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Exploring Nature's Antibiotics; In-vitro antibacterial evaluation of *Psidium guajava* and *Gymnema sylvestre*

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Article Details

ABSTRACT

Keywords: Antibiotic Resistance, *Psidium* The present study was designed to evaluate the antibacterial activity of water, *guajava*, *Gymnema sylvestre*, Inhibitory Zone, methanol, chloroform and petroleum ether extracts of *Psidium guajava* and Plant Extracts, Solvents

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Gymnema sylvestre on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* by using agar well diffusion method. Both plant extracts showed considerable activity against gram positive and gram negative bacteria. Water extract of *Psidium guajava* showed good activity against gram positive bacteria and the methanolic extract against gram negative bacteria while chloroform and petroleum ether extracts have only activity against gram positive bacteria but less than water and methanolic extracts which indicated that its polar component have stronger activity against both gram positive and gram negative bacteria than non polar. *Gymnema sylvestre* extracts also showed activity against both gram positive and gram negative bacteria except water extract which showed no activity against the studied bacteria. The methanolic extract had stronger activity against gram-negative bacteria than chloroform and petroleum ether extracts and had no activity against *Salmonella typhi* at this concentration but had activity against *Escherichia coli* but weaker than chloroform and petroleum ether extracts. This indicated that its polar component (methanolic extract) had good activity against gram positive than gram negative bacteria. While chloroform and petroleum ether extracts have stronger activity against gram negative than gram positive bacteria. This indicated that its non-polar components have stronger activity against gram negative bacteria.

INTRODUCTION

Antibiotic and multi-drug resistance is a worldwide problem in hospitals, long-stay residential centers and in the community (Livermore, 2000). This evolution is generally attributed to the extensive use of antibiotics and selective pressure on the bacterial strains. Bacteria are not only able to acquire resistance through mutation, but also through plasmid spread via different strains. In recent years, there have been a number of responses by national, international and professional bodies, many aimed at curbing this unprecedented growth in resistance, but there is an increasing recognition that a major problem in the management of drug resistant infections is the lack of new drugs, vaccines and diagnostic aids (Finch and Hunter, 2006). Partly this is due to the fact that pharmaceutical companies no longer find this area financially rewarding, while smaller biotechnology companies, normally considered more innovative, are hampered by a lack of funding. Nevertheless, a significant number of new therapeutics is derived from natural sources including plants, as novel drugs (systemic and topical) and antiseptics, to replace or to be used in conjunction with existing products. In the US, regulatory requirements are a barrier to the development of phytotherapeutics and the US pharmaceutical industry prefers the use of synthetic or semi synthetic compounds (Duke, 1993). On the other hand, globally, there is a patient-driven trend towards “natural” remedies. The use of natural medicines has increased by 20% a year since the 1990s in the US and the sale of non-prescribed herbal medicines is estimated at 4 billion dollars per year. In contrast, in Europe and Asia, the regulations are less restricting and in countries such as Japan, the number of natural product patents has surged in recent years. Therefore, there is a clinical need, compliant end-users and appropriate commercial interest in Europe for development of new products from plants to be a viable option.

Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine (Cragg and Newman, 2002). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments. Drug plants that have been of historic importance for centuries include *Opium*, *Physostigma venenosum* (Eserine), *Cinchona bark* and *cocaine* etc. (Trease and Evans., 1991).

With the advancement of science, orthodox medicine was born and the idea of resorting to traditional medicine seemed to vane. However, in many rural settings, scarcity of hospitals or health care units, unaffordability of orthodox medications and inability of these medicaments to provide effective treatment to some human diseases have compelled the rural population to depend on traditional and herbal medicines for their healthcare.

Today according to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases (Azaizeh *et al.*, 2003). Due to less communication means, poverty, ignorance and unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day ailments. Most of these people from the poorest link in the trade of medicinal plants (Khan, 2002). A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al.*, 1999).

In the developed countries, 25% of the medical drugs are based on plants and their derivatives (Principe, 1991). A group of World Health Organization (WHO) experts, who met in Congo Brazzaville in 1976, sought to define traditional African medicine as 'the sum total of practices, measures, ingredients and procedures of all kinds whether material or not, which from time immemorial has enabled the Africans to guard against diseases, to alleviate his/her suffering and to cure him/herself. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future (PEI SJ, 2001).

As a general rule, a given microorganism differs in its resistance to various spices and herbs; bacteria are more resistant than fungi. The effect on spores may be different than that on vegetative cells and Gram-negative bacteria are more resistant than gram-positive bacteria. Moreover, the effect of a spice or herb may be inhibitory or germicidal (Zaika, 1988).

Plants have many different substances like Alkaloids, Tannins, Resins, Flavonoids, Essential oils, Fixed oils, Glycosides, and Steroids etc. These substances have different polarities and different range of antimicrobial activity. The total antimicrobial activity of a plant extract may be either due to its non-polar components, or its polar components or both. "*Successive Extraction By Maceration Using a Wide range of Solvents*" is an effective method by which, it is possible to separate the non-polar, Intermediately polar and highly polar components by extracting plant material in different solvents and the antimicrobial activity of these extracts can be tested to know that the collective antimicrobial effect of the plant material is due to polar or non-polar components. Further, different identification tests can be performed on the extract (with maximum antimicrobial activity) to identify the group of substances (like glycosides or alkaloids or essential oils etc.) in the extract. This approach is quite useful in detecting new

antimicrobial substances.

Many plant extracts and essential oils isolated from plants have been shown to exert biological activity in vitro and in vivo, which justified research on traditional medicine was focused on the characterization of antimicrobial activity of these plants (Martinez *et al.*, 1996).

The plants evaluated in this study have been reported to possess a number of medicinal properties. Leaves of *Psidium guajava* have been used as herbal medicine for the treatment of various human ailments such as wounds, ulcers, cholera, and coughs (Begum *et al.*, 2002, Jaiarj *et al.*, 1999). They also possess antimicrobial, anti-inflammatory, analgesic, spasmolytic, antipyretic, antidiarrheal and antimutagenic activities (Grover and Bala, 1993). While Leaves of *Gymnema sylvestre* are active against glycosuria and many other urinary disorders. The saponin *gymnemic acid*, constituent of the leaves, was shown to suppress sweet taste sensation and to inhibit glucose absorption in the small intestine (Shimizu *et al.*, 1997).

MATERIALS AND METHODS

Collection of Plant Material: Fresh leaves of *Psidium guajava* and *Gymnema sylvestre* were collected from the botanical garden of Govt. College University, Lahore and were authenticated. Leaves were rinsed in distilled water to remove contamination, air dried, ground in pestle and mortar and delivered for the process of extraction by maceration (Afridi *et al.*, 2025).

Maceration: Eight sterile capped bottles of 500mL capacity were used for maceration. Each 500mL of four solvents i.e. Distilled Water; Methanol, Chloroform, and Petroleum Ether were taken in four separate bottles for *Psidium guajava* leaves and in four separate bottles for *Gymnema sylvestre* leaves. Twenty grams of grind leaves of *Psidium guajava* and *Gymnema sylvestre* were weighed accurately using a digital balance and added in 500mL sterile capped bottles. The Bottles were regularly shaken after half an hour manually and placed for three days to allow the plant material to macerate. Extracts were filtered using Whatmann's filter paper. The filtrate obtained was dried in a vacuum desiccator's till dry mass was obtained. The dried mass was weighed and dissolved in respective solvent (1mL of solvent for each 20mg of powder) to get extract of known concentration (Bin Shan *et al.*, 2007).

FILTRATION OF EXTRACT THROUGH 0.2 μ M SYRINGE FILTER

Extracts were subjected to Syringe Filtration, using Filters of Minisart (Sartorius) 0.2 μ m size in safety cabinets for the purpose of sterilization. The extracts were then stored in a refrigerator at 2°C to 8°C till use.

BACTERIAL CULTURES

Bacteria under study were first isolated from the field samples and then identified.

1. *Staphylococcus aureus*
2. *Bacillus Subtilis*
3. *Salmonella typhi*
4. *Escherichia coli*

ISOLATION

Staphylococcus aureus, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* were isolated by using the scheme shown in figure 1 and figure 2

IDENTIFICATION

Identification of bacteria was done by two methods.

1. Slide Method (Gram Staining)
2. Biochemical Testing

SLIDE IDENTIFICATION

A thin smear was prepared and supplied on the surface of a clean dry glass slide with the help of a sterile wire loop. The Glass slide was subjected to Gram staining.

Gram Staining: Thin smear of the bacteria was prepared and heat fixed the smear. The smear was flooded with 1% aqueous solution of crystal violet. Slide was flushed off with lugol's Gram's iodine solution. Slide was covered with a fresh solution of lugol's iodine and left for 2-3 min. Then the slide was washed in water briefly and did not allow the smear to become dry. Slide was Decolorized with acid –alcohol until the decolouriser flowed from the slide almost uncolored (usually 10secs). Then the slide was counter stained by using 2% solution of safranin (10secs). Then the slide was washed briefly with water, and dried quickly. The prepared and the dried slide were observed under the microscope to identify the bacteria.

BIOCHEMICAL TESTING

Biochemical testing of *Salmonella typhi* and *Escherichia coli* was performed by using api 20E Kits (bioMerieux) as described by the manufacturer. Manual testing was performed for the biochemical Identification of *Bacillus subtilis* and *Staphylococcus aureus* as described below.

COAGULASE TEST

One or two colonies were suspended in a drop of sterile water on a slide. If no clumping occurred in 10-20 secs. A straight wire was dipped into human or rabbit plasma. Excess (e.g. a loopful) of plasma was avoided as this may give false positives. Bacterial suspension was stirred with it. Plasma was checked with a known coagulase-positive staphylococcus.

Citrate Test

Solid Simmon's Christensen's medium or fluid medium (Koser) was inoculated with a straight

wire. It was incubated at 30-35°C and growth was observed.

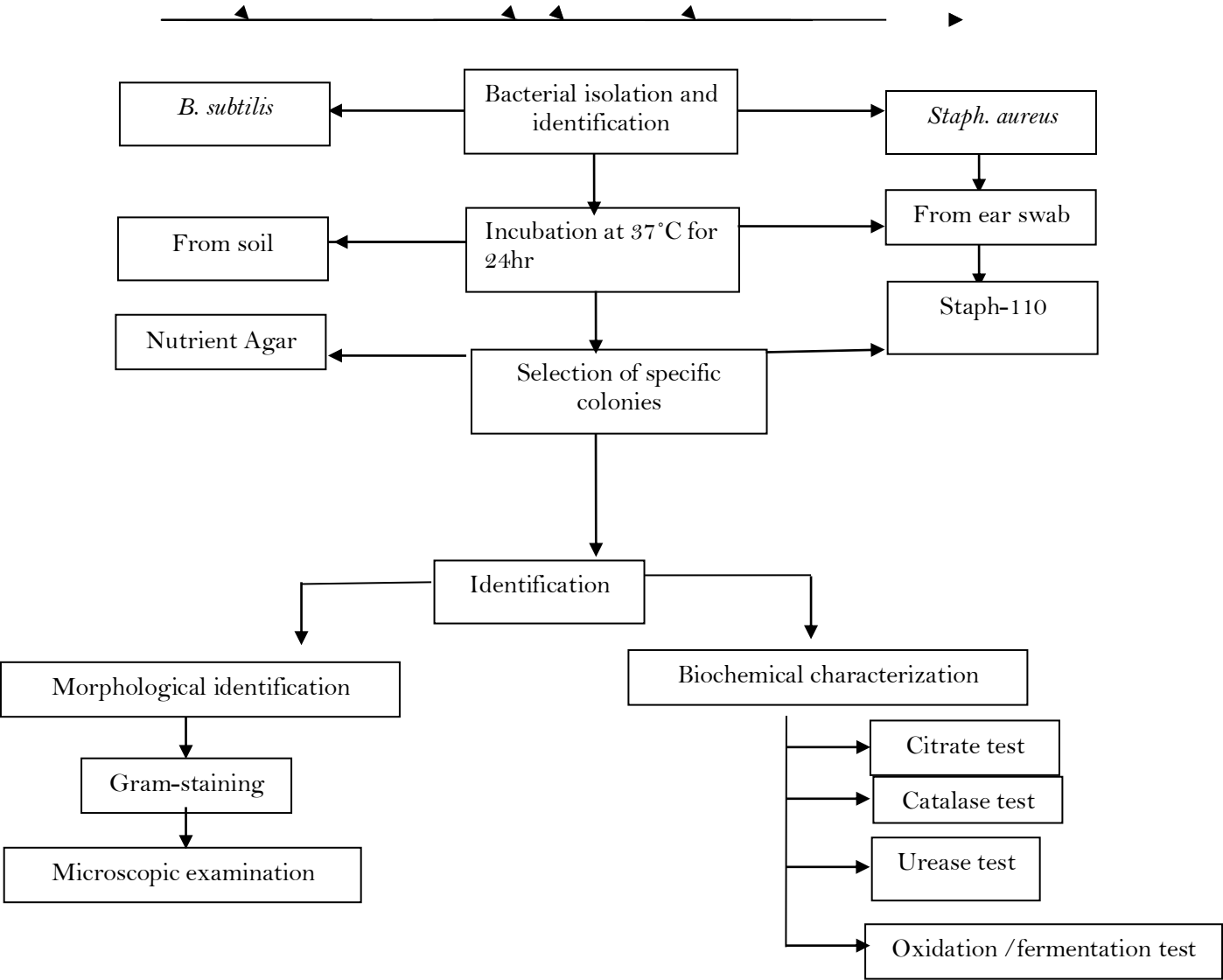


Figure 1- ISOLATION AND IDENTIFICATION OF GRAM POSITIVE BACTERIA

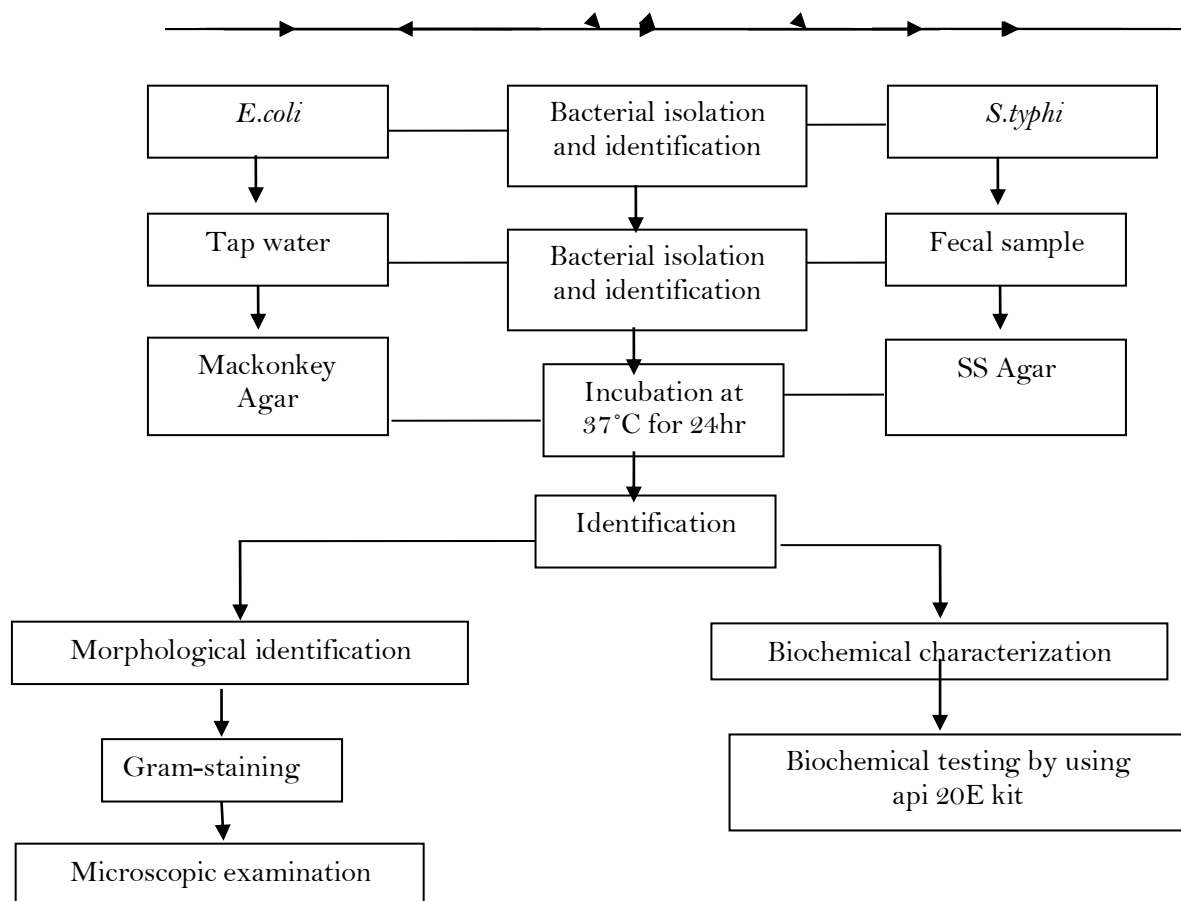


FIGURE 2- ISOLATION AND IDENTIFICATION OF GRAM NEGATIVE BACTERIA
CATALASE TEST

The culture was grown on low-carbohydrate medium (e.g. nutrient agar). Some culture was suspended in 0.5 mL of 1 % solution of Tween-80 in a screw-capped bottle. 0.5% of 20-volume hydrogen peroxide was added and the cap was replaced. Effervescence of oxygen indicated the presence of catalase.

NITRATE REDUCTION TEST

Nitrate broth was inoculated and incubated at 37°C overnight. A dense suspension of the test organism was made in 0.01M NaNO₂ in M/45 phosphate buffer of pH 7.0 and incubated at 37°C for 24 hrs. Acidified with a few drops of 1N HCl and add 0.5 mL each of 0.2% solution of sulphanilamide and 0.1% N-naphthylethylenediamine hydrochloride.

VOGES PROSKAUER TEST (VP TEST)

Inoculated culture into glucose phosphate peptone water. Incubated overnight at 37°C. Added a few drops of Barritt's reagent (5g alpha-naphthol) in 95mL absolute ethyl alcohol. Mixed in

solution containing potassium hydroxide (40.0g) creatine (0.3g). This experiment was designed to evaluate the antibacterial activity of methanolic, aqueous, chloroform and petroleum ether extracts of *Psidium guajava* and *Gymnema sylvestre* leaves against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*.

SUGAR FERMENTATION TEST

10mL of bacteriological peptone was taken by mixing 1g of bacteriological peptone in 100mL of distilled water. Mix the required sugar in 1% of the total weight of the bacteriological peptone. Sterilize the sugar solution by filtration using 0.2µm filter. Pour the sugar solution in the volume of 10mL in 2 screw capped bottles. Culture the bacteria by taking a pure colony in each of the tube. Shake each bottle gently. Incubate one tube for aerobic and the other one an-aerobically. After incubation period of 24 hours, pour a drop of acid-base indicator. Note the results.

ANTIBACTERIAL SENSITIVITY TESTING

Preparation of Bacterial Suspension: 10 autoclave able test tubes were washed and cleaned. 9mL of Phosphate Buffered Saline (PBS) was added in each test tube. The tubes were capped and autoclaved at 121° C and 15 lb pressure for 15-20 mins. The tubes were labeled as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10} . Bacteria were inoculated in Nutrient Broth and incubated at 37°C for 24 hours. 10 fold serial dilution of broth were made in Laminar flow hood. Bacterial colony forming units were determined by Viable Count Method. Briefly, 1mL from each test tube was taken and was poured in separate sterile Nutrient Agar plates. The plates were incubated for 24 hours at 37° C and after that, number of colonies were counted. From that plate which contained 30-300 colonies, number of colony forming units (CFU) per mL of the “Master Suspension” was calculated.

Preparation of Media: Nutrient Agar was weighed and mixed with distilled water in a concentration of 2g per 100mL (as advised by the manufacturer) in an autoclave able conical flask. The media was autoclaved at 15lb pressure and 121°C temperature for 15 to 20 mins. when the temperature was nearly about 50 to 55°C, bacterial suspension from “Master Suspension” was taken (and mixed with the media) in such a quantity that each milliliter of the media contained 10^6 CFU.

Sensitivity Testing: Antibacterial activity of plant extracts was determined by the method used by Bin Shan *et al* (2007) and Munir *et al* (2025). Briefly, Nutrient Agar was prepared and 30 mL of media was poured in each Petri dish. Six wells of 1centimeter diameter were cut in each plate with the help of cork borer and then sealed with Nutrient Agar. In one well, 50µL of extract plus 450 µL of respective solvent (total of 500 µL, Containing 1000µg of plant extract) and in the

second well 250µL of extract plus 250 µL of respective solvent (total of 500 µL, Containing 5000µg of plant extract) and the 3rd well 500µL of extract (containing 10000µg of plant extract) was poured with the help of micropipettes. In 4th well, penicillin G (1600µg per well) and in the 5th well Gentamicin (1000µg per well) was taken as a positive control. In the 6th well, respective solvent without plant extract, were used as negative control. The plates were left open for 20 minutes in Laminar Flow Hood, allowing organic solvents to evaporate and then the plates were closed and incubated at 37 degree Celsius for 24 hours and the diameter of Inhibitory Zones (DIZ) was calculated in millimeters with the help of a scale. Each test was performed in five replicates.

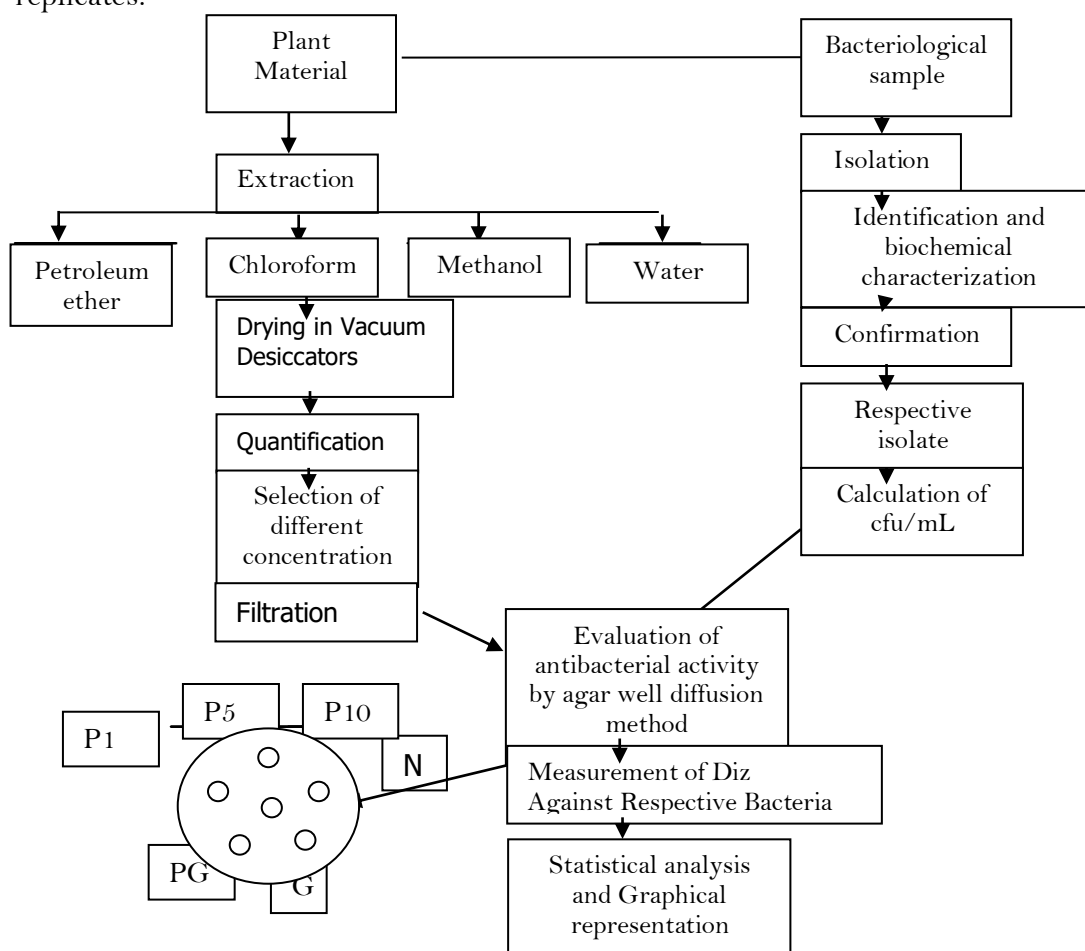


FIG. 3 EXPERIMENTAL DESIGN

P1 Represents Plant Extract 1000 µL /well (Containing 1000µg/well)
 P5 Represents Plant Extract 5000 µL /well (Containing 5000µg/well)
 P10 Represents Plant Extract 10000 µL /well (Containing 10000µg/well)
 N Represents Negative control (Respective solvent)
 PG Represents Penicillin G (1600µg/well)
 G Represents Gentamicin (1000µg/well)

RESULTS

CONCENTRATIONS OF PLANT EXTRACTS

Plant extracts were dried in vacuum desiccators (Millipore). The dried mass obtained after drying was weighed and the findings are given in the table below.

Table 1- WEIGHT OF DRY MASS OBTAINED AFTER DRYING OF EXTRACTS IN DESICCATORS

Extract	<i>Psidium guajava</i> (mg)	<i>Gymnema sylvestre</i> (mg)
Petroleum Ether	527	470
Chloroform	920	1456
Methanol	1190	1510
Water	880	630

IDENTIFICATION TESTS

Two types of Identification tests were performed:

- 1) Slide Identification
- 2) Biochemical testing

SLIDE IDENTIFICATION

Morphological characteristics of the colonies of bacteria were observed. Bacteria were subjected to gram staining. The results of gram staining and the morphological characteristics of the colonies are given in the table below.

TABLE 2- MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL COLONIES AND THE RESULTS OF GRAM STAINING

Sr.#	BACTERIA	MEDIA USED	BACTERIAL SHAPE	COLONY MORPHO- LOGY	GRAM STAINING
1	<i>Staphylococcus aureus</i>	Staph Media	110 Blue clusters of balls	Smooth, with entire edges. Orange in colour	Gram Positive (Blue Stained)
2	<i>Bacillus subtilis</i>	Nutrient Agar	Blue rods	Irregular, dull surface colonies, cream	Gram Positive (Blue Stained)

				coloured		
3	<i>Escherichia coli</i>	MacConkey Agar	Red Rods	Blood colonies	Red	Gram Negative (pink Stained)
4	<i>Salmonella typhi</i>	SS Agar	Red Rods	Opaque colonies of intermediate appearance		Gram Negative (pink Stained)

BIOCHEMICAL TESTS

BIOCHEMICAL IDENTIFICATION OF GRAM POSITIVE BACTERIA

Biochemical identification of Gram positive bacteria was done by performing citrate test, vogus proskaur (VP) test, nitrate reduction test, sugar fermentation test and coagulase test. *Bacillus subtilis* gave positive VP test, positive citrate test, negative coagulase test and negative catalase test. *Staphylococcus aureus* gave positive coagulase test, positive catalase test, negative VP test and negative citrate test. Both bacteria gave positive nitrate reduction test. Sugar fermentation test was performed using four sugars i.e. Glucose, xylose, lactose and mannitol. *Bacillus subtilis* gave glucose and xylose test positive and *Staphylococcus aureus* gave glucose, lactose and mannitol test positive.

TABLE -3 RESULTS OF BIOCHEMICAL TESTS PERFORMED FOR THE IDENTIFICATION OF GRAM POSITIVE BACTERIA

Biochemical Test	Observation (end point)		Inference	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. aureus</i>
VP	Pink Colour	No Change	+	-
Citrate	Changes to Deep Blue Colour	No Change	+	-
Nitrate Reduction	To Red Colour	To Red Colour	+	+
Catalase	No Bubbles	Gas in capillary Tube	-	+
Glucose	Red Colour	Red Colour	+	+
Lactose	Yellow Colour	Red Colour	-	+

<i>Xylose</i>	Red Colour	Yellow Colour	+	-
<i>Manitole</i>	Yellow Colour	Red Colour	-	+
<i>Coagulase</i>	Plasma not Coagulated	Plasma Coagulated	-	+

+ Sign indicates that the bacteria gave positive test

- Sign indicates that the bacteria gave Negative test

Biochemical Identification of Gram Negative Bacteria: Biochemical identification of gram negative bacteria was performed by using api 20E kits according to the directions of kit manufacturer (Biomერიux). Results are given in the table below.

TABLE 4- RESULTS OF BIOCHEMICAL IDENTIFICATION OF GRAM NEGATIVE BACTERIA USING API 20E KITS

BIO- CHEMICAL	Observation		Inference	
	<i>E.coli</i>	<i>S. typhi</i>	<i>E.coli</i>	<i>S. typhi</i>
<i>ONPG</i>	YELLOW	YELLOW	+	+
<i>ADH</i>	ORANGE	RED	+	+
<i>LDC</i>	RED	RED	+	+
<i>ODC</i>	RED	RED	+	+
<i>CIT</i>	GREENISH YELLOW	GREENISH BLUE	-	+
<i>H₂S</i>	YELLOW	WHITE	-	-
<i>URE</i>	YELLOW	RED	-	+
<i>TDA</i>	WHITE	WHITE	-	-
<i>IND</i>	RED	WHITE	+	-
<i>VP</i>	WHITE	WHITE	-	-
<i>GEL</i>	BLACK	BLACK	+	+
<i>GLU</i>	YELLOW	YELLOW	+	+
<i>MAN</i>	YELLOW	GREENISH BLUE	+	-
<i>INO</i>	BLUE	BLUE	-	-
<i>SOR</i>	YELLOW	BLUE	+	-
<i>RHA</i>	YELLOW	BLUE	+	-
<i>SAC</i>	YELLOW	GREEN	+	-

MEL	YELLOW	BLUE	+	-
AMY	GREENISH BLUE	GREEN	-	-
ARA	YELLOW	BLUE	+	-

+ Sign indicates that the bacteria gave positive test

- Sign indicates that the bacteria gave Negative test

TOTAL VIABLE BACTERIAL COUNT

Total viable count was performed as described in material and method. The results of bacterial count for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* are given in table 5, table 6, table 7 and table 8 respectively.

ANTIBACTERIAL ACTIVITY PROFILE

The in vitro antibacterial activity of *psidium guajava* and *gymnema sylvestre* extracts were determined by measuring diameters of inhibitory zones of these extracts against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The results of in vitro antibacterial activity of both plants in methanol extract, water extract, chloroform extracts and petroleum ether extracts, extracts are given in table 9, 10, 11 and 12 respectively.

STATISTICAL ANALYSIS

ANOVA was applied on the results and the ANOVA tables of Diameters of Inhibitory Zones of Plant Extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* are given in Table 13, 14, 15, and 16 respectively.

GRAPHICAL REPRESENTATION

Figure 4 indicates *Psidium guajava* antibacterial activity against *Staphylococcus aureus*. Activity of *Psidium guajava* extracts against *Bacillus subtilis* is given in Figure 5. Activity of extracts of *Psidium guajava* against *Escherichia coli* are given in Figure 6. Figure 7 indicates *Psidium guajava* antibacterial activity against *Salmonella typhi*. Figure 8 shows *Gymnema sylvestre* antibacterial activity against *Staphylococcus aureus*. Figure 9 indicates *Gymnema sylvestre* antibacterial activity against *Bacillus subtilis*. The antibacterial activity of *Gymnema sylvestre* against *Escherichia coli* is given in Figure 10 and the activity of *Gymnema sylvestre* extracts against *Salmonella typhi* are given in Figure 11.

TABLE 5- COUNTING OF CFU/ML OF *BACILLUS SUBTILIS* SUSPENSION USING VIABLE COUNT METHOD

<i>Bacteria</i>	<i>Dilution Of Broth</i>	<i>Volume inoculum's plate</i>	<i>of Number per Colonies Counted</i>	<i>Of CFU/mL of bacterial suspension</i>	<i>Volume of bacterial suspension containing 10⁶ CFU (μL)</i>
<i>B. subtilis</i>	10 ⁻¹	1 mL	More than 300	4.3 × 10 ⁹	0.23
	10 ⁻²	1 mL	More than 300		0.23 μL of broth was added per milliliter of media.
	10 ⁻³	1 mL	More than 300		
	10 ⁻⁴	1 mL	More than 300		
	10 ⁻⁵	1 mL	More than 300		
	10 ⁻⁶	1 mL	More than 300		
	10 ⁻⁷	1 mL	More than 300		
	10 ⁻⁸	1 mL	43		
	10 ⁻⁹	1 mL	Less than 30		
	10 ⁻¹⁰	1 mL	Less than 30		

Colonies were counted only from that plate which contained colonies within 30-300.

TABLE 6- COUNTING OF CFU/ML OF *STAPHYLOCOCCUS AUREUS* SUSPENSION USING VIABLE COUNT METHOD

<i>Bacteria</i>	<i>Dilution Of Broth</i>	<i>Volume of 705 inoculums's per plate</i>	<i>Number of Colonies Counted</i>	<i>Of CFU/mL of bacterial suspension</i>	<i>Volume of bacterial suspension containing 10⁶ CFU (μL)</i>
<i>S. aureus</i>	10 ⁻¹	1 mL	More than 300	5.0 × 10 ⁸	2.00
	10 ⁻²	1 mL	More than 300		2.00 μL of broth was added per
	10 ⁻³	1 mL	More than 300		milliliter of media.
	10 ⁻⁴	1 mL	More than 300		
	10 ⁻⁵	1 mL	More than 300		
	10 ⁻⁶	1 mL	More than 300		
	10 ⁻⁷	1 mL	50		
	10 ⁻⁸	1 mL	Less than 30		
	10 ⁻⁹	1 mL	Less than 30		
	10 ⁻¹⁰	1 mL	Less than 30		

Colonies were counted only from that plate which contained colonies within 30-300.

TABLE 7- COUNTING OF CFU/ML OF *ESCHERICHIA COLI* SUSPENSION USING VIABLE COUNT METHOD

<i>Bacteria</i>	<i>Dilution Of Broth</i>	<i>Volume of inoculum's plate</i>	<i>Number of Colonies per Counted</i>	<i>Of CFU/mL of bacterial suspension</i>	<i>Volume of bacterial suspension containing 10⁶ CFU (μL)</i>
<i>E. coli</i>	10 ⁻¹	1 mL	More than 300	4.2 × 10 ⁹	0.24
	10 ⁻²	1 mL	More than 300		0.24 μL of broth was added per milliliter of media.
	10 ⁻³	1 mL	More than 300		
	10 ⁻⁴	1 mL	More than 300		
	10 ⁻⁵	1 mL	More than 300		
	10 ⁻⁶	1 mL	More than 300		
	10 ⁻⁷	1 mL	More than 300		
	10 ⁻⁸	1 mL	42		
	10 ⁻⁹	1 mL	Less than 30		
	10 ⁻¹⁰	1 mL	Less than 30		

Colonies were counted only from that plate which contained colonies within 30-300.

Table 8- COUNTING OF CFU/ml OF *SALMONELLA TYPHI* SUSPENSION USING VIABLE COUNT METHOD

<i>Bacteria</i>	<i>Dilution Of Broth</i>	<i>Volume inoculum's plate</i>	<i>of Number per Colonies Counted</i>	<i>Of CFU/mL of bacterial suspension</i>	<i>Volume of bacterial suspension containing 10⁶ CFU (μL)</i>
<i>S. typhi</i>	10 ⁻¹	1 mL	More than 300	4.0×10 ⁸	2.5
	10 ⁻²	1 mL	More than 300		2.5μL of broth was added per milliliter of media.
	10 ⁻³	1 mL	More than 300		
	10 ⁻⁴	1 mL	More than 300		
	10 ⁻⁵	1 mL	More than 300		
	10 ⁻⁶	1 mL	More than 300		
	10 ⁻⁷	1 mL	40		
	10 ⁻⁸	1 mL	Less than 30		
	10 ⁻⁹	1 mL	Less than 30		
	10 ⁻¹⁰	1 mL	Less than 30		

Colonies were counted only from that plate which contained colonies within 30-300.

TABLE 9- DIAMETER OF INHIBITORY ZONES (IN MILLIMETERS) OF METHANOL EXTRACTS OF *PSIDIUM GUAJAVA* AND *GYMNEMA SYLVESTRE* AGAINST *STAPHYLOCOCCUS AUREUS*, *BACILLUS SUBTILIS*, *SALMONELLA TYPHI* AND *ESCHERICHIA COLI* BY AGAR WELL DIFFUSION METHOD

Bacteria	Diameter of Inhibition Zone (mm)							
	EXPERIMENTAL GROUPS						STANDARD	
	<i>Psidium guajava</i>			<i>Gymnema sylvestre</i>			Gentamicin	Penicillin G
	(10,000 µg/well)	(5,000 µg/well)	(1000 µg/well)	(10,000 µg/well)	(5,000 µg/well)	(1000 µg/well)	(1000µg/well)	(1600 µg/well)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
<i>Staphylococcus aureus</i>	26.08±0.895 6	20.25±1.238 6	15.63±1.536 8	15.23±0.856 9	11.50±1.021 1	8.02±0.891 8	28.56±1.1123	15.23±0.215 9
<i>Bacillus subtilis</i>	23.15±0.896 3	17.25±0.965 8	12.16±0.256 9	17.12±1.056 3	13.96±0.895 6	9.56±1.108 1	19.11±0.1250	27.85±1.085 6
<i>Salmonella typhi</i>	20.23±0.896 3	16.36±1.023 5	11.12±0.814 5	0±0.0000	0±0.0000	0±0.0000	17.45±1.0235	0±0.0000
<i>Escherichia coli</i>	19.36±1.269 1	15.25±0.236 9	9.36±1.0236	11.45±1.112 0	7.85±0.1456	4.50±1.023 5	25.36±0.9851	0±0.0000

Number of replicates for each test = n= 5

TABLE 10- DIAMETER OF INHIBITORY ZONES (IN MILLIMETERS) OF WATER EXTRACTS OF *PSIDIUM GUAJAVA* AND *GYMNEMA SYLVESTRE* AGAINST *STAPHYLOCOCCUS AUREUS*, *BACILLUS SUBTILIS*, *SALMONELLA TYPHI* AND *ESCHERICHIA COLI* BY AGAR WELL DIFFUSION METHOD

Bacteria	Diameter of Inhibition Zone (mm)							
	EXPERIMENTAL GROUPS						STANDARD	
	<i>Psidium guajava</i>			<i>Gymnema sylvestre</i>			Gentamicin	Penicillin G
	(10,000 µg/well) Mean± SE	(5,000 µg/well) Mean ± SE	(1000 µg/well) Mean ± SE	(10,000 µg/well) Mean ± SE	(5,000 µg/well) Mean ± SE	(1000 µg/well) Mean ± SE	(1000µg/well) Mean ± SE	(1600 µg/well) Mean ± SE
<i>Staphylococcus aureus</i>	29.78±1.0623	22.63±1.2363	16.12±0.2356	0±0.0000	0±0.0000	0±0.0000	28.56±1.1123	15.23±0.2159
<i>Bacillus subtilis</i>	25.36±1.2358	18.29±0.2356	13.15±.8236	0±0.0000	0±0.0000	0±0.0000	19.11±0.1250	27.85±1.0856
<i>Salmonella typhi</i>	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	17.45±1.0235	0±0.0000
<i>Escherichia coli</i>	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	0±0.0000	25.36±0.9851	0±0.0000

Number of replicates for each test = n= 5

TABLE 11- DIAMETER OF INHIBITORY ZONES (IN MILLIMETERS) OF CHLOROFORM EXTRACTS OF *PSIDIUM GUAJAVA* AND *GYMNEMA SYLVESTRE* AGAINST *STAPHYLOCOCCUS AUREUS*, *BACILLUS SUBTILIS*, *SALMONELLA TYPHI* AND *ESCHERICHIA COLI* BY AGAR WELL DIFFUSION METHOD

Bacteria	Diameter of Inhibition Zone (mm)							
	EXPERIMENTAL GROUPS						STANDARD	
	<i>Psidium guajava</i>			<i>Gymnema sylvestre</i>			Gentamicin	Penicillin G
	(10,000 µg/well) Mean± SE	(5,000 µg/well) Mean ± SE	(1000 µg/well) Mean ± SE	(10,000 µg/well) Mean ± SE	(5,000 µg/well) Mean ± SE	(1000 µg/well) Mean ± SE	(1000µg/well) Mean ± SE	(1600 µg/well) Mean ± SE
<i>Staphylococcus aureus</i>	14.17±1.2358	10.36±0.8965	8.25±0.2564	14.56±1.0369	10.89±0.9653	6.23±0.5693	28.56±1.1123	15.23±0.2159
<i>Bacillus subtilis</i>	11.25±0.8965	8.25±0.2356	5.50±1.0256	15.09±1.096	11.26±0.7562	8.36±0.3692	19.11±0.1250	27.85±1.0856
<i>Salmonella typhi</i>	0±0.0000	0±0.0000	0±0.0000	22.56±1.0693	16.49±0.9632	12.37±1.0236	17.45±1.0235	0±0.0000
<i>Escherichia coli</i>	0±0.0000	0±0.0000	0±0.0000	18.96±0.9656	13.75±0.9658	9.67±1.0369	25.36±0.9851	0±0.0000

Number of replicates for each test = n= 5

TABLE 12- DIAMETER OF INHIBITORY ZONES (IN MILLIMETERS) OF PETROLEUM ETHER EXTRACTS OF *PSIDIUM GUAJAVA* AND *GYMNEMA SYLVESTRE* AGAINST *STAPHYLOCOCCUS AUREUS*, *BACILLUS SUBTILIS*, *SALMONELLA TYPHI* AND *ESCHERICHIA COLI* BY AGAR WELL DIFFUSION METHOD

Bacteria	Diameter of Inhibition Zone (mm)							
	EXPERIMENTAL GROUPS						STANDARD	
	<i>Psidium guajava</i>			<i>Gymnema sylvestre</i>			Gentamicin	Penicillin G
	(10,000 µg/well)	(5,000 µg/well)	(1000 µg/well)	(10,000 µg/well)	(5,000 µg/well)	(1000 µg/well)	(1000µg/well)	(1600 µg/well)
	Mean± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
<i>Staphylococcus aureus</i>	6.02±0.5698	0±0.0000	0±0.0000	7.36±0.9632	3.26±0.5623	0±0.0000	28.56±1.1123	15.23±0.2159
<i>Bacillus subtilis</i>	4.27±1.0378	0±0.0000	0±0.0000	9.36±1.2364	4.50±0.9658	0±0.0000	19.11±0.1250	27.85±1.0856
<i>Salmonella typhi</i>	0±0.0000	0±0.0000	0±0.0000	14.26±0.3265	8.23±0.2359	3.13±1.7506	17.45±1.0235	0±0.0000
<i>Escherichia coli</i>	0±0.0000	0±0.0000	0±0.0000	11.96±1.0251	6.27±0.4595	0±0.0000	25.36±0.9851	0±0.0000

Number of replicates for each test = n= 5

TABLE 13- RESULTS OF F-TEST (ANOVA) APPLIED ON THE DIAMETERS OF INHIBITORY ZONES OF PLANT EXTRACTS AND POSITIVE REFERENCE (PENICILLIN G AND GENTAMICIN) AGAINST *STAPHYLOCOCCUS AUREUS*

Dependent Variable: DIZ

		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	2956.16	1	2956.16	1812.162	.000	.695	1812.162	1.000
	Error	5.623	4	1.406(b)					
Extract	Hypothesis	2356.263	23	102.446	113.425	.000	.695	113.425	1.000
	Error	26.356	92	.286(c)					
Replicate	Hypothesis	3.265	4	.816	4.236	0.11	.126	16.233	.621
	Error	41.235	92	.448(c)					
Extract	* Hypothesis	61.235	92	.665	.	.	1.000	.	.
Replicate	Error	.000	0	.(d)					

Computed using alpha = .05

TABLE 14- RESULTS OF F-TEST (ANOVA) APPLIED ON THE DIAMETERS OF INHIBITORY ZONES OF PLANT EXTRACTS AND POSITIVE REFERENCE (PENICILLIN G AND GENTAMICIN) AGAINST *BACILLUS SUBTILIS*

Dependent Variable: DIZ

		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	3673.611	1	3673.611	4118.397	.000	.958	4021.321	1.000
	Error	3.569	4	.892(b)					
Extract	Hypothesis	2311.234	23	100.488	135.562	.000	.944	2356.124	1.000
	Error	72.569	92	.788(c)					
Replicate	Hypothesis	3.956	4	.989	1.056	.465	.051	5.123	.339
	Error	72.569	92	.793(c)					
Extract	* Hypothesis	72.978	92	.756		.	1.000	.	.
Replicate	Error	.000	0	.(d)					

Computed using alpha = .05

TABLE 15- RESULTS OF F-TEST (ANOVA) APPLIED ON THE DIAMETERS OF INHIBITORY ZONES OF PLANT EXTRACTS AND POSITIVE REFERENCE (PENICILLIN G AND GENTAMICIN) AGAINST *ESCHERICHIA COLI*

Dependent Variable: DIZ

		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	1856.112	1	1856.112	7278.870	.000	.981	4562.251	1.000
	Error	1.023	4	.255(b)					
Extract	Hypothesis	2132.215	23	92.705	291.856	.000	.981	6235.213	1.000
	Error	15.456	92	.168(c)					
Replicate	Hypothesis	1.032	4	.258	.856	.356	.033	2.236	.211
	Error	16.336	92	.177(c)					
Extract	* Hypothesis	16.336	92	.177	.	.	.995	.	.
Replicate	Error	.000	0	.(d)					

Computed using alpha = .05

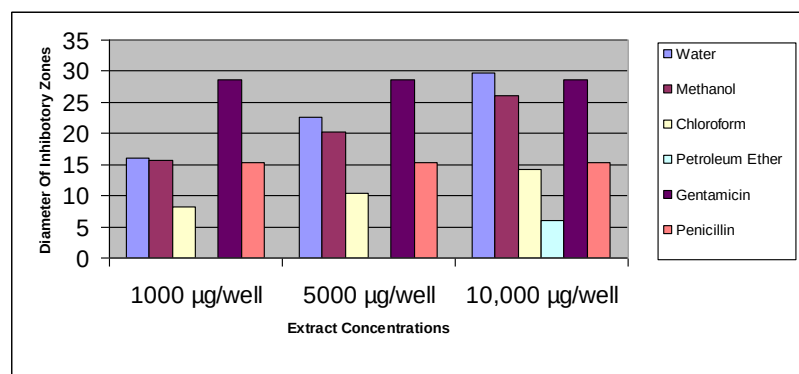
TABLE 16- RESULTS OF F-TEST (ANOVA) APPLIED ON THE DIAMETERS OF INHIBITORY ZONES OF PLANT EXTRACTS AND POSITIVE REFERENCE (PENICILLIN G AND GENTAMICIN) AGAINST *SALMONELLA TYPHI*

Dependent Variable: DIZ

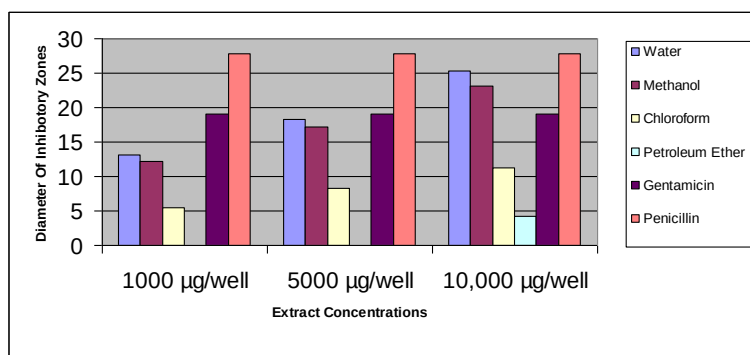
Source		Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	233.125	1	233.125	4562.231	.000	.865	4562.231	1.000
	Error	.235	4	.058(b)					
Extract	Hypothesis	1752.166	23	76.181	1231.236	.000	.895	2135s.123	1.000
	Error	3.263	92	.035(c)					
Replicate	Hypothesis	.122	4	.030	.456	.892	.022	1.023	.236
	Error	4.235	92	.046(c)					
Extract	* Hypothesis	5.422	92	.059	.	.	1.000	.	.
Replicate	Error	.000	0	.(d)					

Computed using alpha = .05

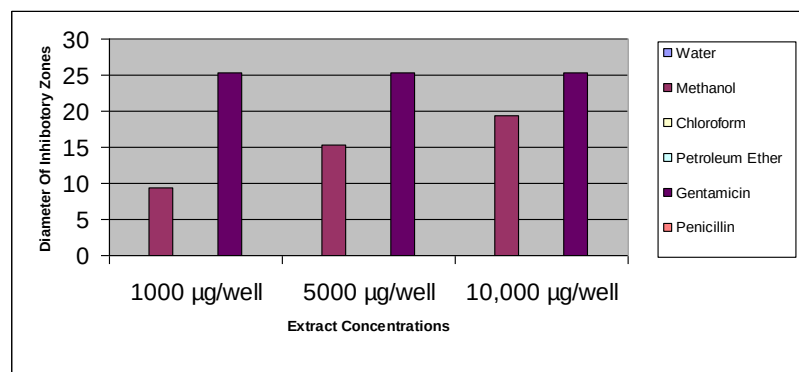
FIGURE 4 – IN VITRO ANTIBACTERIAL ACTIVITY OF *PSIDIUM GUAJAVA* EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS* (a), *BACILLUS SUBTILLIS* (b), *ESCHERICHIA COLI* (c) AND *SALMONELLA TYPHI* (d)



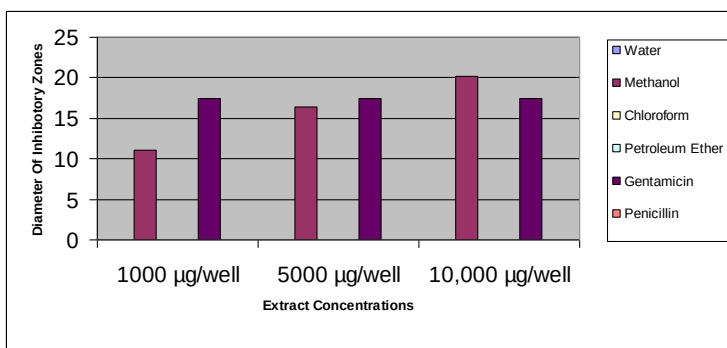
(a)



(b)



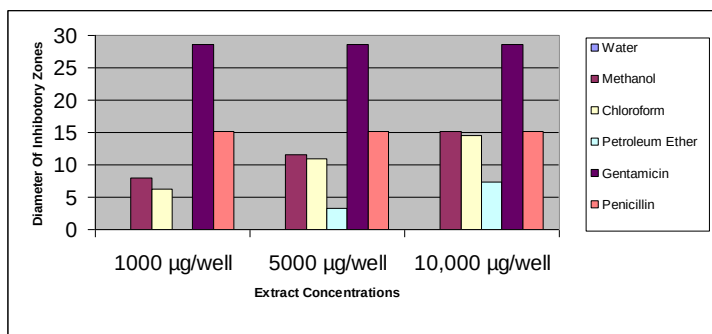
(c)



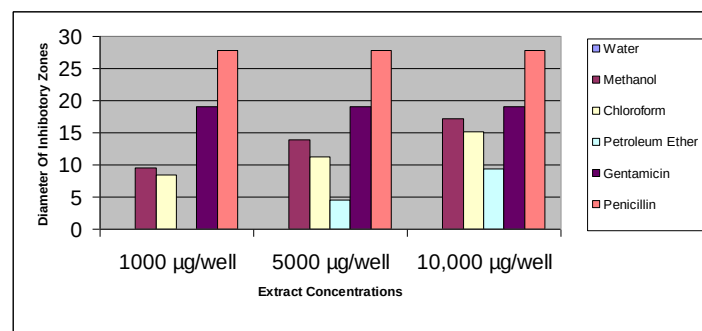
(d)

1000 indicates *PSIDIUM GUAJAVA* Extract 1000 µg/well Concentration
 5,000 indicates *PSIDIUM GUAJAVA* Extract 5,000 µg/well Concentration
 10,000 indicates *PSIDIUM GUAJAVA* Extract 10,000 µg/well Concentration
 PG indicates 1600µg/well Concentration of penicillin G
 G indicates 1000µg/well Concentration of Gentamicin

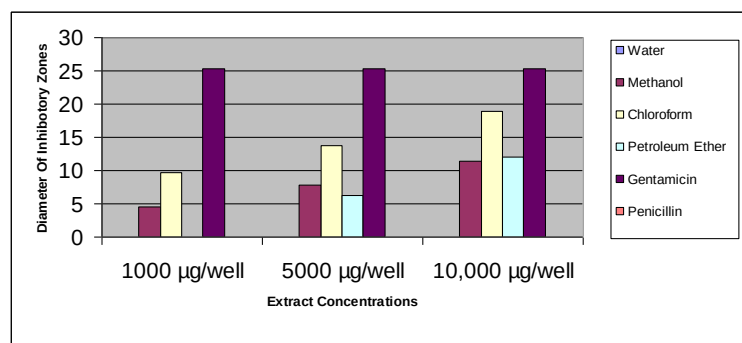
FIGURE 5– IN VITRO ANTIBACTERIAL ACTIVITY OF *GYMNEMA SYLVESTRE* EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS* (a), *BACILLUS SUBTILLIS* (b), *ESCHERICHIA COLI* (c) AND *SALMONELLA TYPHI*(d)



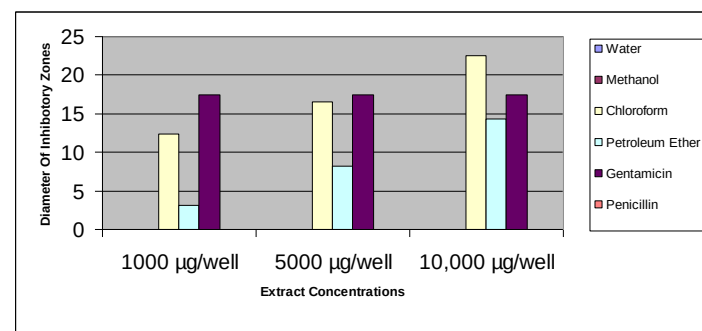
(a)



(b)



(c)



(d)

1000 indicates *GYMNEMA SYLVESTRE* Extract 1000 µg/well Concentration
 5,000 indicates *GYMNEMA SYLVESTRE* Extract 5,000 µg/well Concentration
 10,000 indicates *GYMNEMA SYLVESTRE* Extract 10,000 µg/well Concentration
 PG indicates 1600µg/well Concentration of penicillin G
 G indicates 1000µg/well Concentration of Gentamicin

DICUSSION

The development of antibiotic resistance by bacteria is an evolutionary inevitability, a convincing demonstration of their ability to adapt to adverse environmental conditions (Khan *et al.*, 2025). Antimicrobial activities of herbs have been well known for long time. Many studies reported the activities of spices and herbs or essential oils to food borne pathogenic bacteria. Flavonoids (derivatives of phenylchromone ring) are a large group of compounds naturally occurring in higher and lower plants. Flavonoids have been shown to be able to affect various biological functions: capillary permeability, cellular secretory processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers. The inhibitory activities of flavonoids against bacteria and yeast have been investigated by a number of researchers (Shoukat *et al.*, 2025).

The plants evaluated in this study have been reported to possess a number of medicinal properties. *Psidium guajava* leaves have been used as herbal medicine for the treatment of various human ailments such as wounds, ulcers, cholera, and coughs (Begum *et al.*, 2002, Jaiarj *et al.*, 1999). They also possess antimicrobial, anti-inflammatory, analgesic, Spasmolytic, antipyretic antidiarrheal and antimutagenic activities (Grover and Bala, 1993). While the Leaves of *Gymnema sylvestre* are active against glycosuria and many other urinary disorders. The saponin *gymnemic acid*, constituent of the leaves, was shown to suppress sweet taste sensation and to inhibit glucose absorption in the small intestine (Shimizu *et al.*, 1997).

Water extract of *Psidium guajava* exhibited stronger antibacterial activity against the gram-positive bacteria than all the 3 extracts of *Psidium guajava* and inhibited the growth of gram-positive tested bacteria's with a (DIZ) Diameter of inhibitory zone ranges from (29.78mm, 25.36mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively at a concentration of 10000µg/well) while the water extract did not show any activity against gram –negative bacteria at a concentrations used in this study. Antibacterial activity of methanolic extract was stronger against gram-negative bacteria as compare to other extract that did not show any activity against gram-gram negative bacteria at a concentrations used in this study. While The result indicated that the methanolic extract have stronger antibacterial activity against gram positive bacteria's with a (DIZ) Diameter of inhibitory zone ranges from (26.08mm, 23.15mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively at a concentration of 10000µg/well

) than relatively gram-negative bacteria (20.23mm, 19.36mm against *Salmonella typhi* and *Escherichia coli* respectively at a concentration of 10000µg/well) which shows that its polar components have stronger activity against gram-positive bacteria than gram-negative bacteria. The chloroform and Petroleum ether extracts of *Psidium guajava* also possess the antibacterial activity but only against the gram-positive bacteria with a (DIZ) Diameter of inhibitory zone ranges from (14.17mm, 11.25mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively at a concentration of 10000µg/well) for chloroform and Petroleum ether extract with a (DIZ) Diameter of inhibitory zone ranges from (6.02mm,4.00mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively at a concentration of 10000µg/well), the results indicated that the chloroform extract has stronger antibacterial activity to that of Petroleum ether extract but weaker than that of water and methanolic extracts which shows that polar or highly polar components of *Psidium guajava* had stronger antibacterial activity than non-polar components. While the chloroform and Petroleum ether extracts had no any activity against gram negative bacteria. This shows that chloroform and petroleum ether extracts had no antibacterial activity against gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) at concentrations used in this study. This study also revealed that methanolic extract of *Psidium guajava* possesses remarkable growth inhibitory activities against gram positive and negative bacteria.

The extracts from *Psidium guajava* showed activity against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and only methanolic extract against gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria. This could be expected because the outer membrane of gram-negative bacteria is known to present a barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are able of breaking down foreign molecules introduced from outside. (Duffy and Power, 2001).

These findings showed a correlation with the finding of Jaiarj *et al.*, (1999) who studied to evaluate the antitussive activity of *Psidium guajava* leaf extract. Moreover, growth of *Staphylococcus aureus* and *streptococcus* group A, as determined by the disc diffusion method, was inhibited by water, methanol and chloroform extract of dry guava leaves. Chah *et al.*, (2006) evaluated methanolic extracts of *Psidium guajava* for antibacterial and wound healing properties. Antibacterial properties of the extracts were studied against eleven wound isolates (*Staphylococcus aureus* (four strains), *Escherichia coli* (two strains), *Pseudomonas aeruginosa* (one

strain), *Proteus* spp. (three strains), and *Shigella* spp. (one strain)) using the well diffusion method. *Psidium guajava* and *Anthocleista djalensis* extracts prevented growth of 81.8 and 72.7% of the test organisms, respectively. *Ageratum conyzoides* and *Ocimum gratissimum* extracts did not inhibit growth of any of the test organisms. More than 90% wound healing was recorded in the extract and cicatrin® powder treated groups by 14 days post surgery, where as 72% healing was observed in the distilled water-treated group. Sanches *et al.*, (2005) also evaluated the antibacterial activities of aqueous and ethanol: water extracts of *Psidium guajava*. The antibacterial activities of the extracts against bacteria were tested by using both micro dilution assays. The aqueous extracts of *Psidium guajava* were active against the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and virtually inactive against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*.

Chloroform Extracts from *Gymnema sylvestre* showed activity against all 4 tested organisms but the antibacterial activity of chloroform extract was more pronounced in gram-negative bacteria than gram-positive bacteria. This shows that its non-polar components have more pronounced affect on gram-negative bacteria than gram-positive bacteria with a mean (DIZ) diameter of inhibitory zone ranges from (14.56mm, 15.09mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively and 18.96mm 22.56mm against *Escherichia coli* and *Salmonella typhi* respectively at a concentration of 10000µg/well) for Chloroform extract and petroleum ether extract with a mean (DIZ) diameter of inhibitory zone ranges from (7.36mm, 9.36mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively and 11.96mm,14.26mm against *Escherichia coli* and *Salmonella typhi* respectively at a concentration of 10000µg/well). These finding indicated that chloroform extract have strong antibacterial effect than Petroleum ether extract against both gram positive and gram negative bacteria. While its water extract had no antibacterial activity against the tested organisms. The methanolic extract of *Gymnema sylvestre* had stronger antibacterial activity against gram positive bacteria than chloroform and petroleum ether extract which shows that the polar components (methanolic extract) of *Gymnema sylvestre* had stronger antibacterial activity against gram-positive than gram-negative bacteria with a mean (DIZ) diameter of inhibitory zone ranges from (15.23mm, 17.12mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively and 11.45mm, 0mm against *Escherichia coli* and *Salmonella typhi* respectively at a concentration of 10000µg/well). The

results indicated that the chloroform and petroleum ether extract of *Gymnema sylvestre* have stronger antibacterial activity against gram negative bacteria than methanolic extract. The results of the antibacterial property of *Gymnema sylvestre* extracts showed a correlation with the finding of Poonkothai *et al.*, (2005) who studied the antibacterial activity of chloroform, ethanol and aqueous extracts of *Gymnema sylvestre* leaves on *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* on Muller Hinton agar plates. The results indicate that *gymnemic acid*, an active component of *Gymnema sylvestre*, soluble in both chloroform and ethanol has a strong antibacterial activity. There was no significant effect of aqueous extract because there was no zone of inhibition on the specified microorganisms.

Two antibiotics i.e. Penicillin G and Gentamicin were used as Positive control. Benzyl penicillin (commonly known as penicillin G) showed activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (diameter of inhibitory zone was 15.23mm and 27.85mm against *Staphylococcus aureus* and *Bacillus subtilis* respectively) but the gram negative bacteria were found to be resistant to penicillin-G (DIZ was 0.0mm for both *Escherichia coli* and *Salmonella typhi*). It might be due to the presence of penicillin resistant strains that produce penicillinases. Gentamicin a broad spectrum antibiotic (aminoglycoside) showed activity against both gram positive and gram negative bacteria (diameter of inhibitory zone was 28.56mm, 19.11mm, 17.45mm, and 25.36mm against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli* respectively). The present study showed that the isolated bacteria were sensitive.

CONCLUSION

The results of this study had confirmed the antibacterial potentials of extracts of *Psidium guajava* and *Gymnema sylvestre*. The extracts of these plants can further be developed into phytomedicines for the management of septic wounds. The differences in the findings can be attributed to the seasonal changes in temperature and humidity, also to different stage of plant part collection and metabolism and Polarity changes for soluble components.

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