenish colonial morphologies on PDA and septate, branched and brown hyphae with a long and thick conidiospores arising from the hyphae. *Aspergillus flavus* was confirmed by their septate, branched, pale yellow hyphae with long thin conidiospores arising from the hyphae following a stain with LPCB. *Candida albicans* was confirmed microscopically with spherical buds and pseudo-hyphal appearance with a bluish colour from the LPCB (Patra *et.al.,* 2020; Mandal and Paul 2022).

The modified Kirby Bauer, (2019) disk surface diffusion technique was adopted to test for the antimicrobial potential of the different extracts of *X. americana* against seven different microorganisms (*S. aureus, S. epidermidis, Salmonella sp, Shigella* sp, *A. niger* and *A. flavus*). Prepared gram –ve multi-antibiotic discs by Maxi care Medical Laboratory, Nigeria (Septrin 30µg; Chloramphenicol 30 µg; Sparfloxacin 10µg; Ciprofloxacin 30 µg; Amoxicillin 30 µg; Augmentin 10 µg; Gentamycin 30 µg; Sparfloxacin 30 µg; Tarivid 10 µg and Streptomycin 30 µg) were obtained and used as positive and negative controls for *S. aureus* and *E. coli* respectively while 10mg of clotrimazole was used as a negative control for the fungal test organism. A disc size of 8mm was aseptically cut using a sterile sharp cork borer. Twenty-four hours cultures of the test organisms were prepared on sterile nutrient broth with turbidity corresponding to the 0.5 McFarland standards. Each test organism was picked with a sterile wire loop (previously flamed on the burner) and aseptically streaked evenly to give a uniform and thin layer of the organism on the NA and PDA. The discs were dipped into each concentration of the extracts and gently placed on the surface of the plates. This was covered and incubated at 37°c for 24hrs. Diameters of zones of inhibitions (ZI) for susceptibilities were measured and recorded against each organism and extract concentration. Interpretation of the results was done based using the guidelines from the Clinical Laboratory Standard Institute (CLSI) as described in CLSI, (2024).

Statistical Analysis

Simple descriptive statistics - Microsoft office 2019 Excel version 16.0 was used to organize and present the overall susceptibility outcome of the different concentrations of the ethyl acetate extracts from the three parts -roots, stem bark and leaves- of the *X. americana* plant.

RESULTS AND DISCUSSION

Results

The outcome of the general bioactive screening done on the ethyl acetate extracts of different plant parts (roots, stem bark and leaves) of *X. americana* indicated the presence of some glycosides (saponins, flavonoids); polyphenols (tannins, steroids) and alkaloids presented in table 4.1. While tables 4.2a, 4.2b and 4.2c indicated the different susceptibility profiles of the extracts from the roots, stem bark and leaves of the plant to selected gram positive (*S. aureus* and *S. epidermidis*) and gram negative bacterial (*E. coli, Salmonella* sp and S*higella* sp) pathogens at different concentrations of 20mg/ml, 25mg/ml, 30mg/ml, 35mg/ml and 40mg/ml respectively. Tables 4.3a, 4.3b and 4.3c indicated the susceptibility profiles of the same *X. americana* plant parts to selected fungal species (*C. albicans*, *A. niger* and *A. flavus*) whereas table 4.4 presented the combined minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) of the different extracts from the three different parts of *X. americana*. Finally Figure 1 shows the overall susceptibility points on the selected microbial pathogens by the ethyl acetate extract from the different parts of *X. americana*.

Table 1: Phytochemical screening outcome of the different parts of *X. americana* plant parts

S/N	PCC	Extracts		
		Roots	Stem bark	Leaves
1	Alkaloids	++++	++++	++++
2	Tannins	+++	++	++++
3	Flavonoids	++++	++++	++++
4	Saponins	++++	++	++++
5	Steroids	++++	++++	++++

KEY: ++++ = Deeply intense; +++ = Intense; ++ = fairly intense; + = poorly intense and PCC= Phytochemical components

Table 2a: Antibacterial susceptibility profile of ethyl acetate root extract of *X. americana* by surface disc diffusion (Disc size: 8mm)

Test Organisms	Zone of Inhibition (mm) Against Concentrations of Extracts in (mg/ml)								
	20	25	30	35	40	Control			
						G +ve		G -ve	
						Drug	ZI	Drug	ZI
Escherichia coli	R(8)	R(8)	S(10)	R(8)	R(8)	CPX	S(16)	PEF	S(16)
Salmonella sp	S(9)	S(10)	S(10)	R(8)	R(8)	OFX	S(14)	RO	S(13)
Shigella sp	R(8)	S(9)	S(9)	R(8)	R(8)	SP	S(14)	SXT	S(11)
Staphylococcus aureus	R(8)	R(8)	R(8)	R(8)	R(8)	GN	S(12)	E	S(12)
Staphylococcus epidermidis	R(8)	S(10)	S(9)	S(10)	R(8)	AM	S(11)	CPX	S(16)

KEY: S- Susceptible/Sensitive; R= Resistant; CPX=Ciprofloxacilin (10µg); OFX=Tarivid (10µg); SP=Sparfloxacin (10µg); GN=Gentamycin (10µg); S=Streptomycin (30µg); E=Erythromycin (10µg); AM=Amoxicillin (30µg); RO=Rocephine; PEF= Peflacilin (10µg); ZI = Zone of inhibition (mm); G-ve = Gram negative; G+ve = Gram positive

Table 2b: Antibacterial susceptibility profile of ethyl acetate stem bark extracts of *X. americana* by Surface disc Diffusion (Disc size: 8mm)

Test Organisms	Zone of Inhibition (ZI) in mm against Concentrations of Extracts in (mg/ml)								
	20	25	30	35	40	Control			
						G +ve		G -ve	
						Drug	ZI	Drug	ZI
Escherichia coli	R(8)	S(10)	S(10)	R(8)	R(8)	CPX	S(16)	PEF	S(16)
Salmonella sp	S(10)	R(8)	R(8)	R(8)	S(10)	OFX	S(14)	RO	S(13)
Shigella sp	R(8)	S(9)	R(8)	S(9)	R(8)	SP	S(14)	SXT	S(11)
Staphylococcus aureus	S(9)	R(8)	S(9)	S(9)	R(8)	GN	S(12)	E	S(12)
Staphylococcus epidermidis	S(10)	R(8)	R(8)	R(8)	R(8)	AM	S(11)	CPX	S(16)

KEY: S- Susceptible/Sensitive; R= Resistant; CPX=Ciprofloxacilin (10µg); OFX=Tarivid (10µg); SP=Sparfloxacin (10µg); GN=Gentamycin (10µg); S=Streptomycin (30µg); E=Erythromycin (10µg); AM=Amoxicillin (30µg); RO=Rocephine; PEF= Peflacilin (10µg); ZI = Zone of inhibition (mm); G-ve = Gram negative; G+ve = Gram positive

Table 2c: Antibacterial Susceptibility profile of ethyl acetate leave extracts of *X. americana* by surface disc diffusion (Disc size: 8mm)

Test Organisms	Zone of Inhibition (ZI) in mm against concentrations of extracts in (mg/ml)								
	20	25	30	35	40	Control			
						G +ve		G -ve	
						Drug	ZI	Drug	ZI
Escherichia coli	S(10)	R(8)	R(8)	R(8)	R(8)	CPX	S(16)	PEF	S(16)
Salmonella sp	S(10)	S(9)	S(9)	S(10)	S(10)	OFX	S(14)	RO	S(13)
Shigella sp	S(10)	S(9)	S(9)	S(9)	S(9)	SP	S(14)	SXT	S(11)
Staphylococcus aureus	S(12)	S(9)	S(9)	S(9)	S(9)	GN	S(12)	E	S(12)
Staphylococcus epidermidis	S(11)	S(10)	R(8)	R(8)	R(8)	AM	S(11)	CPX	S(16)

KEY: S- Susceptible/Sensitive; R= Resistant; CPX=Ciprofloxacilin (10µg); OFX=Tarivid (10µg); SP=Sparfloxacin (10µg); GN=Gentamycin (10µg); S=Streptomycin (30µg); E=Erythromycin (10µg); AM=Amoxicillin (30µg); RO=Rocephine; PEF= Peflacilin (10µg); ZI = Zone of inhibition (mm); G-ve = Gram negative; G+ve = Gram positive

Table 3a: Antifungal Susceptibility profile of ethyl acetate leave extracts of *X. americana* by Surface disc Diffusion (Disc size: 8mm

Test Organisms	Zone of Inhibition (mm) Against Concentrations of Extracts in (mg/ml)					CNT (CMX)
	20	25	30	35	40	10
Candida albicans	R(8)	S(9)	S(9)	S(9)	S(9)	
Aspergillus flavus	R(8)	R(8)	R(8)	R(8)	R(8)	S(14)
Aspergillus niger	S(10)	S(9)	S(9)	S(9)	S(9)	

KEY: CNT=Control; CMX= clotrimazole (10mg)

Table 3b: Antifungal susceptibility profile of ethyl acetate stem bark of *X. americana* by Surface disc Diffusion (Disc size: 8mm)

Test Organisms	Zone of Inhibition (mm) Against Concentrations of Extracts in (mg/ml)					CNT (CMX)
	20	10	30	35	40	10
Candida albicans	S(9)	S(14)	S(9)	R(8)	R(8)	
Aspergillus flavus	S(9)	S(9)	S(9)	S(9)	S(9)	S(14)
Aspergillus niger	S(9)	R(8)	R(8)	R(8)	R(8)	

KEY: CNT=Control; CMX= clotrimazole (10mg)

Table 3c: Antifungal susceptibility profile of ethyl acetate roots of *X. americana* by Surface disc Diffusion (Disc size: 8mm)

Test Organisms	Zone of Inhibition (mm) Against Concentrations of Extracts in (mg/ml)					CNT (CMX)
	20	25	30	35	40	10
Candida albicans	R(8)	R(8)	R(8)	R(8)	R(8)	
Aspergillus flavus	R(8)	R(8)	S(9)	R(8)	R(8)	S(14)
Aspergillus niger	R(8)	R(8)	R(8)	R(8)	R(8)	

KEY: CNT=Control; CMX= clotrimazole 10mg

Table 4: Combined Minimum Inhibitory, Bactericidal and Fungicidal Concentrations (MIC and MBC) of the different extracts of parts of *Ximenia americana* used

Test Organism	MIC(mg/ml)			MBC(mg/m)l			MFC(mg/ml)		
	Root	Stem bark	Leave	Root	Stem bark	Leave	Root	Stem bark	Leave
Escherichia coli	30	25	20	30	30	-			
Salmonella sp	20	20	25	25	40	30			
Shigella sp	25	25	20	30	35	30			
Staphylococcus aureus	NS	20	20	NS	30	25			
Staphylococcus epidermidis	25	20	20	30	-	25			
Candida albicans							NS	25	35
Aspergillus flavus							30	35	NS
Aspergillus niger							NS	20	35

KEY: MIC= Minimum Inhibitory Concentration (mg/ml); MBC= Minimum Bactericidal Concentration (mg/ml); MFC= Minimum Fungicidal Concentration (mg/ml) and NS= No Susceptibility

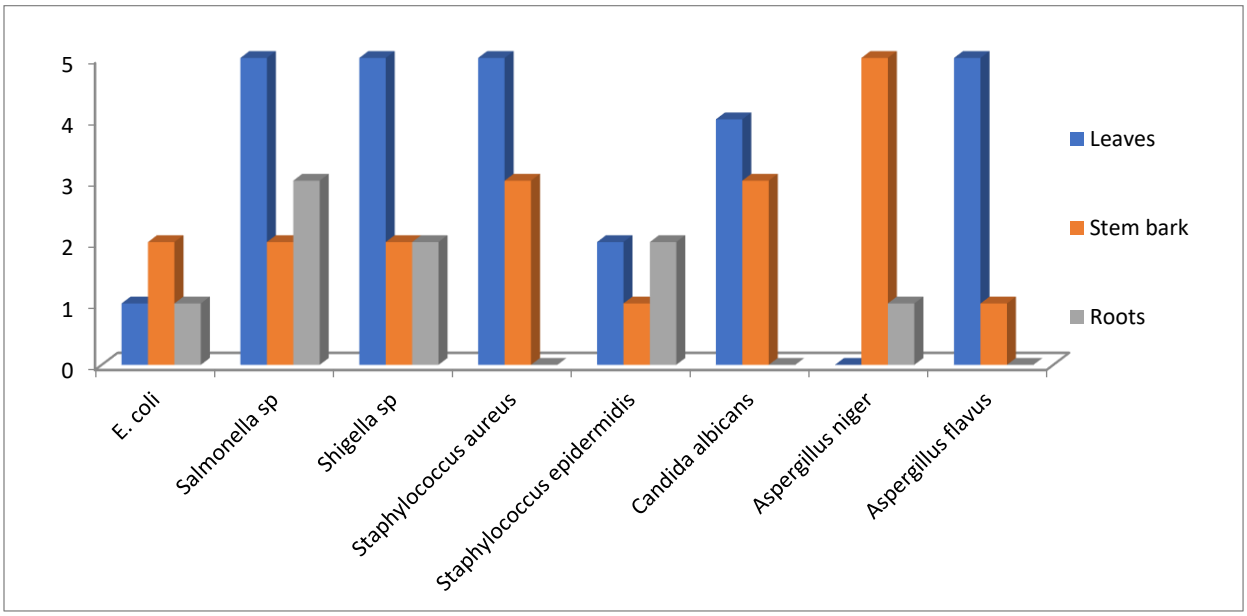

Figure 1: The Overall susceptibility points on the selected microbial pathogens by the Ethyl acetate extracts from the different parts of *X. americana*

Discussion

The outcome of the Ethyl acetate extraction of the roots, stem bark and leaves of *Ximenia americana* showed a yield of 42.75g of crude extract from pulverized dry weight of 342g of the leaves, 11.9g crude from 95g pulverized dry weight from the roots and a 4.7g crude from a 37.4g pulverized dry weight of the stem bark representing an overall 12.5% yield. However, the respective yields from their works differ significantly with this study which could be due to plant age, maturity, environmental factors and solvent purity.

Similarly, five different bioactive components were reported from the screening of *X. americana* plant parts. Extracts from the roots, stem bark and leaves revealed the presence of some phytochemicals ranging from glycosides (saponins and flavonoids), polyphenolics (tannins and steroids) to alkaloids where alkaloids, flavonoids and steroids were found to be present in the same degree in both parts (roots, stem bark and leaves) while the leaves showed presence of alkaloids, tannins, flavonoids steroids and steroids at varying degrees. This is in agreement with the findings of Shetter *et.al.,* (2015); De Menezes *et.al.,* (2019); Mubarak *et al.,* (2022) and Bakrim *et.al.,* (2022) and Dahiru *et.al.,* (2024) where they reported similar phytochemicals were found in the Ethyl acetate extracts of the roots, stem bark and leaves of *X. americana*.

All the bacterial pathogens used in this study showed some form of susceptibility to varying concentrations of the different ethyl acetate extracts of the roots, stem bark and leaves of *X. americana* plant. However, the highest susceptibility was exhibited from the leaf extracts where three of the test bacterial pathogens (*Salmonella* sp, *Shigella* sp, *Salmonella* sp and *Staphylococcus aureus*) showed varying degrees of susceptibility through different zones of inhibitions (ZI) in millimeter from 20mg/ml, 25mg/ml, 30mg/ml, 35mg/ml and 40mg/ml respectively which conforms with the findings of Muhammad *et al.,* (2021) where they reported a significant improvement in laboratory animals treated with different doses of ethyl acetate fractions of the stem bark of *X. americana* after being challenged with 1.5x 10^4 load of *Salmonella typhii*. Furthermore, *Staphylococcus aureus* within this category showed the highest ZI of S (12mm) indicating an increase in the disc size of 8mm which conforms with Gentamycin (10µg) and Erythromycin (10µg) controls of commercially available antibiotic multi disks with ZIs of S (12mm) each. Dahiru, Abaka and Ya'u *et al.,* (2024) reported similar potentiality of *X.americana* to bacterial pathogens in their work on molecular docking, dynamics and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) predictions. Ngeiywa *et al.,* (2024) also reported the efficacious potentiality of *X. americana* to bacterial infectious diseases such as digestive disorders, dysentery, diarrhea and other gastrointestinal diseases which further agrees with the outcome of this study. In contrast, the ethyl acetate leaves extract showed the highest ability to inhibit the bacteria in this study followed by the roots and stem bark extracts which differed slightly with the works of De Menezes *et.al.*, (2019) and Uchoa *et al.,* (2022) attributing differences in extractible bioactive components to solvent type, age of the plant, season of harvest and test organisms used.

Similarly, *Aspergillus niger* and *Aspergillus flavus* were inhibited by all the concentrations (20mg/ml, 25mg/ml, 30mg/ml, 35mg/ml and 40mg/ml) of the leaves and stem bark extracts respectively at ZIs of S (9) showing a weaker comparison to the control (clotrimazole 10µg) which does

not agree with the works of Hassan *et al.,* (2021) which reported a higher activity of *X. americana* extracts against *C. albicans*. This study further reveals that the root extracts of the plant had no inhibitory effects on *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* at concentrations of 20mg/ml. 25mg/ml, 30mg/ml, 35mg/ml and 40mg/ml. Generally, this work shows the minimum bactericidal concentration to be above the inhibitory concentrations indicating that the higher the concentration of the extracts on the potential antigen, the more bactericidal effects it confers. Uchoa *et al.,* (2022) also reported variations in the minimum inhibitory and bactericidal concentrations in their work.

CONCLUSION

The qualitative analyses of the ethyl acetate extracts of *X. americana* plant parts (roots, stem bark and leaves) from this study revealed the presence of bioactive components ranging from glycosides (saponins and flavonoids), polyphenolics (tannins and steroids) to alkaloids at varying degrees. These extracts also showed varying inhibitions on bacterial and fungal pathogens at different concentrations. This finding shows substantially, the suitability of this plant to potentially contain anti-bacterial and anti-fungal properties which can be harnessed and used to develop pharmaceuticals drugs to prevent and treat a variety of chronic diseases. This study presents researchers with insights into the different extractable bioactive components of the parts of *X. americana* and their potentialities, which are important for its exploitation and utilization. Therefore, the possible antimicrobial therapeutic efficacy of *X .americana* is no longer in doubt. It is anticipated that this study will revalidate researchers and herbal medical practitioners to improve on the plant's optimal utilization for its several antimicrobial benefits.

ACKNOWLEDGEMENTS

Our profound gratitude goes to the Management of the Central, Zoology and Microbiological Laboratories of the Kaduna State University, College of Computing, Engineering and Sciences, Faculty of Life Sciences where the phytochemical and microbiological analyses of this work were done. We acknowledge the immense contributions of Mallam Sani Mohammed, Chief Technologist, Microbiology Department and Mr. Benedict David, Senior Technologist, Central Laboratory, Kaduna State University who provided us with the basic technical assistance, isolation and confirmation of the test organisms used in this study. The contribution of Mallam Shehu Gallah, Technologist (Curator), Biological Sciences Department, Kaduna State University in the identification and authentication of the plant used is highly appreciated and acknowledged.

REFERENCES

Aguilera-Correa, J. J., Esteban, J., & Vallet-Regí, M. (2021). Inorganic and polymeric nanoparticles for human viral and bacterial infections prevention and treatment. Nanomaterials, 11(1), 137.

Adomat, Y., & Grischek, T. (2021). Sampling and processing methods of microplastics in river sediments-a review. Science of the Total Environment, 758, 143691.

Adams, J., Olowolafe, E., & Dabi, D. (2024). A Classification and Analysis of Metal Content of some Irrigated Crops in Southern Kaduna, Kaduna State. African journal of geographical sciences, 5(1), 54-63.

Bakrim, W. B., Nurcahyanti, A. D. R., Dmirieh, M., Mahdi, I., Elgamal, A. M., El Raey, M. A., & Sobeh, M. (2022). Phytochemical profiling of the leaf extract of ximenia americana var. caffra and its antioxidant, antibacterial, and antiaging activities in vitro and in Caenorhabditis elegans: a cosmeceutical and dermatological approach. Oxidative Medicine and Cellular Longevity, 2022(1), 3486257.

Bakrim, W. B., Nurcahyanti, A. D. R., Dmirieh, M., Mahdi, I., Elgamal, A. M., El Raey, M. A., & Sobeh, M. (2022). Phytochemical profiling of the leaf extract of ximenia americana var. caffra and its antioxidant, antibacterial, and antiaging activities in vitro and in Caenorhabditis elegans: a cosmeceutical and dermatological approach. Oxidative Medicine and Cellular Longevity, 2022(1), 3486257.

Barrow, P. A. (2021). The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. Journal of Chemical Technology & Biotechnology, 76(7), 677-682.

Bazezew, A. M., Emire, S. A., & Sisay, M. T. (2021). Bioactive composition, free radical scavenging and fatty acid profile of Ximenia americana grown in Ethiopia. Heliyon, 7(6).

Beveridge, T. J. (2021). Use of the Gram stain in microbiology. Biotechnic & Histochemistry, 76(3), 111-118.

Batu, A., Sadiq, O., Dadan-Garba, A., & Namadi, M. M. (2019). Assessment of some chemical contaminants in river Kaduna, Nigeria. African Journals of Earth and Environmental Science, 49-62.

Clinical Laboratory standard Institute, CLSI (2025). Performance Standards for Antimicrobial Susceptibility testing. 34th Edition.

Dahiru, M. M., Abaka, A. M., & Ya'u, I. (2024). Antibacterial Potential of Ximenia americana L. Olacaceae: Molecular Docking, Molecular Dynamics, and ADMET Prediction. Journal of Pharmacy, 4(1), 51-67.

De Menezes I. R. A., Da Costa R. H. S., Augusti Boligon A., (2019). Ximenia americana L. enhances the antibiotic activity and inhibit the development of kinetoplastid parasites.

Comparative Immunology, Microbiology and Infectious Diseases. 2019;64:40–46. https://doi.org/10.1016/j.cimid.2019.02.007. [PubMed] [CrossRef] [Google Scholar]

De Menezes, I. R. A., da Costa, R. H. S., Boligon, A. A., Rolón, M., Coronel, C., Vega, C., ... & Quintans-Júnior, L. J. (2019). Ximenia americana L. enhances the antibiotic activity and inhibit the development of kinetoplastid parasites. Comparative immunology, microbiology and infectious diseases, 64, 40-46.

Dejene, T., Mohamed S. A., Dolores A., Pablo M. P. (2020) Ethnobotanical survey of wild edible fruit tree species in lowland areas of Ethiopia. Forests; 2020:17. [Google Scholar]

Dunachie, S. J., Day, N. P., & Dolecek, C. (2020). The challenges of estimating the human global burden of disease of antimicrobial resistant bacteria. Current Opinion in Microbiology, 57, 95-101.

Deusenbery, C., Wang, Y., & Shukla, A. (2021). Recent innovations in bacterial infection detection and treatment. ACS Infectious Diseases, 7(4), 695-720.

Fatope, M.O., Adoum, O.A. & Takeda, Y. (2000). Oleanene palmitate from Ximenia americana. Pharmaceutical Biology, 38(5): 391–393. https://doi.org/10.1076/phbi.38.5.391.5972.

Foning, L. M., Fombang, É., & Clergé, T. (2022). Evaluation of antioxidant potentialities of whole fruit juices from Ximenia americana Linn., Vitex doniana sweet. and Annona senegalensis Pers.

Gerace, E., Mancuso, G., Midiri, A., Poidomani, S., Zummo, S., & Biondo, C. (2022). Recent advances in the use of molecular methods for the diagnosis of bacterial infections. Pathogens, 11(6), 663.

Hassan, A. U., Madu, A. H., Ozojiofor, U. O., Galadanci, A. H., Mato, I. B., & Jafaru, R. (2021). Antimicrobial activities of Cymbopogon citratus and Ximenia americana leaf extracts against some selected bacterial and yeast clinical isolates. Asian Journal of Biochemistry, Genetics and Molecular Biology, 9(1), 1-10.

Heinzelmann, M., Scott, M., & Lam, T. (2022). Factors predisposing to bacterial invasion and infection. The American journal of surgery, 183(2), 179-190.

Izah, S. C. (2018). Some determinant factors of antimicrobial susceptibility pattern of plant extracts. Res Rev Insight, 2(3), 1-4.

Kirby, B. (2019). Antibiotic Susceptibility . https://bio.librates.org/bookshelves/ancillary_materials/laboratoryexperiment/microbiologylaboratories. Retrieved on 6th January 2024 at 9:36pm

Kefelegn, G. A., & Desta, B. (2021). Ximenia americana: Economic importance, medicinal value, and current status in Ethiopia. The Scientific World Journal, 2021(1), 8880021.

López-Bascón, M. A., & De Castro, M. L. (2020). Soxhlet extraction. In Liquid-phase extraction (pp. 327-354). Elsevier.

Lompo, O., Dimobe, K., Mbayngone, E., Savadogo, S., Sambaré, O., Thiombiano, A., & Ouédraogo, A. (2021). Climate influence on the distribution of the yellow plum (Ximenia americana L.) in Burkina Faso. Trees, Forests and People, 4, 100072.

Mandal, S. M., & Paul, D. (2022). Isolation of Normal Microbiota from the Human Body and Microbial Identification. In Automation and Basic Techniques in Medical Microbiology (pp. 93-121). New York, NY: Springer US.

Martin, B., & Linacre, A. (2020). Direct PCR: A review of use and limitations. Science & Justice, 60(4), 303-310.

Meis, J. F., & Verweij, P. E. (2021). Current management of fungal infections. Drugs, 61, 13-25.

MacInnes, J. I., Van Immerseel, F., Boyce, J. D., Rycroft, A. N., & Vázquez-Boland, J. A. (2022). Pathogenesis of bacterial infections in animals. J. F. Prescott (Ed.). John Wiley & Sons, Incorporated.

Mubarak, M., Dahiru, E. B. B. and Neksumi,M. (2022) Phytochemistry, GS-MS analysis, and heavy metals composition of aqueous and ethanol stem bark extracts of Ximenia americana GSC Biological and Pharmaceutical Sciences, 21(03), 145-156.

Muhammad, H. L., Garba, R., Abdullah, A. S., Muhammad, H. K., Busari, M. B., Hamzah, R. U., & Makun, H. A. (2021). In vivo anti-typhoid and safety evaluation of extracts of Ximenia americana on experimental rats. Pharmacological Research-Modern Chinese Medicine, 1, 100009.

Ngeiywa, M., Rotich, C., Makwali, J., & Wetungu, M. (2024). Medicinal uses and pharmacological activity of Ximenia americana L.-A systematic review. African Journal of Education, Science and Technology, 8(1), 179-200.

Olanrewaju Timothy, O., Odumosu Patricia, O., & Eyong Kenneth, O. (2019). Anti-trypanosomal evaluation of Ximenia americana root bark and chromatographic-mass spectrometric profile. GSC Biological and Pharmaceutical Sciences, 7(2).

Ounyambila, L., Anne M. L., Benjamin L., Amadé, O. (2018) Influence of climate on fruit production of the yellow plum, Ximenia americana, in Burkina Faso, West Africa. Journal of Horticulture and Forestry; 10(4):36–42. https://doi.org/10.5897/jhf2017.0517 . [CrossRef] [Google Scholar]

Patra, J. K., Das, G., Das, S. K., Thatoi, H., Patra, J. K., Das, G., ... & Thatoi, H. (2020). Isolation, culture, and biochemical characterization of microbes. A Practical Guide to Environmental Biotechnology, 83-133.

Sauvêtre, A., Eichhorn, P., & Pérez, S. (2021). Metabolism of pharmaceuticals in plants and their associated microbiota. Interaction and Fate of Pharmaceuticals in Soil-Crop Systems: The Impact of Reclaimed Wastewater, 221-264.

Sethi, S., & Murphy, T. F. (2001). Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. Clinical microbiology reviews, 14(2), 336-363.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2), 603-608.

Shettar, A., Katrahalli, K., Kaliwal, B., Vedamurthy, A. (2015). Evaluation of in vitro antioxidant and anti-inflmmatory activities of Ximenia americana extracts. Asian Pacific Journal of Tropical Disease. 5. 918-923. https://doi.org/10.1016/S2222-1808(15)60957-4.

Shubham, S., Mishra, R., Gautam, N., Nepal, M., Kashyap, N., & Dutta, K. (2019). Phytochemical analysis of papaya leaf extract: screening test. EC Dental Science, 18(3), 485-490.

Suresh, A., & Abraham, J. (2020). Phytochemicals and their role in pharmaceuticals. Advances in Pharmaceutical Biotechnology: Recent Progress and Future Applications, 193-218.

Suwardi, A. B., Navia Z. I., Harmawan T., Syamsuardi, Mukhtar E.(2020) Wild edible fruits generate substantial income for local people of the gunung leuser national park, aceh tamiang region. Ethnobot Research Application; 20:1–13. https://doi.org/10.32859/era.20.11.1 -[CrossRef] [Google Scholar]

Tang, K. W. K., Millar, B. C., & Moore, J. E. (2023). Antimicrobial resistance (AMR). British Journal of Biomedical Science, 80, 11387.

Uchôa, V. T., Rai, M., Carvalho, G. F. S., Sousa, H. G., & da Silva Carneiro, R. (2022). Antimicrobial Activity of Extracts and Fractions of Ximenia americana L.(Olacaceae). In Eco-Friendly Biobased Products Used in Microbial Diseases (pp. 58-69). CRC Press.

Van Wyk, B.-E. & Gericke, N. (2000). People's plants, a guide to useful plants of southern Africa. Briza Publications, Pretoria.

Var, S.K., Hadi, R,. Khardori, N.M. (2019). Evaluation of regional antibiograms to monitor antimicrobial resistance in Hampton Roads, Virginia. Annals of clinical microbiology and antimicrobials. 09: 14-22. Public Medical Centre: 4397712.

Walsh, T. R., Gales, A. C., Laxminarayan, R., & Dodd, P. C. (2023). Antimicrobial resistance: addressing a global threat to humanity. PLoS medicine, 20(7), e1004264.

Yu, T., Jiang, G., Gao, R., Chen, G., Ren, Y., Liu, J., ... & Busscher, H. J. (2020). Circumventing antimicrobial-resistance and preventing its development in novel, bacterial infection-control strategies. Expert Opinion on Drug Delivery, 17(8), 1151-1164.