WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 10, 675-681. Research Article ISSN 2277–7105

COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF GUAVA WITH GENTAMYCIN AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Article Received on
16 July 2017,
Revised on 04 August 2017,
Accepted on 25 August 2017
DOI: 10.20959/wjpr201710-9319

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ABSTRACT

Psidium guajava Linn (Guava) commonly known for its food and nutritional values. The medicinal properties of leaves of Psidium guajava Linn are also well known in traditional system of medicine. Five grams of powder were used for crude solvent extraction in Chloroform, Ethanol, Petroleum Ether and Water. The solvents were evaporated to dryness and compound was used for the antibacterial assay by disc diffusion method. The bacterial pathogens were used as Staphylococcus aureus and Escherichia coli. The result was found that, the extracts of Guava leaves were inhibited the growth of Escherichia col to maximum extent than other pathogens. Finally, it was concluded that leaves extract of Psidium guajava Linn plant was shown effective and efficient result against bacterial pathogen used. Psidium guajava leaves could serve as good source of antibacterial agents.

KEYWORDS: Psidium guajava, E. coli, S. aureus.

INTRODUCTION

Guavas (singular guava) are common tropical fruits cultivated and enjoyed in many tropical and subtropical regions.

Psidium guajava (common guava, lemon guava) is a small tree in the *Myrtle* family (*Myrtaceae*), native to Mexico, Central America, and northern South America. Although related species may also be called guavas, they belong to other species or genera, such as the "pineapple guava."

Escherichia coli

Escherichia coli is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections. All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative

Staphylococcus aureus

Members of the genus Staphylococcus (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters.

EXPERIMENTAL

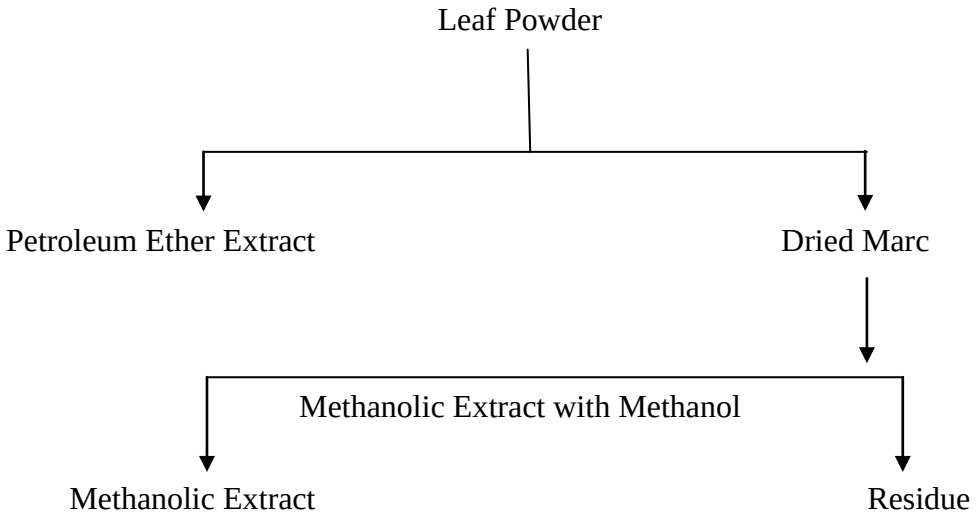
Materials and method

2.1 Collection of Plant Materials and Leaves

The leaves were collected from medicinal garden of Manoharbhai Patel Institute of Pharmacy, Gondia. The collected leaves were shade dried for 7 days and finally pulverized in to coarse powder. It was stored in a well closed container free from environmental climatic changes till usage.

Preparation of Extracts

Successive extraction of plant material show in flow chart.



Plant Extraction

Soxhlet extraction

Different components which used in Soxhlet extraction like thimble, water cooling system, and reservoir, by pass tube, siphon tube and condenser can be seen in figure 2. We will take

10 mg of solid material of leaves keep in thimble which is loaded into soxhlet vessel having flask containing extractor solvent. Solvent vapor moves up to the column and floods into the chamber housing the thimble of solid. Some part of non volatile compounds dissolves in solvent. Process repeats many times until we get desired concentrated compounds in flask. Process has been done at boiling temperature of solvent and extraction has been done in 100 ml ethanol for 3.5 hours.

Microorganism

The test organisms include Gram negative bacteria: Escherichia coli (E. coli), and one Gram positive bacteria Staphylococcus aureus (S. aureus).

They were previously isolated, identified and stored in the Department of Microbiology of Manoharbhai Patel Institute of Pharmacy, Gondia.

Agar Well Diffusion Assay

The antimicrobial activity of the leaf extracts was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth to match the turbidity of 0.5 McFarland standards to be inoculated on Muller-Hinton agar.[7] After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Once wells were formed, they were filled with plant extracts and blank. Commercially available gentamycin (10 µg) discs were used as a positive control in this study. Plates were incubated for 24 h at 37 °C to allow leaf extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different leaf extracts against different bacteria were measured in milliliter for further analysis.

Antibacterial Activity

Antimicrobial susceptibility testing was done using the well-diffusion method according to the standard of the National Committee for Clinical Laboratory Standards The plant extracts were tested on Mueller Hinton II plates to detect the presence of antibacterial activity. Prior to streaking the plates with bacteria, 5 mm diameter wells were punched into the medium using a sterile borer. All plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 McFarland standard solution; a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculums. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of

inoculums with a final swab around the rim. The plates are allowed 3 to 5 min to dry the excess moisture. Fifty ml aliquots of each test extract was dispensed into each well after the inoculation of the plates with bacteria. The wells were also arranged in a triangle formation 2 inches apart. The same extract was used on each plate, with a total of three plates used for each extract for selecting bacterium. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with parafilm, labeled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments.

Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the guava were carried out with the extracts using the standard procedure as described. For each test, 1 ml of each solvent extract was used for analysis, in exception for the saponin test in which 3 ml solvent extract was used.

Test for Saponins

Extract was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Phenols and Tannins

Extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Terpenoids (Salkowski's Test)

Extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase was formed to show positive results for the presence of terpenoids.

Test for Flavonoids (Shinoda Test)

Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

Test for Glycoside

Extract was mixed with 2 ml of glacial acetic acid containing 2 drops of 2% $FeCl_3$. The mixture was poured into another tube containing 2 ml of concentrated sulfuric acid. A brown ring at the interphase indicates the presence of glycosides.

OBSERVATION

1) Soxhlet Extraction

Table 1 :- Extraction of Powder Of Guava Leaf

Type Of Extract	Amount of Extract (gm)	Yield (ml) after extraction	Appearance
Petroleum Ether	Petroleum Ether-900 ml Powder- 500 gm	250ml	Greenish Brown
Methanolic extract	900ml	200ml	Brownish Black

2) Agar Well Diffusion Method: Table.2.Plant Extract.

Plant Extract	Zone of Inhibition	
	S.Aureus	E.Coli
Methanol	23 mm	9mm

Table.3: Antibiotic.

Antibiotic	Zone of Inhibition	
	S.Aureus	S.Aureus
Gentamycin	21mm	7mm

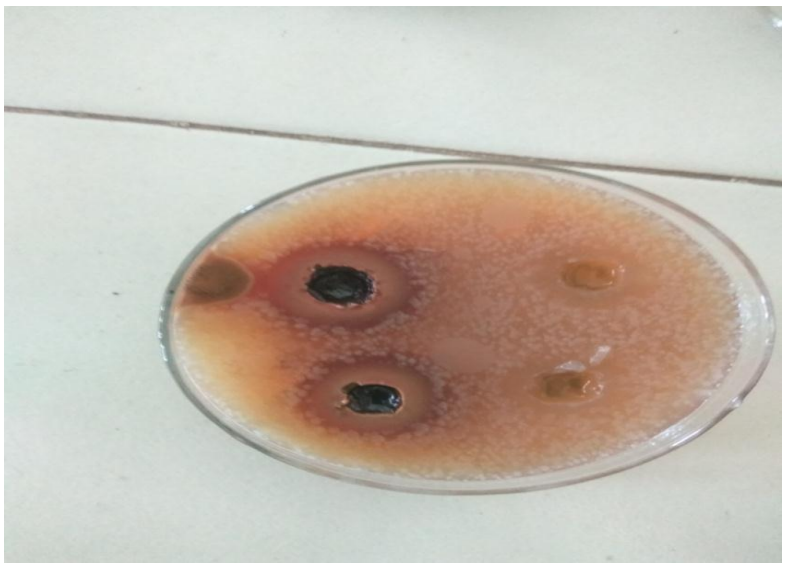


Fig: Methanolic extract.

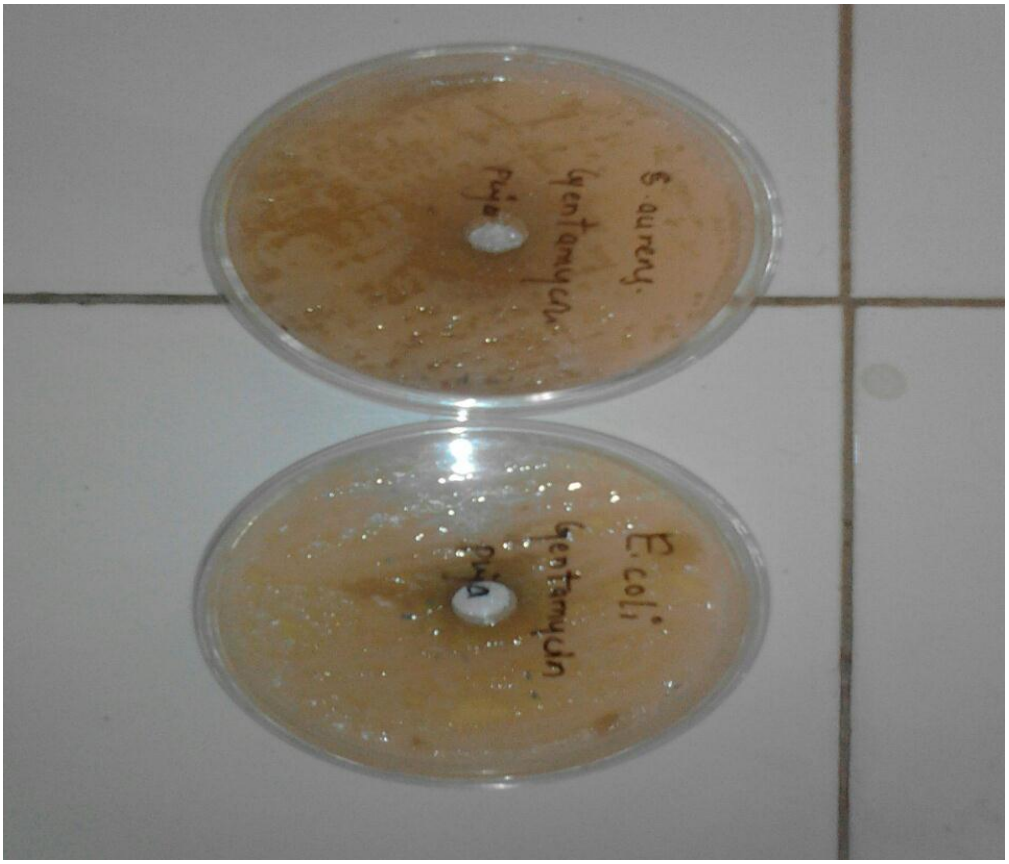


Fig. Antimicrobial activity of drugs with bacteria.

Phytochemicals Test

Table. 4.

Chemicals Test	Petroleum Ether Extract	Extract
Alkaloids		
Mayer's Reagent	-	-
Drangendorff'sReagent	-	+++
Wagner's Reagent	-	-
Hager's Reagent	-	+++
Saponin		
Froth test	-	-
Sterols		
Salkowasld test	-	++
Leibermann's Reagent	-	++
Leibermann's Burchards	-	++
Carbohydrates		
Molish Test	-	++
Fehling's test	-	++
Anthraquinone glycoside		
Borntrager's test	-	-
Cardiac Glycosides		
Legal's test	-	-
Killer killani test	-	-

Tannins		
Lead acetate solution	-	+++
Ferric chloride test	-	++
Proteins		
Xanthoproteic test	-	-
Biuret test	-	-
Flavonoids		
Ammonia test	-	++
Alkaline Reagent test	-	++
Magnesium ribbon test	-	++

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