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EFFECT OF DIFFERENT EXTRACTS OF PSIDIUM GUAJAVA LEAVES ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Madhuri Shinde[1]*, Abhilasha Sawant[2], Pallavi Dhekale[3] and Ajay Sharma[4]

Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara 415015, Maharashtra, India.

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***Corresponding Author**
Madhuri Shinde
Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara 415015, Maharashtra, India.

ABSTRACT

The present study was carried out to evaluate the antimicrobial and antifungal activity of *Psidium guajava* against three clinically important bacteria and one fungus namely Staphylococcus aureus, E. coli, Bacillus and Candida glaberata, the antimicrobial and antifungal activity of different extracts was done with agar well diffusion assay in plates containing nutrient agar media. The antimicrobial activity of methanolic extracts showed that all the combinations of extracts were effective against the test microorganisms. The patterns of inhibition varied with the different solvent extracts as the tested microorganisms. The methanolic extract of *Psidium guajava* showed effective antibacterial and antifungal activity.

KEYWORDS: *Psidium guajava*, Antibacterial and antifungal activity, agar well diffusion assay.

INTRODUCTION

Antibiotics provide the basis for the fungal and bacterial infections therapy. The discovery of antibiotics and making use of them as chemotherapeutic agents has made the medical fraternity to believe that they will eradicate various infectious diseases. However, indiscriminate use of antibiotics in human and veterinary healthcare systems has led to the emergence of multi-drug resistant (MDR) strains of different groups of microorganisms. The emergence and dissemination of MDR bacteria has made chemically synthesized antibiotics ineffective for the treatment of infectious diseases caused by such bacteria. These circumstances have propelled the researchers and scientists to explore new antimicrobial substances from various sources such as medicinal plants. There are many studies that have

described different type of plants such as herbs, shrubs and trees with the aim of knowing their phytoconstituents and using them for the treatment of various diseases as possible alternatives to the synthetic drugs. The screening of plants for medicinal purposes represents a serious effort to discover newer, safer, and possibly more effective drugs with the potential of fighting pathogenic bacteria and fungi. The green medicines are widely believed as safe and dependable in contrast with expensive synthetic drugs that have undesirable side effects along with beneficial effects. The plants have been in use in traditional medicine worldwide since long time but are still understudied, particularly in clinical microbiology. In past few decades, the curiosity to evaluate plants possessing antimicrobial, antifungal, anti-inflammatory activity for various diseases has grown many folds and a large number of biologically active compounds have been characterized. Several studies have established that many plants contain substances like peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others, which have antimicrobial properties.

Psidium guajava is member of the family Myrataceae, which is very common in the tropical countries and known by the English name Guava. 2 The Guava is a phytotherapic plant, which contains active components that help to treat various diseases like malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions. The components present in Guava include lectins, phenols, tannins, flavonoids, essential oils, fatty acids, vitamins, etc. However, most of the medicinal properties of the Guava are attributed the presence of flavonoids. Hence, the present study was initiated to evaluate the antibacterial and antifungal activity of methanol, chloroform and aqueous leaves extracts of P. guajava against some gram-positive and gram-negative bacterial and fungal strains.[6,7,8,9,10]

MATERIALS AND METHODS

Sample collection and extraction[1,2,11,13,14]

The shade dried 100 gm coarse powdered of leaves of P. guajava plant was immersed in 200 ml of different solvents (methanol, chloroform and aqueous) contained in 500 ml sterile conical flasks and covered with cotton wool separately. It was placed aside with intermittent shaking for one week. They were first filtered with double layered muslin cloth and then through Whatman No. 1 filter paper, and the march was discarded. The filtrate was subjected to evaporation by treating at 40°C in an oven to obtain a dried extract. The dried extract was stored at 4°C until used for further study31.

Preparation of stock solutions: The dry powdered extracts of P. guajava dissolved separately in methanol, chloroform and water to get the final concentration of 0.5 mg/ml for each extract.

Test organisms: Microbial strains were obtained from the microbiology laboratory of GIPER, Limb, Satara. In total four microorganisms were tested against the above mentioned plant extracts in which was Gram positive Staphylococcus aureus, bacillus, Gram negative namely Escherichia coli and Candida glaberata fugal strains. The strains were maintained on nutrient agar slants at 4°C.

Preparation of culture media: Nutrient Agar media was prepared by suspending 38 g in 1000 ml of distilled water. The media was sterilized by autoclaving at 121°C for 15 min and poured into sterile Petri plates at around 50°C. To observe the effect of pH on the growth of tested bacteria, pH was adjusted by adding 0.1M HCL or 0.1M NaOH into the media.

Antibacterial susceptibility Test: All the plant extract were screened against pathogenic bacterial strains. The tested organisms were Escherichia coli, staphylococcus aureus and Bacillus. The disc diffusion method was used to test the antimicrobial activity of the plant Extract. 20ml of sterilized nutrient agar medium for E. coli, staphylococcus aureus, Bacillus were poured into each sterile petridish. The plates were allowed to solidify for 5min and 0.1 % inoculum suspension was swabbed uniformly. The entire agar surface of the each plate was inoculated with this swab first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper disc soaked in 1ml of the plant Extract or loaded with 5mg/disc, of dry extract and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5min and then the plates were incubated at 37°c for 24hr. At the end of incubation, inhibition zone formed around the disc were measured with transparent ruler in millimetre. These studies were performed in triplicate.[1,3,4,9]

Antifungal susceptibility Test[1,2,11,13,14]

All the plant extract were screened against pathogenic fungal strains. The tested organism was candida glaberata. The disc diffusion method was used to test the antimicrobial activity of the plant Extract. 20ml of sterilized nutrient agar medium for candida glaberata were poured into each sterile petridish. The plates were allowed to solidify for 5min and 0.1 % inoculum suspension was swabbed uniformly. The entire agar surface of the each plate was

inoculated with this swab first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper disc soaked in 1ml of the plant extract or loaded with 5mg/disc, of dry extract and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5min and then the plates were incubated at 37 °c for 24hr. At the end of incubation, inhibition zone formed around the disc were measured with transparent ruler in millimetre. These studies were performed in triplicate.[1,3,4,9] Agar plates wells were then filled with the plant extract solution (ranging 10 µl–25 µl) in the combinations. Petri plates were placed for 30 min in refrigerator for diffusion of extracts and then incubated at temperatures 37°C for 24 hours. At the end of the incubation period, the zone of inhibition (including well diameter) was measured. The experiment was carried out thrice independently in duplicate and the mean of all the readings is mentioned in the results.[5]

RESULT AND DISCUSSION[1,2,11,13,14]

The different extracts of *P. guajava* were tested for their antimicrobial activity in combinations of concentration. The tested plants have been in use as folk medicine and were familiar to the local people. The results of the antimicrobial activity of methanolic extracts showed that all the concentrations were effective against tested microorganisms with varying zones of inhibition. The methanolic extract of *P. guajava* was found to be inhibition against all bacteria and fungi. The diameter of zones of inhibition exhibited by S. aureus were 19 mm,E. coli 23 mm, Bacillus19mm and Candida glaberata 21 mm. The chloroform extract of *P. guajava* was found most inhibitory to S. aureus showing zone of inhibition of 20 mm (Table 1). This combination further inhibited growth of Candida glaberata fairly well (19 mm zone diameter). The water extract of *P. guajava* was found to be inhibition against all bacteria and fungi. The diameter of zones of inhibition exhibited by S. aureus was 21 mm, E. coli 22 mm, Bacillus 24 mm and Candida glaberata 19 mm.

Table. 1: Zone of inhibition: Antifungal and Antibacterial activity.

Sr. No.	Organism	Inhibition zone		
		Methanol	Chloroform	Water
1	Bacillus	26mm	21mm	24mm
2	E.coli	23mm	23mm	22mm
3	C. glabereta	21mm	19mm	19mm
4	S.Aureus	19mm	20mm	21mm

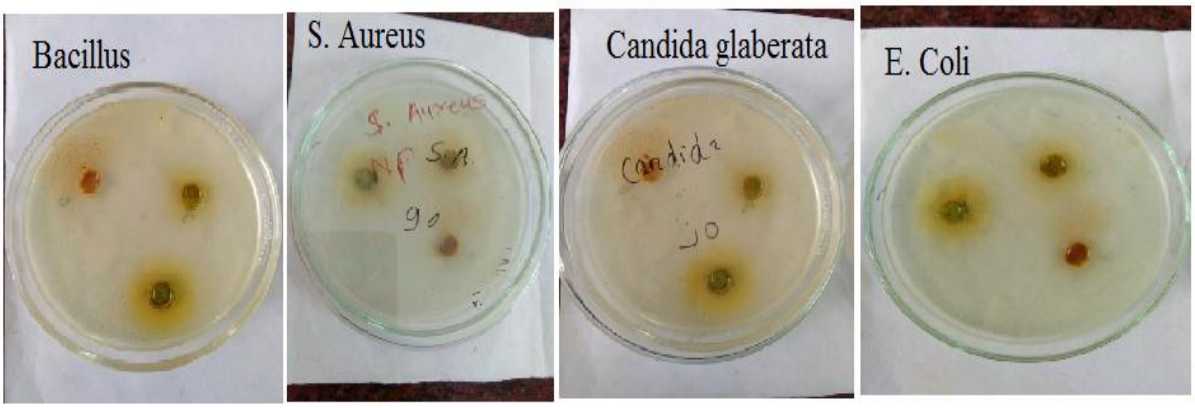

Figure. 1: Typical agar plates showing the growth of inhibition zones by C. officinalis and P. guajava.

CONCLUSION

The result of present study indicates that the methanolic extract of P. guajava leaves possessed good antimicrobial potential. Both extract showed antifungal & antibacterial activity that is comparable high antifungal activity was showed in the leaves.

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