EVALUATION OF ANTIBACTERIAL ACTIVITY OF MUSA SP AND TRIGONELLA FOENUM-GRAECUM EXTRACT AGAINST HUMAN PATHOGENIC BACTERIA

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Abstract: Alcoholic and aqueous extract exhibited a higher degree of antimicrobial activity tested against various infections causing by bacterial species. Banana peel and fenugreek seed extract contains large amount of tannins, flavonoids, glycosides, saponins and alkaloids. The antibacterial activity may be indicating the presence of these secondary metabolites. The antibacterial activity may be indicating the presence of these secondary metabolites. Alcoholic and aqueous extract exhibited a higher degree of antimicrobial activity tested against various infections causing by bacterial species. The zone of inhibition of the growth of the isolates was obtained to be a function of the relative antibacterial potency of the extracts. The zone of inhibition decreased as the concentration of the extract decreased. The highest zone of inhibition was obtained at a concentration of 1000µg/ml. Banana peel and fenugreek seed extract shows ZOI more than the standard. combined extract at a concentration of 50 µg/ml shows less ZOI when compared with std and the results are shown in the above table. The potent activity of extract at a concentration of 1000 µg/ml shows ZOI more than the std in well diffusion method against four pathogenic bacteria. Comparison of our results with those of the literature showed that the antibacterial activity of the plant extracts was very variable depending on the phytochemical composition of the plant, the solvents used for the extraction, and the bacteria tested. These plants seem to have a broad spectrum of antibacterial activity. As a result, these extracts would present major targets, safe and effective in antibacterial therapy and for the preservation of food, and can be used in antiseptic and disinfectant formulations, as well as in chemotherapy.

Keywords: Antibacterial activity, Trigonella foenum-graecum extract, Human pathogenic bacteria

INTRODUCTION:
A number of new antibiotics have produced by pharmacological industries in the last 30 years, resistance to these drugs by microorganisms has been increased day by day. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the medical world. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. This indigenous knowledge, passed down from generation to generation in various parts of the world, has significantly contributed to the development of different traditional systems of medicine[1] as well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency[2]. In the last few years, a number of studies have been conducted in different countries to prove such efficiency[3],[4]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils as well as in tannin[5],[6].

Musa sp. (Musaceae) also known as banana is a familiar tropical fruit and important source of food in the world. From its native South western Pacific home, the banana plant spread to India by about 600 BC and later on it spread all over
the tropical world. It is possibly the world's oldest cultivated crop[7]. It possesses efficient medicinal values such as stem juice is also used in nervous affectations like epilepsy, hysteria and in dysentery and diarrhoea. Several oligosaccharides comprising fructose, xylose, galactose, glucose and mannose occur naturally in banana[8] making it an excellent prebiotic for the selective growth of beneficial bacteria in the intestine.

Trigonella foenum-graecum commonly known as fenugreek is an annual herb indigenous to the countries touching on the eastern shores of the Mediterranean and widely cultivated in India, Egypt, and Morocco [8]. The plant parts like leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations and as an ingredient in traditional medicines [9]. A wide range of uses was found for fenugreek in ancient times. Fenugreek being rich in antioxidants and phytochemicals has been traditionally used as food, forage and medicinal plant [10, 11]. Medicinally it was used in the treatment of wounds, abscesses, arthritis, bronchitis, ulcer and digestive problems. Thus, fenugreek is food and a spice commonly eaten in many parts of the world for many years. Fenugreek is also used traditionally as a demulcent, laxative, lactation stimulant and exhibits hypcholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans [9]. The pharmacological uses of different plant parts of fenugreek have been reported by different researchers. The seeds of fenugreek have been reported to have anti-diabetic [12, 13], anti-cancerous [14], anti-inflammatory [15] and antioxidant activity [16]. Its leaves have been reported to possess potential antibacterial activity [7, 17-20], antifungal activity [21], anti-diabetic [22] and antioxidant property [18, 23, 24]. Fenugreek is an ancient medicinal plant as the plant contains active constituents such as alkaloids, flavonoids, steroids, saponins, etc. [8]. The aim of the present study is to explore evaluation of anti-bacterial activity of musa spp and trigonella foenum –graecum extract against human pathogenic bacteria.

MATERIAL AND METHODS

1. MATERIALS

A. Ingredients of bacteriological media

I. Agar -agar type I (Hi - media lab Pvt. Ltd.)

II. Beef extract (Glaxo - Lab Chemical Division)

III. D - Mannitol (Hi - media Laboratories)

IV. Dextrose sugar (Hi - media Lab Pvt. Ltd)

V. D - lactose (Sarabhai M. Chemicals)

VI. Eosin Water Soluble Yellowish (George T. Gurr Ltd) VII. Mc - conkey Agar Base (Hi - MediaLab. Pvt. Ltd)

VIII. Methylene Blue M. S. (S. D. Fine Chem. Pvt Ltd.)

IX. Peptone- Bacteriological (Glaxo Lab Chemical Division)X Sodium Chloride (Glaxo Lab Chemical Division)

2. Preparations of stains:

A large number of coloured compounds (dye) are available for staining microorganisms. These compounds are generally rather complex in terms of molecular structure. Fixed staining preparation are most frequently used for the observation of the morphological characteristics of bacteria.

The advantages of this procedure are that;

the cells are made more clearly visible after they are coloured.

Differences between cells of different species and within the same species can be determined by use of appropriate staining solutions (differential or selective staining).

Stain for Gram's staining:
Gram staining is one of the most important and widely used differential techniques. This technique was introduced by Christian Gram in 1884. In this process the fixed bacterial smear is subject to the following staining reagents in the order listed.

(a). violet (Primary stain)

1 % crystal violet aqueous solution. W / V

(b). Neutral red

1 gm of neutral red dissolved in 2ml of 1 % acetic acid solution made to 1000 ml in distilled water.

(c). Gram’s iodine solution

20 gm of potassium iodide and 10 gm of iodine crystals dissolved in 1 L of distilled water.

Lacto phenol cotton blue stain:

Phenol crystal 20.0 g, Glycerin 20.0 g, Lactic acid 20.0 g and Water 20.0 ml were mixed with gentle heating. Cotton blue 0.05 g added and dissolved.

3. Preparation of bacteriological media:

I. Nutrient agar

10 g of peptone was mixed with 5 g of beef extracts and 5 g of sodium chloride. These ingredients were mixed to 1000 ml of distilled water and pH was adjusted to 7.2. Agar - agar type I was added at the rate of 2 %. The media was autoclaved at 121 oC and 15 lb pressure, dispersed in Petri dish and stored in refrigerator at 4 oC till use.

II. Blood agar

Nutrient agar basal media was prepared and autoclaved. The temperature of the medium was brought to 50 oC and sheep blood was added at rate of 5 % and then dispersed in Petri dishes.

III. MacConkey agar base media

Readymade MacConkey agar base media (Hi Media) was used. Lactose was sterilized at 10 lb pressure in autoclave and added to basal media.

IV. Mannitol salt agar

Beef extract 1 g; Peptone 10g, Sodium chloride 75 g and Phenol red 0.025 g were mixed and dissolved in 1000 ml of distilled water, then pH was adjusted to 7.4. Then 36 10 g of mannitol was sterilized in water bath at 90 oC for 30 minutes and added to basal media.

V. Hugh and Leifson’s medium

2 g of Peptone, 5 g of Sodium chloride, 0.3 g of di - basic Potassium phosphate, and 1 %Bromothymol blue (3ml) were mixed in 1000 ml of distilled water. Agar - agar at rate of 0.5 % was added and then pH was adjusted to 7.1. Glucose was added to the final concentration of 10 %. This medium was autoclaved and distributed in the tubes.
VI. Eosin methylene blue agar

Peptone 10 g, lactose 10 g, Di potassium hydrogen phosphate 2 g, Eosin yellow 0.4 g, and Methylene blue 0.065 g were dissolved in 1000 ml of distilled water. Agar - agar was added at rate of 2 % and final pH was adjusted to 6.8. The media was autoclaved at 121 oC for 15 minutes at 15 lb pressure.

VII. Sabouraud's dextrose agar

Dextrose sugar 10 g, Peptone 10 g, and Agar 20 g mixed in 1000 ml distilled water, and then pH was adjusted to 5.0 - 6.0 and the media was autoclaved at 121 oC for 10 minutes at 10 lb pressure.

4. Methodology

The proceeding of the methodology was as follow:

A. Collection of banana peels and fenugreek seeds.
B. Antimicrobial activity of plants was carried out by testing them against gram positive and gram-negative bacteria.

The following plants were selected for determination of antimicrobial activity.

Musa sp

Trigonella foenum-graecum

All the above-mentioned plants were collected from the local market of the Bapatla. The following parts from the plant were collected for determination of antimicrobial activity.

Musa sp: peels

Trigonella foenum-graecum: seeds

Collected plant parts were washed and cleaned by muslin cloth and kept for drying for 7 days at 37oC. Then plant parts were ground in to a powder form.

Extraction of plant phytochemical Extraction:

process removal of desirable soluble constituent from a substance, leaving out those which are not wanted, with the aid of solvent and standardized processes.

Extraction is a process in which generally a part is treating with solvent for separating out the active constituents completely or partially.

Plant contains chemical substances some of which provide relief and a variety of diseased conditions. The isolation of active constituent may be an extremely difficult and expensive process.

If the other constituents have no undesirable effect, the administration of the unprocessed drug or its partially purified extracts may provide the 38 desired therapeutic effect.

In recent years active principles from both plants have been isolated or obtained as purified products of precisely known potency and stability.

The solvent used for extraction is known as ~Menstruuml and the undissolved residue left behind after the process is called ~Marcell.

The process of drug extraction can be summarized in to these steps.

1. Penetration of the solvent in to the drug.

2. Dissolution of the constituent.
3. Outward diffusion of the solutions from the cells.

**Maceration**
Maceration was a popular and inexpensive homemade technique for the preparation of tonics since a long time. Moreover, this technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration procedure consists of multiple steps in extraction. The whole or coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent. This process is done in a closed vessel where an appropriate solvent (menstruum) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known as marc to recover an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes: (1) promotes diffusion, (2) separates concentrated solution from the samplesurface by adding new solvent to the menstruum for increasing the extraction yield.

**PRELIMINARY PHYTOCHEMICAL INVESTIGATION**

The present study was aim to prepare alcoholic extracts from seeds. The prepared extracts were subjected to qualitative chemical examination to identify the different phytoconstituents present in Trigonella foenum-graecum.

**Collection of plant material:**

The plant Trigonella foenum-graecum was collected from its natural habitat i.e., from the compound surroundings of Bapatla college of pharmacy, Bapatla district, Andhra Pradesh, India. **Preparation of the plant material:**

The seeds were separated from the collected aerial parts of the Trigonella foenum-graecum and subjected to shade drying for complete removal of moisture from the selected parts and for the better identification of the components by phytochemical screening in extract. The process was continued till both the selected parts were absolutely dried in order to facilitate their proper grinding. The completely dried seeds were grinded into optimal coarse powder.

![Fig 1. Dried seed powder](image-url)
Successive solvent extraction:

The seeds were subjected to successive solvent extraction by using Maceration process. The extraction was carried out for 6 days with the solvent methanol.

Preparation of methanolic extract:

About 60g of the dried powdered seeds were extracted with methanol by maceration for 6 days. The concentrated extracts was taken in a beaker and evaporated to thick paste on water bath, maintained at around 50 to get methanolic extracts of seeds.

Fig 2. Successive solvent extraction by Maceration

Table 1: The physical characteristics and percentage yield of successive solvent extracts of seeds of Trigonellafoenum-graecum.

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>Extractname</th>
<th>Nature</th>
<th>Color</th>
<th>Odour</th>
<th>Weight of extract (%W/W) ingms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds</td>
<td>Methanol</td>
<td>Thick pasty</td>
<td>Dark Brown</td>
<td>Characteristic</td>
<td>9.16</td>
</tr>
</tbody>
</table>
Qualitative chemical examination for phytoconstituents:
Qualitative tests were conducted for all the above prepared extracts of Trigonella foenum-graecum to identify the various phytoconstituents present in each extract. The various tests and reagents used for examination are given below.

Phytochemical Screening

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extracts

PHYTOCHEMICAL SCREENING OF BANANA PEEL EXTRACT:

The present study was aim to prepare alcoholic extracts from seeds. The prepared extracts were subjected to qualitative chemical examination to identify the different phytoconstituents present in Musa Spp.

Collection of plant material:

The plant Musa Spp was collected from its natural habitat i.e., from the compound surroundings of Bapatla college of pharmacy, Bapatla district, Andhra Pradesh, India.

MATERIALS AND METHOD

CHEMICALS: All the chemicals used were of analytical grade Methanol, Ethanol, Folin’s phenol reagent, Anthrone, Bovine serum albumin, Alkaline copper solution, Silica gel-G, Acetic acid, DPPH, 2, 4 dinitrophenyl hydrazine (2,4DNPH), Trichloro acetic acid, Sulphuric acid (85%), Sodium carbonate.

PLANT SOURCE:

The musa paradisiaca (banana) were purchased from the local market. The fruit was washed thoroughly with tap water and then with distilled water. The peels of the fruits were air dried in the sunlight for three days. The dried peel was ground into uniform powder using milling machine. The powder used for extraction preparation.

PREPARATION OF SAMPLE:

Ethanol Extraction:

6gm of musa paradisiaca peel powder was dissolved in 120ml of ethanol. The extract is prepared using soxhlet extractor. The supernatant was collected.

Aqueous Extraction:

6gm of musa paradisiaca peel powder was dissolved in 120ml of distilled water and boiled on slow heat for two hours. It was then filtered through filter paper and the supernatant was collected.

Chloroform Extraction:

6gm of musa paradisiaca peel powder was dissolved in 120ml of chloroform. The extract was prepared using soxhlet extractor. The supernatant was collected.

ANTIMICROBIAL ACTIVITY AND MINIMUM INHIBITION CONCENTRATION OF BANANA PEEL

MATERIALS AND METHODS:

A. Sample collection and preparation

The bananas were purchased from a local super market in Chennai, Tamil Nadu. The banana peels were removed and air-dried for two weeks and ground into powder with a mechanical blender and sieved with a mesh of size less than 0.5 mm. The powdered samples were stored at room temperature for further studies.

B. Preparation of methanol extract
The banana peel powder was washed with distilled water to remove any adherent particles and shade dried. 25g of banana peel sample was mixed with 300 ml of methanol by continuous hot percolation with the help of soxhlet apparatus for 10 hrs. The extract was filtered and concentrated using a rotary evaporator in the temperature range of 50°C-60°C. The concentrated extract was stored in the refrigerator.

C. **Source of microorganisms**

Pure culture of pathogenic bacteria *serratia marcescens*, *pseudomonas aeroginosa*, *bacillus subtilis*, *shigella flexneri*, *vibrio parahaemolyticus* were obtained from Life Tech Research Center, Chennai, Tamil Nadu, India. The organisms were subcultured in a nutrient broth and incubated at 37°C for 24 hr.

D. **Antibacterial activity assay**

Antibacterial activity of extracts was determined by agar disc diffusion method on Muller Hinton agar (MHA) (Nazrul et. al., 1984). The organisms to be tested was inoculated in stock cultures, and maintained at 4°C on a nutrient broth. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tube containing nutrient broth, and were incubated for 24 hrs at 37°C. Muller Hinton agar (MHA) was poured into a petriplate. After the agar solidified, the inocula were spread on the solid plates with sterile swab moistened with the bacterial suspension. The discs were placed in MHA plates and 20 micro litre of samples were added (concentrations: 1000 μg/ml, 750 μg/ml, 500 μg/ml) were placed in the disc. The plates were incubated at 37°C for 24 hr. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition. The inocula tube and the 0.5 McFarland standard were compared against a card with a white background and contrasting black lines. Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension was diluted in a broth. After inoculation, each tube contains approximately 5 x 10⁵ CFU/ml. This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1 x 10⁶ CFU/ml. The subsequent 1:2 dilution in step 3 brings the final inoculum to 5 x 10⁵ CFU/ml. 1 mg of sample was taken and mixed with 1 ml of DMSO obtaining a concentration of 1 mg/ml.

E. **Minimum Inhibitory concentration (MIC) determination**

Minimum inhibition concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The inoculums are prepared by making a direct broth suspension of isolated colonies selected from an 18 - to 24 -hour agar plate (use a nonselective medium, such as blood agar). The suspension was adjusted to achieve a turbidity equivalent of a 0.5 McFarland turbidity standard. This results in a suspension containing approximately 1 to 2 x 10⁸ colony forming units (CFU)/mL for bacterial cultures viz., *Pseudomonas aeroginosa*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus*. This assay consists the determination of chemical agent spectrum of action, according to resistance of studied microorganisms. 1 ml of sterile LB broth was distributed for every tube and was submitted to autoclave under constant pressure at a temperature of 1210°C. After the broth reaches room temperature 1 ml of diluted samples added in tube 1. 1 ml was transferred from tube 1 to tube 2. The transfer was repeated successively until tube 8. 100 μl of bacterial cultures were added to all the tubes from 1 to 8. Incubation was done at 37°C for 24 hrs. After incubation, the turbidity was observed. MIC is the concentration of higher dilution tubes in which there was no bacterial growth.

**Antimicrobial assay by well diffusion method for fenugreek seeds:**

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants extracts. A loop full of gram positive and gram negative bacterial strains such as *E. coli*, *B. subtilis*, *Ps. aeroginosa*, *S.aureus* were inoculated in nutrient broth in a conical flask and inoculated for 24 hrs to activate the strain in agar well diffusion method. The agar plate surface is inoculated by spreading a volume of the microbial inoculums over the entire agar surface. Before the assay the bacteria were sub cultured and used in assay. Then, a hole with adiometer of 6 to 8 mm is punched aseptically with a sterile cork borer the volume of plant extract solution is introduced into the wells. Then, agar plates are incubated for 24 hr. at 34°C. 24 hr. after the introduction of solvent extracts with different dilutions the anti-bacterial activity were observed.

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
RESULTS AND DISCUSSION:

**TABLE 2: PHYTOCHEMICAL CONSTITUENTS OF BANANA PEELEXTRACTS**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>PHYTOCHEMICALS</th>
<th>WATER</th>
<th>ETHANOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Anthroquinon</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**TABLE 3: PHYTOCHEMICAL CONSTITUENTS OF FENUGREEK SEED EXTRACT**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>PHYTOCHEMICALS</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>quinones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>terpenoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Alcoholic and aqueous extract exhibited a higher degree of antimicrobial activity tested against various infections causing by bacterial species. Banana peel and fenugreek seed extract contains large amount of tannins, flavonoids, glycosides, saponins and alkaloids. The antibacterial activity may be indicating the presence of these secondary metabolites.

**TABLE 4: PHYTOCHEMICAL CONSTITUENTS OF COMBINED EXTRACT(FENUGREEK SEED AND
BANANA PEEL

<table>
<thead>
<tr>
<th>S.NO</th>
<th>PHYTOCHEMICALS</th>
<th>WATER</th>
<th>ETHANOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test for Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Test for Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Test for Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Test for Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Test for Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Test for Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Test for Anthroquinon</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Test for cardiaglycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Test for steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Test for quionons</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The antibacterial activity may be indicating the presence of these secondary metabolites. Alcoholic and aqueous extract exhibited a higher degree of antimicrobial activity tested against various infections causing by bacterial species.

**ANTI BACTERIAL ACTIVITY OF BANANA PEEL EXTRACT**

In vitro anti bacterial activity of methanol extracts of banana peel was done by disc diffusion method against four bacterial strains. The result of antibacterial activity of the methanolextracts of banana peel as shown in Table-1. The zone of inhibition of the growth of the isolates was obtained to be a function of the relative antibacterial potency of the extracts. The zone of inhibition decreased as the concentration of the extract decreased. The highest zone of inhibition was obtained at a concentration of 1000µg/ml.

**TABLE 5: Zone of inhibition of banana peel extract by well diffusion method**

<table>
<thead>
<tr>
<th>Name of microorganisms</th>
<th>Standard Ampicillin(mm)</th>
<th>1000 µg/ml (mm)</th>
<th>50 µg/ml (mm)</th>
<th>500 µg/ml (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.Coli</td>
<td>12</td>
<td>15</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Pseudomonasaeroginoua</td>
<td>06</td>
<td>09</td>
<td>07</td>
<td>07</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>20</td>
<td>27</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>S.aureus</td>
<td>06</td>
<td>12</td>
<td>07</td>
<td>8</td>
</tr>
</tbody>
</table>

**Table 6: Zone of inhibition of fenugreek seed extract by well diffusion method**

<table>
<thead>
<tr>
<th>Name of microorganisms</th>
<th>Standard Ampicillin(mm)</th>
<th>1000 µg/ml (mm)</th>
<th>50 µg/ml (mm)</th>
<th>500 µg/ml (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.Coli</td>
<td>10</td>
<td>14</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>
Banana peel and fenugreek seed extract shows ZOI more than the standard. Combined extract at a concentration of 50 µg/ml shows less ZOI when compared with std and the results are shown in the above table. The potent activity of extract at a concentration of 1000 µg/ml shows ZOI more than the std in well diffusion method against four pathogenic bacteria.

CONCLUSION:
Some of the extracts had a good potential for therapeutic uses against some pathogens. It appears that extracts with high antibacterial activity against Gram-negative bacteria do not necessarily have high activity against other Gram–negative bacteria compared to Gram-positive bacteria. This may mean that the activity is not related to the differences in cell wall structure.

Because there is such a wide range of MICs for different strains of the same bacterial species, it is dangerous to generalize these results for one strain of each of the bacteria although these strains were the strains recommended by the National Committee for Clinical Laboratory Standards to compare different antibiotics. Further investigation is underway on the two species E.coli and Staphylococcus aureus that had promising potency and safety.

Our result suggest that the Methanolic extract of banana peel & fenugreek seeds is having potential source of antimicrobial activity. Further phytochemical analysis is initiated to isolate the elements of peel that show a wide spectrum of pharmacological activity.

The potency of many of the extracts on the test bacteria was apparently not due to the presence of a general metabolic toxin but possibly through another mechanism of action. It may be interesting to investigate the mode of action of the extracts against test bacteria and resistant clinical strains. Comparison of our results with those of the literature showed that the antibacterial activity of the plant extracts was very variable depending on the phytochemical composition of the plant, the solvents used for the extraction, and the bacteria tested. These plants seem to have a broad spectrum of antibacterial activity. As a result, these extracts would present major targets, safe and effective in antibacterial therapy and for the preservation of food, and can be used in antiseptic and disinfectant formulations, as well as in chemotherapy.

REFERENCES:
7. Yussoff NAB. Penang: Universiti Sains Malaysia; 2008. Correlation between total phenolics and mineral content with antioxidant activity and determination of bioactive compounds in various local bananas (Musa sp.)