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Effects of cotton plant (*Gossypium herbaceum* Linn.) extracts on some vancomycin and methicillin-resistant pathogenic bacteria

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Abstract

Bacterial resistance to antibiotics is a serious global problem, hence attention is being turned to plants as alternative source of antimicrobial to combat the menace of antibiotic resistance among pathogenic microorganisms. This study explores the antibacterial properties of *Gossypium herbaceum* leaf extracts against a spectrum of antibiotic-resistant bacteria such as *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, sourced from clinical isolates at Don Bosco Catholic Laboratory and Obafemi Awolowo University Teaching Hospital in Nigeria. The research utilized cold extraction methods with methanol and n-hexane solvents to prepare the extracts, which were then analyzed for their phytochemical composition, revealing the presence of flavonoids, steroids, tannins, terpenoids, and glycosides. Antibacterial activity was assessed using the disc diffusion method, with the extracts showing varying zones of inhibition; n-hexane extracts produced zones between 21.35 mm and 4.10 mm, while methanol extracts showed zones between 18.75 mm and 5.05 mm at a concentration of 200mg/mL. Minimum inhibitory concentrations (MIC) were determined, with n-hexane extracts ranging from 25 mg/mL to 6.25 mg/mL and methanol extracts from 50 mg/mL to 6.25 mg/mL. Further analysis of the bioactive fractions was conducted through Fourier Transform Infrared Spectroscopy (FTIR), which identified six distinct bioactive compounds in the methanol extract, including alkenes, alcohols, amines, phenols, nitro compounds, and alkanes. Plasmid profiling indicated the presence of plasmids in clinically significant pathogens like *P. aeruginosa* and *S. aureus*, suggesting a potential mechanism for their antibiotic resistance. Overall, the study highlights the significant antibacterial activity of *G. herbaceum* extracts against resistant bacteria and suggests their potential as alternative antimicrobial agents in combating antibiotic resistance, which is a growing global health concern.

Keywords: *Gossypium herbaceum*; Antibacterial Properties; Antibiotic Resistance; Fourier Transform Infrared Spectroscopy (FTIR); Plasmid Profiling

1. Introduction

Bacterial and fungal pathogens are some of the etiological agents of human infections (Negi et al., 2012) which have raised concern in the healthcare field over the years, particularly those which have adopted resistance towards antimicrobial agents (Chuah et al., 2014). Antibiotics discovery and clinical use is undoubtedly one of the pillars of modern medicine (Andrei et al., 2018). They are considered as miracle drugs that have saved the lives of millions of people from many serious microbial diseases and eased human pain and suffering for decades. However, on the other side, scientists ignored the fact that microorganisms have acquired a wonderful metabolic power and survived millions of years on our changing earth, even with the administration of antibiotics (Bérdy, 2012). Microorganisms have successfully evaded the effects of antibiotics through developed antibiotic resistance (Abdallah, 2016).

Antimicrobial resistance poses an escalating global health crisis, diminishing the efficacy of established therapies and elevating the risk associated with infections, particularly those caused by bacteria that have acquired resistance to

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multiple drug classes. This phenomenon represents a significant barrier to the continued success of antibiotic treatments, which have historically played a pivotal role in managing infectious diseases and reducing associated morbidity and mortality. The emergence of formidable pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB), and carbapenem-resistant *Enterobacteriaceae* (CRE) highlights the urgent need for new antimicrobial strategies. These organisms have evolved mechanisms that render them resistant to nearly all available antimicrobial agents, posing severe challenges to public health.

In response to the diminishing returns from current antibiotic discovery and development pipelines, which have failed to keep pace with the spread of resistance, there is an imperative to explore alternative sources of antimicrobial agents. Plant-derived compounds, with their diverse pharmacological activities, offer a promising reservoir of novel bioactive molecules. This study focuses on the potential of *Gossypium herbaceum* leaf extracts to combat antibiotic-resistant pathogens. Utilizing advanced phytochemical techniques, this research aims to isolate and characterize the compounds within these extracts that exhibit antibacterial activity. *Gossypium herbaceum*, commonly known as cotton plant has been reported as traditional medicine plant with the unique properties like antifertility (Garratt et al., 2001), antispermatogenic, antitumor (Lee and Lin 1998), abortifacient, contraceptive (Al-Snafi, 2018), antidiabetic, antiviral (Sharma et al., 2005) and antibacterial activity (Agarwal et al., 2012). It has also been reported in the treatment of tooth pain (Hebber et al., 2004). Our choice of this plant is based on its prevalence in the South western part of Nigeria and its common usage in delicacies and local medications.

Hence, this research will specifically isolate and identify pathogenic bacteria that are resistant to vancomycin and methicillin from clinical samples; examine the plasmid content of these bacteria to understand the genetic basis of their resistance; evaluate the antibacterial efficacy of *G. herbaceum* extracts against these pathogens; and perform detailed phytochemical profiling to determine the active constituents of the extracts. The outcomes of this study are expected to provide valuable insights into the potential of *Gossypium herbaceum* as a source of new antimicrobial agents, contributing to the broader efforts to mitigate the impact of antibiotic resistance on global health.

2. Materials and Methodology

2.1. Collection of *Gossypium herbaceum* Plant and Clinical Isolates

Fresh, mature leaves of *Gossypium herbaceum* Linn. (cotton) were harvested from their natural environment in Aponmu, Ondo State, during November and December of 2017. Post-collection, the plant was authenticated at the Department of Crop, Soil, and Pest Management at The Federal University of Technology, Akure. The leaves were then air-dried at room temperature until thoroughly dried. Clinical isolates from various sources, including urine, wounds, semen, stool, sputum, and high vaginal swabs (HVS), were sourced from the stock culture of Don Bosco Catholic Laboratory in Akure, Nigeria, and the Microbiology and Parasitology Department at Obafemi Awolowo University Teaching Hospital in Ile-Ife, Nigeria.

2.2. Culture, Isolation and Identification of Bacteria from Clinical Isolates

Media were prepared as per the manufacturer's instructions. Specifically, nutrient agar (NA) was prepared by dissolving 2.8 grams in 100 mL of distilled water, while Mueller Hinton agar (MHA) involved dissolving 3.8 grams in 100 mL of distilled water. All media were sterilized at 121°C for 15 minutes.

2.2.1. Biochemical Tests

A 22-hour-old bacterial culture was used to prepare a smear on a grease-free glass slide, which was air-dried. The slide underwent staining with crystal violet for 60 seconds, washed, treated with Lugol's iodine, decolorized with absolute alcohol, and counterstained with safranin. Post-staining, the slide was observed under a microscope using a 100x oil immersion objective. **Catalase test** was performed according to the method reported in Fawole and Oso (2007). Briefly, colony from 24 hours old bacteria culture was mixed with 3% hydrogen peroxide on a slide, and the presence of oxygen bubbles (effervescence) indicated catalase activity. A colony from 24-hour old culture was emulsified in normal saline on clean grease free slide and an equal volume of plasma was added and mixed together aseptically. Clumps or precipitate in the mixture indicates a positive **coagulase test**, this shows that the organism produces coagulase enzyme while the absence of clumps gives a negative result (Fawole and Oso, 2007).

The **Oxidase test** was performed as described in Fawole and Oso (2007). Briefly, a piece of filter paper was placed in a sterile petri dish and 2-3 drops of freshly prepared oxidase reagent was added. Using a sterile inoculating loop, a colony

of the test bacterium was picked and smeared on the filter paper and was observed for 10 seconds. The presence of blue-purple color indicates a positive oxidase while no blue-purple color indicates a negative oxidase test.

The **Indole test** was performed according to the method described in Olutiola et al. (2000). After incubating the culture in tryptone water for five days, Kovac's reagent was added. A deep red color indicated **indole presence**. A **motility test** involved inoculating sterilized nutrient broth with the bacteria and observing under a microscope after 24 hours of incubation.

The fermentation test and McFarland Turbidity standard to measure the density of the bacteria cells were conducted according to the method described in Cheesbrough (2006).

2.3. Antibiotic Susceptibility Test

Each bacterial isolate was cultured in separate tubes containing Mueller-Hinton broth (Oxoid) at 37°C for 16-18 hours with agitation. The cultures were then diluted to achieve an optical density of 0.1, equivalent to 0.5 McFarland Standard, at a wavelength of 625 nm and stored at 4°C. Susceptibility testing was performed following the protocols of the Clinical and Laboratory Standards Institute (CLSI, 2017). Antibiotic disks, each containing 500 µg/mL of Vancomycin and Methicillin, were used to assess the susceptibility of the bacterial isolates. The disks were applied to the inoculated agar plates using sterile forceps and pressed down gently yet firmly to ensure contact. The plates were then incubated at 37°C for 24 hours, after which the zones of inhibition were measured and interpreted using the 2017 CLSI guidelines.

2.4. Plasmid Analysis of Bacterial Isolates

2.4.1. Plasmid Extraction

A single bacterial colony was incubated in 5 ml of Muller-Hinton broth for 24 hours, then centrifuged at 10,000 rpm for 2 minutes. The cell pellet was resuspended in 150 µl of EDTA-Tris buffer and vortexed. Next, 175 µl of 2% SDS and 0.4N NaOH were added, followed by 250 µl of cold 5M potassium acetate. After centrifugation at 12,000 rpm for 5 minutes, the supernatant was transferred to a new tube, mixed with an equal volume of cold isopropanol, and centrifuged at 12,000 rpm for 10 minutes. The pellet was washed with 650 µl of cold 70% ethanol, air-dried for 30 minutes, and resuspended in 40 µl of sterile deionized water.

2.4.2. Agarose Gel Electrophoresis

Agarose gel was prepared by dissolving 0.8 g in 100 ml of 1X TBE, microwaved for 3 minutes, cooled, then mixed with 10 µl of ethidium bromide. The gel was set in an electrophoresis tank, covered with TBE buffer, and loaded with samples and a DNA ladder. Electrophoresis ran at 80 V for 1 hour, and DNA fragments were visualized under UV light.

2.4.3. Plasmid Curing

To determine if drug resistance markers were plasmid-borne or chromosomal, each isolate was cultured with 0.01 ml of acridine orange for 24 hours. Cured cultures (10 µl aliquots) were inoculated into fresh nutrient broth and incubated for 24 hours at 37ºC. Antibiotic sensitivity was tested to assess the location of resistance markers.

2.5. Leaf extractions and Phytochemical Quantifications

Dried leaves from *G. herbaceum* from were finely ground using an industrial grinder, and 500 g of the resulting powder was measured out. This was combined with two liters of methanol and mixed intermittently using a magnetic stirrer. After resting for 72 hours in a sealed container, the mixture was stirred again and then filtered using muslin cloth and Whatman filter paper. The filtrate was left to evaporate at room temperature to yield the solid crude extract, which was then stored in sealed plastic containers at 4 °C for subsequent use in antimicrobial activity screenings (Handa et al., 2008). Extracts were purified by Thin Layer Chromatography as described by Manik et al., 2017.

The phytochemical composition of the methanol and n-hexane extracts from *G. herbaceum* Linn. leaves were analyzed both qualitatively and quantitatively using established methods (Evans, 2002; Sofowora, 2008). Phytochemicals determined include tannin, phlobatannin, anthraquinone, flavonoids, steroid, terpenoid and cardiac glycosides.

2.6. Antibacterial Assay of *Gossypium herbaceum* Leaf Extracts on Test Organisms.

The antibacterial activity of *G. herbaceum* leaf extracts was evaluated using the agar well diffusion method. Bacterial isolates were cultured in nutrient broth at 37°C to a density equivalent to 0.5 McFarland standard (approx. 1.5 x 10^8 cfu/ml). The media plates were inoculated with these cultures and allowed to stabilize for 40 minutes before wells were

created using an 8 mm sterile cork borer. The extracts, diluted to 200 mg/mL in 20% Tween-20, were added to the wells, with 20% Tween-20 serving as a negative control, and vancomycin and methicillin as positive controls. After incubating at 37°C for 24 hours, zones of inhibition were measured to determine the bacterial sensitivity to the extracts.

For the determination of Minimum Inhibitory Concentration (MIC), the extracts were tested at varying concentrations from 200 mg/mL to 3.125 mg/mL, again using the agar well diffusion method. Mueller Hinton agar plates were seeded with the test organisms, and the extracts were introduced into wells on the plates. The MIC was identified as the lowest concentration that inhibited bacterial growth after 24-hour incubation at 37°C.

3. Results

3.1. Morphological and Biochemical Characteristics of Isolates

The characteristics of clinical bacterial isolates, derived from various sources such as stool, urine, high vaginal swabs, semen, wounds, and sputum, are detailed in Table 1. These include *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Acinetobacter baumannii,* and *Salmonella typhi.*

Table 1 Morphological characteristics and biochemical characteristics of the bacterial isolates

Cultural Characteristics		**Organism**							
	A	B	C	D	E	F	G	H	I
Color	Pale yellow	Creamy	Mucoid white	Creamy	Creamy	Green	Pale yellow	Creamy	Green
Edge	Entire	Entire	Entire	Lobate	Lobate	Entire	Entire	Entire	Entire
Elevation	Raised	Flat	Convex	Flat	Flat	Flat	Raised	Conves	Raised
Surface	Smooth	Rough	Smooth	Rough	Rough	Smooth	Smooth	Rough	Smooth
Shape	Cocci	Rod	Cocci	Rod	Rod	Rod	Rod	Cocci	Cocci
Gram reaction	±	-	±	-	-	+	±	+	+
Catalase	±	±	±	±	±	±	±	-	-
Coagulase	±	-	±	-	-	-	-	+	+
Oxidase	-	-	-	-	-	±	-	-	+
Indole	-	±	-	-	±	-	-	-	+
Motility	-	±	-	±	±	±	-	-	-
Sugar Fermentation									
Glucose	AG	AG	AG	AG	AG	AG	AG	A	A
Galactose	AG	AG	AG	-	-	A	AG	-	AG
Sucrose	A	A	AG	-	AG	-	AG	-	AG
Lactose	A	AG	A	-	-	-	-	A	AG
Mannitol	AG	AG	AG	AG	-	AG	AG	AG	AG

Keys: A-Staphylococcus aureus, B- Escherichia coli, C- Streptococcus pnuemoniae, D- Salmonella typhi, E- Proteus mirabilis, F- Pseudomonas aeruginosa, G- Acinetobacter baumannii,; H- *Streptococcus faecalis, I- Streptococcus pyogenes.;* A- acid, G- gas, - (negative), ± (positive).

3.2. Antibiotics Sensitivity Testing

Table 2 documents the antibiotic sensitivity of Gram-positive bacteria; some strains of *S. aureus* were susceptible, while all strains of *Streptococcus faecalis* and *Haemolytic streptococcus* were resistant.

Table 2 Antibiotics sensitivity screening on Gram positive bacteria

Bacteria	SOURCE	VANCOMYCIN	METHICILLIN
Staphylococcus aureus	ECS	10.00±1.00b	11.50±0.70c
Staphylococcus aureus	HVS	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	URINE	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	SEMEN	12.00±0.57c	13.50±0.50d
Staphylococcus aureus	ASPIRATES	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	SEMEN	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	SWAB	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	ECS	11.00±1.00b	4.50±0.50b
Staphylococcus aureus	WOUND	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	EAR SWAB	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	URINE	0.00±0.00a	0.00±0.00a
Streptococcus pyogenes	SPUTUM	0.00±0.00a	0.00±0.00a
Streptococcus pyogenes	SEMEN	0.00±0.00a	0.00±0.00a
Streptococcus pyogenes	HVS	0.00±0.00a	0.00±0.00a
Streptococcus faecalis	SFA	0.00±0.00a	0.00±0.00a
Streptococcus faecalis	Sputum	0.00±0.00a	0.00±0.00a

Data are presented as Mean ± S.E (n=3). Values with the same letter(s) along the same column are not significantly different (P<0.05). **Keys:** ECS-Endocervical swab, SFA-Seminal Fluid Analysis and HVS- High Vaginal Swab.

Table 3 covers the sensitivity of Gram-negative bacteria; *Pseudomonas aeruginosa* from semen, *Proteus mirabili*s from urine, and *Escherichia coli* from urine were susceptible. However, most strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumannii, and Salmonella typhi* exhibited resistance. Resistant strains, both Gram-positive and Gram-negative, were selected for further screening.

Table 3 Antibiotics sensitivity of Gram-negative bacteria

Bacteria	SOURCE	VANCOMYCIN	METHICILLIN
Klebsiella pneumoniae	Urine	0.00±0.00a	0.00±0.00a
Klebsiella pneumoniae	Sputum	0.00±0.00a	0.00±0.00a
Klebsiella pneumoniae	Wound	0.00±0.00a	0.00±0.00a
Klebsiella pneumoniae	Csf	0.00±0.00a	0.00±0.00a
Pseudomonas aeruginosa	Urine	0.00±0.00a	0.00±0.00a
Pseudomonas aeruginosa	Wound	0.00±0.00a	0.00±0.00a
Pseudomonas aeruginosa	Semen	11.50±0.50b	5.50±0.50b
Proteus mirabilis	Sputum	0.00±0.00a	0.00±0.00a
Proteus mirabilis	Urine	0.00±0.00a	0.00±0.00a
Proteus mirabilis	Urine	11.50±0.50b	15.00±1.00d
Escherichia coli	Urine	0.00±0.00a	0.00±0.00a
Escherichia coli	HVS	0.00±0.00a	0.00±0.00a

Escherichia coli	Urine	11.50±1.00b	14.00±1.00c
Escherichia coli	Semen	0.00±0.00a	0.00±0.00a
Escherichia coli	Wound	0.00±0.00a	0.00±0.00a
Escherichia coli	Stool	0.00±0.00a	0.00±0.00a
Escherichia coli	ECS	0.00±0.00a	0.00±0.00a
Acinetobacter baumannii	Urine	0.00±0.00a	0.00±0.00a
Salmonella typhi	Stool	0.00±0.00a	0.00±0.00a

Data are presented as Mean±S.E (n=3). Values with the same letter(s) along the same column are not significantly different (P<0.05). **Keys: CSF: Cerebrospinal Fluid, ECS: Endocervical Swab, HVS: High Vaginal Swab.**

3.3. Plasmid Profile

Table 4 shows the two methods of extraction (the conventional and the kit method) usedto test for the presence of plasmid in the antibiotic resistant bacteria. The conventional method revealed the presence of plasmid in *Pseudomonas aeruginosa* alone while the kit method revealed the presence of plasmid in both *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Table 4 Plasmid Profile of Test Organism

Organism	Method of Extraction	Nucleic Acid (ng/µl)	Unit	260/280	260/230	Sample Type	Factor
S. aureus	Conventional	20.4	1.3	0.5	DNA	50	No
S. pyogenes	Conventional	16.9	1.19	0.46	DNA	50	No
P. aeruginosa	Conventional	28.3	1.4	0.63	DNA	50	Yes
P. mirabilis	Conventional	29	1.43	0.55	DNA	50	No
S. faecalis	Conventional	15.4	1.16	0.44	DNA	50	No
Salmonella typhi	Conventional	30	1.36	0.54	DNA	50	No
K. pneumonia	Conventional	20.1	1.16	0.42	DNA	50	No
E. coli	Conventional	27.5	1.29	0.47	DNA	50	No
S. aureus	Kit	22.4	1.32	0.55	DNA	50	Yes
S. pyogenes	Kit	20.6	1.29	0.46	DNA	50	No
P. aeruginosa	Kit	34	1.38	0.65	DNA	50	Yes
P. mirabilis	Kit	27.7	1.43	0.54	DNA	50	No
S. faecalis	Kit	18.8	1.2	0.43	DNA	50	No
Salmonella typhi	Kit	26	1.35	0.56	DNA	50	No
K. pneumonia	Kit	21	1.17	0.42	DNA	50	No
E. coli	Kit	17.4	1.12	0.42	DNA	50	No

3.4. Phytochemical Screening of *Gossypium herbaceum* Leaf

Figure 1 indicates the detection of tannins, flavonoids, steroids, terpenoids, and cardiac glycosides in both methanol and N-hexane leaf extracts of *Gossypium herbaceum* Linn. Conversely, compounds such as saponins, phlobatannins, anthraquinones, and alkaloids were absent in both extracts. The methanol extract exhibited a higher concentration of these phytochemicals overall, with the exception of terpenoids, which were more abundant in the N-hexane extract.

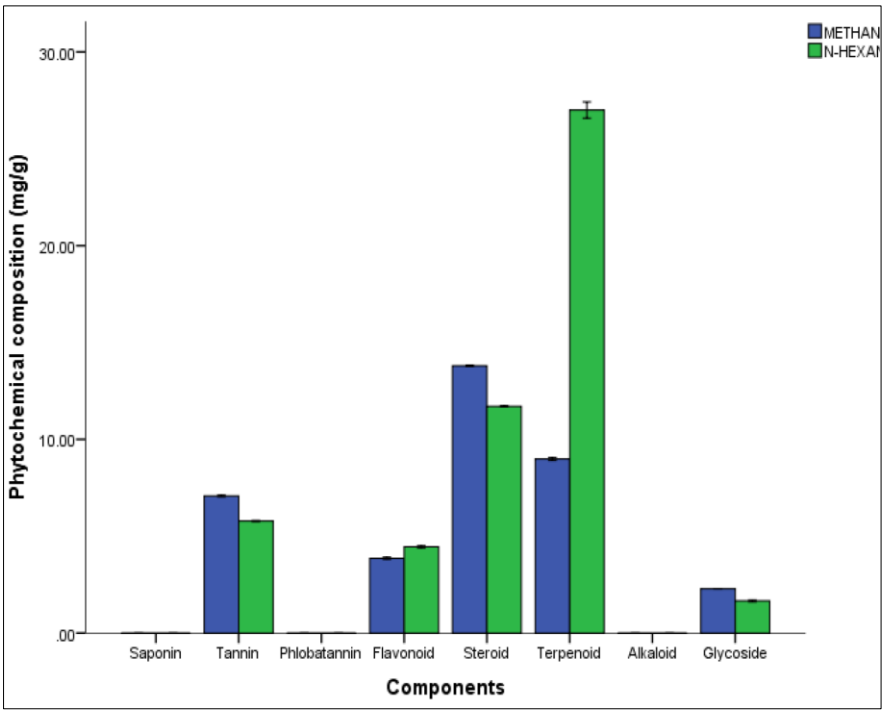

Figure 1 Phytochemical composition of *Gossypium herbaceum* leaf extracts

3.5. Antibacterial Susceptibility Pattern of *G. herbaceum* Leaf Extracts on Bacterial Isolates

Tables 5 and 6 illustrate the antibacterial susceptibility of resistant bacterial isolates, revealing the methanol extract to be more effective than the n-hexane extract. For the methanol extract, *S. aureus* from urine was the most susceptible Gram-positive bacterium, showing a 9.65±0.35 mm zone of inhibition at 200 mg/mL and a MIC of 50 mg/mL. Conversely, *Streptococcus pyogenes* was the least susceptible, with an 18.45±0.55 mm zone at 200 mg/mL and a MIC of 6.25 mg/mL, exhibiting an 8.60±0.06 mm zone. Among the Gram-negative bacteria, *Proteus mirabilis* from urine was the most susceptible with a 5.05±0.95 mm zone at 200 mg/mL and a MIC of 100 mg/mL. P. aeruginosa from urine and E. coli from urine followed, with zones of 18.75±0.25 mm and 18.25±0.75 mm at 200 mg/mL, respectively, and MICs at 25 mg/mL and 6.25 mg/mL, respectively.

For the n-hexane extract, *Streptococcus faecalis* was the most susceptible Gram-positive bacterium with a 4.10±0.11 mm zone at 200 mg/mL and a MIC at 50 mg/mL. *S. aureus* from semen was the least susceptible, with a 19.20±0.80 mm zone at 200 mg/mL and a MIC of 6.25 mg/mL. *Acinetobacter baumanii* showed no susceptibility to the n-hexane extract. For Gram-negative bacteria, *Proteus mirabilis* was the most susceptible with an 8.60±0.40 mm zone at 200 mg/mL and a MIC at 50 mg/mL, while *E. coli* from urine was the least susceptible with a 21.35±0.65 mm zone at 200 mg/mL and a MIC at 6.25 mg/mL.

Table 5 Antibacterial Effect of *G.herbaceum* Methanol Leaf Extract on the Test Organisms

Bacteria	Sources	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL
Staphylococcus aureus	Urine	9.65±0.35^{b}	6.65±0.35^{b}	4.50±0.05^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
S. aureus	Semen	15.15±0.85^{f}	14.65±0.35^{f}	12.70±0.30^{f}	9.45±0.55^{b}	8.65±0.50^{b}	6.85±0.45^{b}	0.00±0.00
Streptococcus faecalis	Semen	13.15±0.85^{d}	12.95±0.05^{e}	11.65±0.35^{e}	9.50±0.50^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
Streptococcus pyogenes	HVS	18.45±0.55^{g}	16.40±0.60^{g}	15.65±1.55^{g}	14.30±0.70^{d}	11.60±0.40^{d}	8.60±0.06^{d}	0.00±0.00
Salmonella typhi	Stool	11.20±0.80^{c}	9.25±0.75^{c}	8.55±0.45^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
Klebsiella pneumoniae	Urine	12.10±0.90^{d}	11.65±0.35^{d}	10.20±0.80^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
Acinetobacter baumannii	Urine	15.10±0.90^{f}	11.20±0.80^{d}	9.25±0.75^{cd}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
Proteus mirabilis	Urine	5.05±0.95^{a}	2.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
P. aeruginosa	Semen	14.25±0.75^{e}	11.50±0.50^{d}	9.00±1.00^{cd}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
P. aeruginosa	Urine	18.75±0.25^{g}	16.20±0.80^{g}	15.25±0.75^{g}	12.10±0.90^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00
E. coli	Urine	18.25±0.75^{g}	16.30±0.70^{g}	15.25±0.75^{g}	12.00±1.00^{c}	9.35±0.65^{c}	7.75±0.25^{c}	0.00±0.00

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Table 6 Antibacterial Effect of *G. herbaceum* N-hexane Leaf Extract on the Test Organisms.

Bacteria	Source	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL
Staphylococcus aureus	Urine	11.50±1.50^{f}	2.00±0.00^{b}	1.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
S.aureus	Semen	19.20±0.80^{i}	17.20±0.80^{e}	16.22±0.77^{f}	14.76±0.24^{c}	11.22±0.77^{c}	7.75±0.24^{c}	6.70±0.30^{b}
Streptococcus faecalis	Semen	4.10±0.11^{b}	2.00±0.00^{b}	1.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
Streptococcus pyogenes	HVS	18.50±0.50^{h}	16.00±1.00^{e}	12.00±1.00^{e}	11.25±0.75^{b}	9.40±0.60^{b}	6.70±0.30^{b}	0.00±0.00
Salmonella typhi	Stool	10.50±0.50^{e}	7.50±0.50^{cd}	6.25±0.75^{d}	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
Klebsiella pneumoniae	Urine	10.20±0.80^{e}	8.55±0.45^{d}	5.60±0.40^{c}	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
Acinetobacter baumannii	Urine	0.00±0.00^{a}	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
Proteus mirabilis	Urine	8.60±0.40^{c}	6.70±0.30^{c}	3.60±0.40^{b}	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
P. aeruginosa	Semen	9.25±0.75^{d}	7.20±0.80^{cd}	1.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
P. aeruginosa	Urine	12.25±0.75^{g}	9.70±0.30^{d}	5.70±0.30^{c}	0.00±0.00a	0.00±0.00a	0.00±0.00	0.00±0.00
E. coli	Urine	21.35±0.65^{j}	19.35±0.65^{f}	17.75±0.25^{g}	14.10±0.90^{c}	11.25±0.75^{c}	7.70±0.30^{c}	0.00±0.00

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

3.6. Antibacterial Susceptibility Pattern of Isolates To Purified *G.herbaceum* Leaf Extracts

The antibacterial susceptibility pattern of resistant Gram-positive bacterial isolates to purified Gossypium herbaceum leaf extracts is depicted in Figure 2a. Eight purified fractions were tested against *Staphylococcus aureus, Streptococcus faecalis, and Streptococcus pyogenes*. None of the fractions exhibited activity against *S. aureus* and *S. faecalis* showed susceptibility to five fractions (F3H, F1H, F2M, F2H, and F4M), while only fraction 1 from the n-hexane extract was active against *S. pyogenes.* Figure 2b illustrates the antibacterial susceptibility pattern of resistant Gram-negative bacterial isolates to methanol and n-hexane fractions. Eight purified fractions were tested against *Salmonella typhi, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus mirabilis, Pseudomonas aeruginosa,* and *Escherichia coli*. None of the fractions demonstrated activity against *S. typhi, K. pneumoniae, A. baumannii,* and *P. aeruginosa*. Four fractions (F1M, F1H, F2M, and F4H) inhibited *P. mirabilis*, while two fractions (F1M and F1H) inhibited *E. coli*.

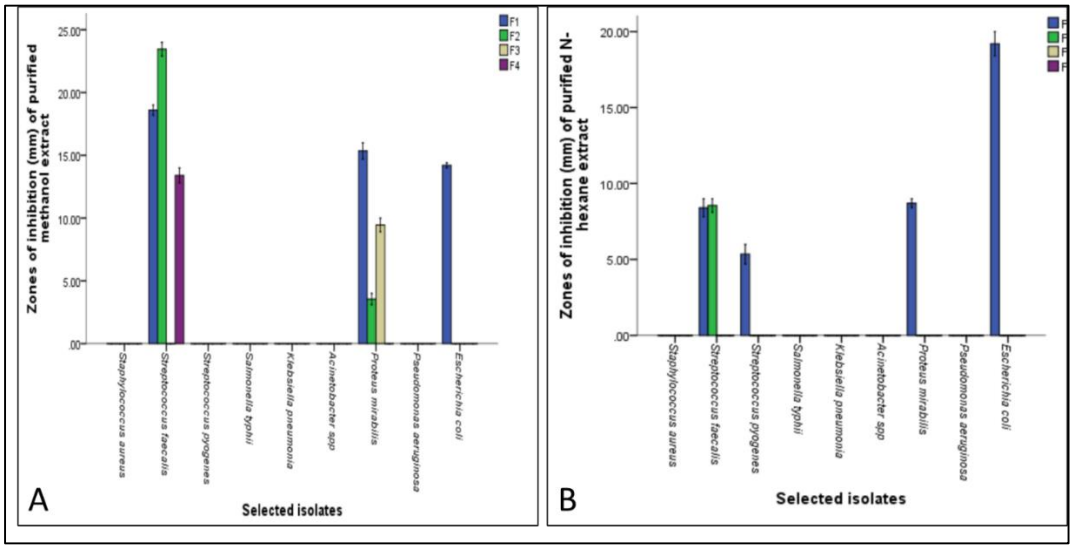

Figure 2 Antibacterial susceptibility pattern of antibiotic resistant bacterial isolates to purified A. methanol fractions and B. n-hexane fraction. Bars represent percentage ± standard error. Significant difference was taken at (P ≤ 0.05) according to Duncan's New Multiple Range tests.

Key: F=Fraction.

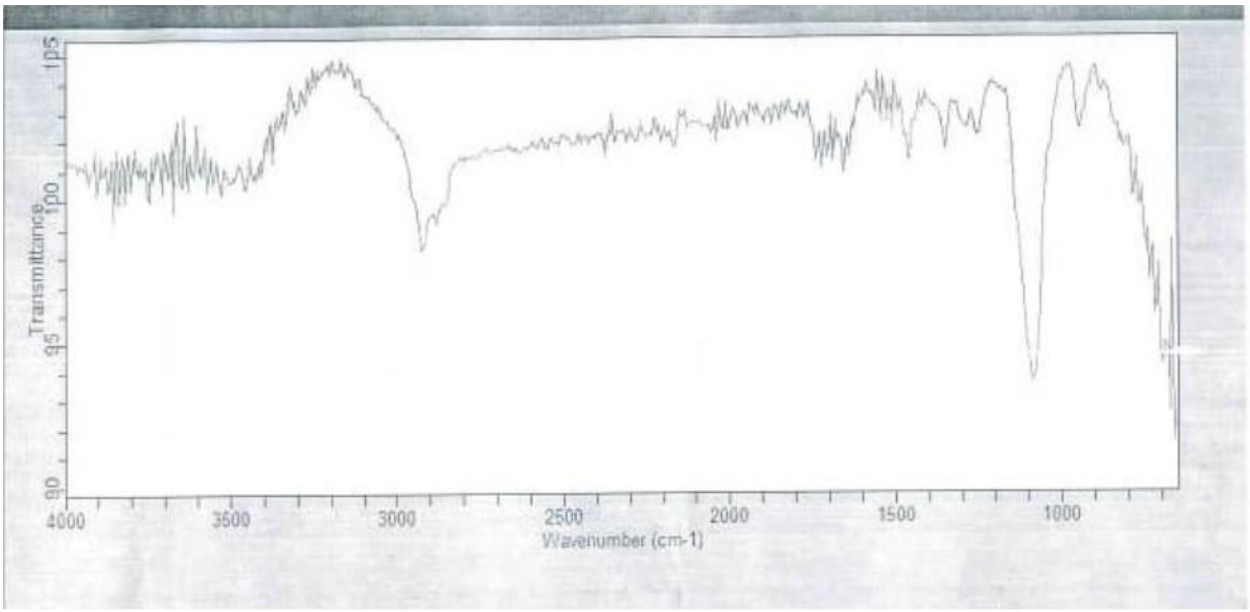

Figure 3 Infrared spectrum of the methanol fraction

4. Discussion

This study examined the antibacterial properties of Gossypium herbaceum extracts against vancomycin and methicillin-resistant pathogens such as Staphylococcus aureus and Escherichia coli, highlighting their prevalence in clinical samples (Muluye et al., 2014; Abera and Kibret, 2011). The findings demonstrate that while the bacterial isolates exhibited resistance to conventional antibiotics, they varied in susceptibility to the methanol and n-hexane extracts of G. herbaceum, with methanol extract showing greater efficacy.

Particularly, none of the extracts were effective against *S. aureus*, but *S. faecalis* was susceptible to several fractions (F3H, F1H, F2M, F2H, and F4M). In contrast, only one n-hexane fraction was active against *S. pyogenes*. The methanol extract, especially, demonstrated higher inhibitory rates against Gram-positive bacteria compared to Gram-negative ones, possibly due to differences in cell wall structures which affect permeability of the extracts (Kakad et al., 2015).

Furthermore, the study confirmed the presence of significant bioactive compounds such as tannins, flavonoids, and terpenoids in the extracts, known for their antimicrobial properties (Mehta et al., 2017; Al-snafi, 2018; Monika and Mishra, 2018). Plasmid analysis indicated resistance mechanisms in Pseudomonas aeruginosa and Staphylococcus aureus were likely not plasmid-mediated, suggesting intrinsic resistance factors at play (Gill et al., 2016; Ashwag, 2017). Overall, the concentrated methanol extract demonstrated greater bacteriostatic activity, suggesting potential for therapeutic applications. This aligns with previous research indicating the effectiveness of phytochemical-rich plant extracts in combating resistant bacterial strains (Senthilkumar and Reetha, 2009; Yousef et al., 2018). This underscores the importance of G. herbaceum as a valuable source of medicinal compounds with potential in treating infections resistant to standard antibiotics.

5. Conclusion

In conclusion, this study has highlighted the significant antibacterial activities of methanol and n-hexane leaf extracts from Gossypium herbaceum Linn., underscoring their potential as alternative antibacterial agents. The effectiveness of these extracts against a range of antibiotic-resistant pathogens suggests that the bioactive compounds within the plant, such as flavonoids, phenolics, tannins, and steroids, contribute to its antimicrobial properties. These findings support the use of *G. herbaceum* extracts in the development of new treatments for infections that do not respond to conventional antibiotics.

Recommendations

There should be more enlightenment on the use of antibiotics only after laboratory test has confirmed infections to be of bacterial origin. This is to reduce the rate of antibiotics resistance of bacteria as a result of indiscriminate use of antibiotics.

The potential of *G. herbaceum leaf* as antiviral, antifungal, anticancer as well as an antidiuretic, antihypertensive and tranquilizing agent should be further evaluated.

Toxicity studies should be carried out on the crude active fractions to establish their safe levels for use by humans. This should help in the calculation of the safety dosage required for treatment of bacterial infections. The dosage if calculated would help in improving the safety of *G. herbaceum* leaf based herbal drugs for bacterial infection treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Abbasi-Montazeri, E., et al. (2013). The prevalence of methicillin-resistant Staphylococcus aureus (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. Burns, 39: 650–654.

[2] Abdallah, E.M. (2016). Medicinal plants as an alternative drug against methicillin-resistant Staphylococcus aureus. International Journal of Microbiology and Allied Sciences, 3(1): 35-42.

[3] Abdelbasset, M. and Djamila, K. (2008). Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk "Raïb". African Journal of Biotechnology, 7(16), pp. 2908-2914, ISSN 1684–5315.

[4] Abera, B. and Kibret, M. (2011). Bacteriology and antimicrobial susceptibility of otitis media at Dessie Regional Health Research Laboratory, Ethiopia. Ethiop J Health Dev., 25(2): 161-167.

[5] Adnan, S. J. (2015). Antimicrobial Resistance Pattern and Plasmid Profile of Some Salmonella spp. Isolated from Clinical Samples in Riyadh Area. European Scientific Journal. ISSN: 1857 – 7881.

[6] Adwan, G. and Mhanna, M. (2008). Synergistic Effects of Plant Extracts and Antibiotics on Staphylococcus aureus Strains Isolated from Clinical Specimens. Middle-East Journal of Scientific Research, 3(3): 134-139, ISSN 1990-9233.

[7] Agarwal, B.B., et al. (2012). Identification of novel anti-inflammatory agents from Ayurvedic Medicine for Prevention of Chronic Diseases: "Reverse Pharmacology" and "Bedside to Bench" Approach. Curr Drug Targets, 12(11): 1595–1653.

[8] Agarwal, P., et al. (2016). Antibacterial Activity of Plants Extracts against Methicillin-Resistant Staphylococcus aureus and Vancomycin-Resistant Enterococcus faecalis. J Microb Biochem Technol, 8: 404-407. doi: 10.4172/1948-5948.1000316.

[9] Aja, A.M., et al. (2002). Plasmid profiling and antibiotic resistance of Vibrio strains isolated from cultured penaeid shrimp. FEMS Microbiol. Lett., 213: 7-12.

[10] Akhtar, N., Ali, M., and Alam, M.S. (2005). Herbal drugs used in dental care. The Pharma Review, 10: 61-68.

[11] Akiyama, H., et al. (2001). Antibacterial action of several tannins against Staphylococcus aureus. Journal of Antimicrobial Chemotherapy, 48(4): 487-491.

[12] Al-Snafi, A.E. (2018). Chemical constituents and pharmacological activities of Gossypium herbaceum and Gossypium hirsutum. IOSR Journal of Pharmacy www.iosrphr.org (e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219 Volume 8, Issue 5 Version. I, PP. 64-80.

[13] Aminov, R.I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. Front Microbiol., 1:134.

[14] Andrei, S., et al. (2018). New FDA approved antibacterial drugs: 2015-2017. Discoveries January-March; 6(1); e81. DOI:10.15190/d.2018.1.

[15] Andrew, D. (2015). 6 Factors That Have Caused Antibiotic Resistance. https://infectoncontrol.tips/2015/11/8.

[16] Ashokkumar, R., and Ramaswamy, M. (2013). Determination of DPPH free radical scavenging oxidation effects of methanolic leaf extracts of some Indian medicinal plant species. Journal of Chemical, Biological and Physical Sciences, 3(2):1273-1278.

[17] Ashwag, S. (2017). The role of mobile genetic elements in the Emergence of superbugs. American-Eurasian Network for Scientific Information. ISSN: 1995-0772 EISSN: 1998-1090.

[18] Babu, U., et al. (2010). Antibiotic Sensitivity and Plasmid Profiles of Escherichia coli Isolated from Pediatric Diarrhea. J Glob Infect Dis., 1(2): 107–110.

[19] Ben, E. and Ziv, M. (2004). Complementary medicine and psoriasis: linking the patient's outlook with evidence-based medicine. Int J Dermatol, 43(7):552.

[20] Bérdy, J. (2012). Thoughts and facts about antibiotics: Where we are now and where we are heading. The Journal of Antibiotics, 65: 385–395.

[21] Berry, P.E. (2012). Malvaceae. Encyclopædia Britannica Online. Encyclopædia Britannica Inc.

[22] Boucher, H., Miller, L.G., and Razonable, R.R. (2010). Serious Infections Caused by Methicillin-Resistant Staphylococcus aureus. Clinical Infectious Diseases, 51(S2): S183–S197.

[23] Bruniera, F.R., et al. (2015). The use of vancomycin with its therapeutic and adverse effects: a review. European Review for Medical and Pharmacological Sciences, 19: 694-700.

[24] Brunner, J.H. (1994). Direct spectrophotometer determination of saponin. Animal Chemistry, 34: 1314-1326.

[25] Buricova, L. and Reblova, Z. (2008). Czech medicinal plants as possible sources of antioxidants. Czech Journal of Food Science, 26: 132-138.

[26] Chambers, H. F. (2010). Antimicrobial agents: Protein Synthesis Inhibitors and miscellaneous antibacterial agents. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics 11th edition. pp. 1074-1077.

[27] Chatterjee, A. and Pakrashi, S.C. (2006). The treatise on Indian medicinal plants. Vol.2, National Institute of Science Communication and Information Resources, New Delhi: pp.177-178.

[28] Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, UK., ISBN-13:9781139449298.

[29] Cheng, G., et al. (2016). Antimicrobial Drugs in Fighting against Antimicrobial Resistance. Front Microbiol., 2016;7:470.

[30] Christenhusz, M. and Byng, J. (2016). The number of known plant species in the world and its annual increase. Phytotaxa, 261: 201–217.

[31] Chuah, E. L., et al. (2014). Antimicrobial Activities of Plant Extracts against Methicillin-Susceptible and Methicillin-Resistant Staphylococcus aureus. Journal of Microbiology Research, 4(1): 6-13.

[32] CLSI (Clinical and Laboratory Standards Institute). (2017). Performance Standards for Antimicrobial Susceptibility Testing.

[33] Cox, J.A., et al. (2017). Antibiotic stewardship in low- and middle-income countries: the same but different? Clinical Microbiology and Infection., 23(11): 812-818.

[34] Dehority, W. (2010). Use of vancomycin in pediatrics. Pediatr Infect Dis J, 29: 462-464.

[35] Demilie, T., et al. (2012). Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in Northwest Ethiopia. Ethiop J Health Sci., 2012;22:121–128.

[36] Edeoga, H.O., Okwu, D.E., and Mbaebie, B.O. (2005). Phytochemical Constituents of Some Nigerian Medicinal Plants. Afr. J. Biotechnol., 4: 685-688.

[37] Esimone, C.O., et al. (2012). Antimicrobial activity of Psidium guajava Linn. stem extracts against methicillin-resistant Staphylococcus aureus. African Journal of Biotechnology, 11(89): 15556-15559.

[38] Evans, W.C. (2002). Trease and Evans Pharmacognosy. Elsevier, India. 15th edition. pp. 137-393.

[39] Farnsworth, N.R., et al. (1985). Medicinal Plants in Therapy. Bull WHO, 63(5).

[40] Fawole, M.O. and Oso, B.A. (2001). Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan, Nigeria.

[41] Feng, Y., et al. (2013). Recent Progress on Anticancer Candidates in Patents of Herbal Medicinal Products. Recent Patents on Food, Nutrition & Agriculture, 30-48.

[42] Friedman, N.D., Temkin, E. and Carmeli, Y. (2016). The negative impact of antibiotic resistance. Clin Microbiol Infect., 22: 416–422.

[43] Garratt, L.C., et al. (2001). Haemoglobin-Stimulated growth and antioxidant activities in cultured cotton cells. Free Radic Biol Med, 31: 1156-1162.

[44] Gibbons, S. (2005). Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochemistry Rev., 4: 63-74.

[45] Giedraitiene, A., et al. (2011). Antibiotic resistance mechanisms of clinically important bacteria. Medicina (Kaunas), 47: 137–46.

[46] Gill, S., et al. (2016). "Prevalence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant Pseudomonas aeruginosa from a tertiary level Intensive Care Unit." Journal of Global Infectious Diseases, 8(4): 155-159.

[47] Gladwin, M. and Trattler, B. (2004). Clinical Microbiology Made Ridiculously Simple. 3rd edition. Miami: MedMaster, Inc.

[48] Gledhill, D. (2008). The Names of Plants. 4th edition. Cambridge: Cambridge University Press; p.182.

[49] Gonzalez, C., et al. (1999). "Bacteremic pneumonia due to Staphylococcus aureus: a comparison of disease caused by methicillin-resistant and methicillin-susceptible organisms." Clin Infect Dis., 29(5): 1171–7.

[50] Graham, D. (2009). Intellectual Property Rights and the Life Science Industries: Past, Present and Future. World Scientific. pp. 140. ISBN 978-981-283-227-6.

[51] Gujjeti, P.R. and Mamidala, E. (2013). Phytochemical Analysis and TLC Profile of Madhuca indica Inner Bark Plant Extract. International Journal of Scientific & Engineering Research, 4(10): 1507-1510.

[52] Gupta, A., et al. (2011). Vancomycin nephrotoxicity: myths and facts. Neth J Med, 69: 379-383.

[53] Hamburger, M. and Hostettmann, K. (1991). Bioactivity in Plants: The Link between Phytochemistry and Medicine. Phytochemistry, 30: 3864-3874.

[54] Handa, S. S., et al. (2008). Extraction Technologies for Medicinal and Aromatic Plants. (1st edn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology.

[55] Harborne, J. B. and Williams, C. A. (2007). Advances in flavonoid research since 1992. Phytochemistry, 55: 481-504.

[56] Hebber, S. S., et al. (2004). Ethnomedicine of Dharwad district in Karnataka, India; Plants used in oral health care. Journal of Ethnopharmacology, 94:261

[57] Huttner, A., et al. (2013). Antimicrobial resistance: A global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrobial Resistance and Infection Control.

[58] Iwu, M. M., Duncan, A. R. and Okunji, C. O. (1999). New antimicrobials of plant origin. In Janick J (ed). Perspectives on New Crops and New uses. ASHS Press, Alexandria, VA, pp. 457-462.

[59] Jeyaprakash, K. (2007). Herbal therapy for depression. Herbal Tech Industry, 3(7):19-25.

[60] Kakad, S. L., Tungikar, V. V., and Dhembare, A. J. (2015). Evaluation of antibacterial activity of plant extracts against bacterial pathogens. Der Pharmacia Sinica, 2015, 6(1):1-5.

[61] Kalyani, P. and Ratna, J. (2013). Department of Pharmaceutical Sciences, Dibrugarh University Dibrugarh, Assam, India. International Journal of Herbal Medicine, 1(3): 86-89.

[62] Kamboj, V.P. (2000). Herbal medicine. Current Science, 78(1):35-39.

[63] Khaleequr, R., Arshiya, S. and Shafeequr, R. (2012). Gossypium herbaceum Linn: An Ethnopharmacological Review. J Pharm Sci Innov., 1(5): 1-5.

[64] Koche, D., Shirsat, R., Syed, I. and Bhadange, D. G. (2010). Phytochemical screening of eight ethnomedicinal plants from Akola District (MS). International Journal of Pharma and Bioscience, 1(4):256-259.

[65] Koche, D., Shirsat, R. and Kawale, M. (2016). An overview of Major Classes of Phytochemicals: Their Types and Roles in Disease Prevention. Hislopia Journal, 9(1):1-11.

[66] Krishnanda, P.I., et al. (2007). Phytochemicals: Extraction Methods; Identification and Detection of Bioactive Compounds from Plant Extracts. Journal of Pharmacognosy and Phytochemistry, 6(1): 32-36.

[67] Lanfranco, G. (1992). Popular Use of Medicinal Plants in the Maltese Islands. Insula, 1: 34 – 35.

[68] Lee, R. and Lin, J.Y. (1998). Antimutagenic Activity of extracts of anticancer drugs in Chinese medicine. Mutation Research, 204(2): 229-234.

[69] Lewis, D.A. (1989). Anti-inflammatory drugs from plants and marine sources. Basel: Bikhauser Verlag.

[70] Li, B. and Webster, T. J. (2018). Bacteria Antibiotic Resistance: New Challenges and Opportunities for Implant-Associated Orthopaedic Infections. J Orthop Res., 36(1): 22–32. doi:10.1002/jor.23656.

[71] Low, D. T. (2009). Smart talk on supplements and botanicals. Alternative and Complementary Therapies, 15:101-102.

[72] Luepke, K. H., et al. (2017). Past, present, and future of antibacterial economics: increasing bacterial resistance, limited antibiotic pipeline, and societal implications. Pharmacotherapy, 37: 71–84.

[73] Mahady, G. B. (2005). Medicinal plants for the prevention and treatment of bacterial infections. Curr Pharm Des, 11:2405-2427.

[74] Maisonneuve, E. and Gerdes, K. (2014). Molecular mechanisms underlying bacterial persisters. Cell, 157: 539–48.

[75] Mama, M., Abdissa, A. and Sewunet, T. (2014). Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University specialized Hospital, South West Ethiopia. Ann Clin Microbiol Antimicrob., 13:14. doi:10.1186/1476-0711-13-14.

[76] Manik, S., Abid, R. and Mehak, S. (2017). Phytochemical Screening and Thin Layer Chromatography of Ficus car