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# ANTIBACTERIAL ACTIVITY OF *PSIDIUM GUAJAVA* L. LEAVES EXTRACTS AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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## ABSTRACT

Antibacterial efficiency of *Psidium guajava* L. leaves extracts in different solvents (methanol, ethanol and aqueous) were studied against seven different bacterial strains, including four gram-positive (*Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus* sp.) and three gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) using agar well diffusion assay (AWDA) method. In the present study, aqueous extract gave the highest yield percentage as compared to methanol as well as ethanol extracts. The patterns of inhibition varied with the solvent as well as the tested microorganisms. The results showed that *M. luteus* among the gram-positive and *E. coli* among the gram-negative were highly susceptible as compared to other tested organisms. The methanol extract of *P. guajava* was the most effective as the widest inhibitory zone was observed as compared to the ethanol and aqueous extracts. The minimum inhibitory concentrations (MIC) as well as the minimum bactericidal concentration (MBC) of the crude leaves extracts were determined for the various organisms which ranged between 12.5 to 100 mg/ml. The remarkable antibacterial activity of the leaves extracts against tested gram-positive and gram-negative bacteria suggests that the *P. guajava* plant leaves could be a possible source of novel broad spectrum drug for treating infectious diseases.

**KEY WORDS**: Antibacterial; *Psidium guajava*; Solvents; Zone of Inhibition.

## INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the three levels of biodiversity, namely species, genetic and habitat diversity. In India thousands of plant species are known to have medicinal value (Mahalingam, *et al*., 2011). Medicinal plants have been used to sustain human health and to treat various maladies including infectious diseases since antiquity (Sachin *et al*., 2011). The infectious diseases, caused by bacteria, viruses, fungi, or parasites have led to significant morbidity and mortality to population worldwide (Mahady, 2005; Lana and Julia, 2008).

Synthetic antimicrobial agents such as antibiotics are widely used to cure infections, but their indiscriminate use causes antimicrobial drug resistance, necessitating the use of medicinal plants as the alternative therapeutic agents. Medicinal plants and plant-derived products are cost-effective and easily obtainable and have promising efficacy to treat intractable infectious diseases, and thus they may be useful in eradicating new emerging microbial strains (Chanda and Baravalia, 2010). In addition, they have profound safety profile because they cause fewer side effects such as hypersensitivity, allergic reactions, and immunosuppression as compared to commercial antimicrobials (Sachin *et al*., 2011).

Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines have already formed the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developing world too (De and Ifeoma, 2002). Plants provide an alternative strategy in the search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties (Kayode and Kayode, 2011). It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved drugs (Shah *et al*., 2006).

Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases (Natarajan *et al*., 2003).

*Psidium guajava* L. (Myrtaceae) is an evergreen shrub native to tropical America that has naturalized in southeast Asia. It is a medium sized tree with evergreen, opposite, aromatic, short-petiolated leaves. It is a phytotherapic plant used in folk medicine that has been used for the management of various disease conditions and is believed to act. Various parts of this plant has been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Abdelrahim *et al*., 2002; Kumar, 2015). Thus its uses in traditional medicine are well established against enteric human bacteria. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity (Begum *et al*., 2004; Sunagawa *et al*., 2004). Leaves and bark of *P. guajava* plant has a long history of medicinal uses that are still employed today (Kumar, 2012). Hence, the present study was initiated to evaluate the antibacterial activity of methanol, ethanol and aqueous leaves extracts of *P. guajava* against some gram-positive and gram-negative bacterial strains.

**Figure 1: Photo of *Psidium guajava* L. plant**

## MATERIALS AND METHODS

### Sources of bacterial strains

Bacterial strains, including both gram-positive and gram-negative obtained from M.D. University, Rohtak, Haryana and Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh. The bacterial strains include *Bacillius subtilis*, *Micrococcus luteus* (MTCC106), *Staphylococcus aureus* (MTCC6908), *Streptococcus* sp. (MTCC9724), *Escherichia coli* DH5α, *Pseudomonas aeruginosa* (MTCC4673) and *Salmonella typhimurium* (MTCC3224) have been selected for the present study.

### Culture of bacterial strains

The bacterial strains were propagated in the nutrient broth medium (5g/l peptone, 3g/l beef extract, 5g/l NaCl, and pH 7.0) incubated for 18hr at a respective growing temperature. Slants were prepared from the separated colonies of bacteria, stored at 4°C temperature and sub-cultured in a nutrient broth medium before testing the activity. The chemicals were purchased from Hi-media, Mumbai, India.

### Preparation of plant material

The collected leaves were thoroughly washed under tap water, dried in the shade for one month and then ground into coarse powdered with the help of mortar and pestle. These powders were stored in airtight brown bottles at 4°C until needed for future use.

### Extraction of plant material (Maceration)

The shade dried 100 gm coarse powdered of leaves of *P. guajava* plant was immersed in 200 ml of different solvents (methanol, ethanol 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 study (Atata *et al*., 2003; Jeyaseelan *et al*., 2012).

### Yield percentage of solvent extracts

After the drying, yield of each extraction was measured separately and the extraction efficiency was quantified by determining the weight each of the extracts and the yield percentage was calculated as dry weight/dry material weight ×100 (Parekh and Chanda, 2007).

### Antibacterial activity by agar well diffusion assay method

The antibacterial activity of crude solvents (methanol, ethanol and aqueous) leaves extracts of *P. guajava* against gram-positive as well as gram-negative bacterial strains were evaluated by agar well diffusion assay (AWDA) method (Parekh and Chanda, 2007; Kumar and Gitika, 2014). The diameters of the inhibition zones were measured in millimeters (mm). For this, a well (6mm diameter) was made with the help of a borer in cooled nutrient agar plate, overlaid with soft agar (5ml), seeded with a target strain (~$10^6$ cfu/ml). Aliquots of the test compound (100µl) were introduced into the well and the plates were incubated for overnight at 37ºC. For each bacterial strain, the dissolving solvent 10% DMSO and streptomycin (50 µg/ml) were used as negative and positive controls respectively. To test the antibacterial activity of all extracts were dissolved in 10% DMSO solvent to make a final concentration 200 mg/ml.

### Determination of minimum inhibitory concentration (MIC)

The MIC is the concentration giving the least inhibitory activity and below which there is no further inhibition were determined by using the Broth dilution method (Adesokan *et al*., 2007). Briefly, 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100 mg/ml. 1.0 ml of this dilution was transferred to another test tube till the $7^{th}$ test tube was reached. The $8^{th}$ test tube did not contain any extract, but a solution of pure

solvent and served as negative control. Then 1ml of 18hr grown cultures of each of bacterial strains, adjusted at ~ $1x10^6$ cfu/ml was put into each tube and thoroughly mixed by vortex mixer. The tubes were incubated at 37°C for 18hr and observed the growth in the form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection considered the MIC's value.

**Determination of minimum bactericidal concentration (MBC)**

The MBC values were determined by removing 100 µl of bacterial suspension from the MIC positive tube as well as one above and one below the same tube, spread on nutrient agar plates and incubated at 37°C for 18hr. After incubation, the plates were examined for colony growth and MBC's were recorded (Rahman *et al*., 2008; Nand *et al*., 2012).

**Statistical analysis**

The experiments were carried out in three independent sets, each consisting of 3 replicates. Values shown here represent mean ± standard error of the mean (SEM).

**RESULTS**

In the present investigation, after the drying, the percentage yield of *P. guajava* plant leaves extracts with the various solvents (methanol, ethanol and aqueous) was measured separately and quantified the efficiency of extraction. The results of the present investigation, aqueous extraction gave the highest yield percentage (16.37%) followed by methanol (14.22%) and ethanol (12.40%) shown in Table 1.

**Table 1: Yield percentage of *P. guajava* plant leaves extracts in different solvents**

| Solvent | Yield percentage of extracts (gms) | | |
|---|---|---|---|
| | Weight of dry powder | Weight of dry extracts | Yield percentage |
| Methanol | 100 | 14.22 | 14.22 |
| Ethanol | 100 | 12.40 | 12.40 |
| Aqueous | 100 | 16.37 | 16.37 |

The various solvents (methanol, ethanol and aqueous) leaves extracts produced the inhibitory activity against all the tested seven bacterial strains, including both gram-positive and gram-negative as shown in Figure 2. The maximum zone of inhibition was recorded for the methanol extracts against *B. subtilis* (21), *M. luteus* (23), *S. aureus* (20), *Streptococcus* sp. (18), *E. coli* (22), *P. aeruginosa* (14) and *S. typhimurium* (17). The ethanol extracts showed against *B. subtilis* (18), *M. luteus* (21), *S. aureus* (19), *Streptococcus* sp. (16), *E. coli* (20), *P. aeruginosa* (12) and *S. typhimurium* (15). Similarly, aqueous extracts produced inhibitory zone against *B. subtilis* (16), *M. luteus* (20), *S. aureus* (15), *Streptococcus* sp. (14), *E. coli* (17), *P. aeruginosa* (10) and *S. typhimurium* (12).

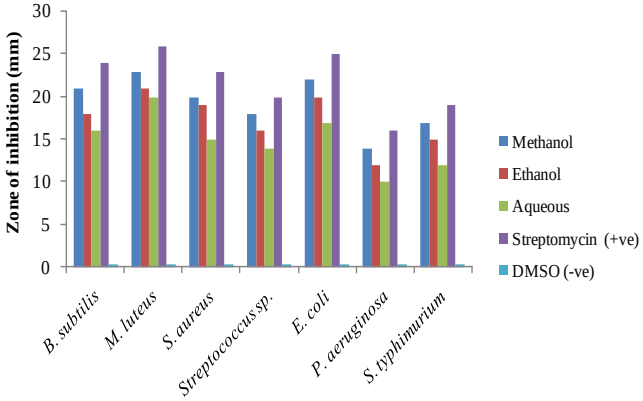


**Figure 2: Antibacterial activity of *P. guajava* leaves extracts**

However, streptomycin used as a positive control exhibited higher inhibition as compared to used different solvent extracts with the zones against *B. subtilis* (24), *M. luteus* (26), *S. aureus* (23), *Streptococcus* sp. (20), *E. coli* (25), *P. aeruginosa* (16), and *S. typhimurium* (19), while DMSO doesn't produced activity.

In the present study, MIC values were calculated for methanol, ethanol and aqueous leaves extracts by broth dilution method, shown in Figure 3. The methanol extract exhibited the MIC values 12.5mg/ml against *B. subtilis*, *M. luteus*, *S. aureus* and *E. coli*; 25mg/ml against *Streptococcus* sp. and *S. typhimurium*; 50mg/ml against only one bacterial strain *P. aeruginosa*. Ethanol extract showed 12.5mg/ml against *M. luteus* and *E. coli*; 25mg/ml against *B. subtilis*, *S. aureus* and *Streptococcus* sp.; 50mg/ml against *S. typhimurium* and *P. aeruginosa*. Similarly, samples of aqueous extract possessed 12.5mg/ml against only *M. luteus;* 25mg/ml against *B. subtilis* and *E. coli*; 50mg/ml against *S. aureus*, *Streptococcus* sp. and *S. typhimurium*; 100mg/ml against only *P. aeruginosa*.

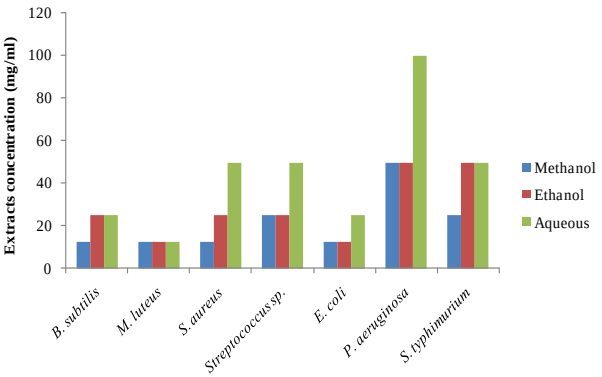


**Figure 3: MIC (mg/ml) values of *P. guajava* leaves extracts**

The MBC values of methanol, ethanol and aqueous leaves extracts as shown in Figure 4. Methanol extract produced MBC values 12.5mg/ml against *B. subtilis*, *M. luteus* and *E. coli*; 25mg/ml against *S. aureus*, *Streptococcus* sp. and *S. typhimurium*; 50mg/ml against

*aeruginosa*. The ethanol extract exhibited 12.5mg/ml against only *M. luteus*; 25mg/ml against *B. subtilis*, *S. aureus* and *E. coli* 50mg/ml against *Streptococcus* sp., and *S. typhimurium*; 100mg/ml against only *P. aeruginosa*. Similarly, aqueous extract possessed MBC values 25mg/ml against *M. luteus* and *E. coli*; 50mg/ml against *B. subtilis*, *S. aureus* and *Streptococcus* sp.; 100mg/ml against *P. aeruginosa* and *S. typhimurium*.

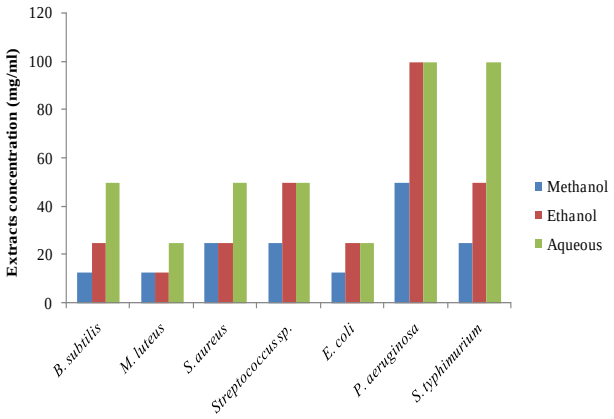


**Figure 4: MBC (mg/ml) values of *P. guajava* leaves extracts**

## DISCUSSION

Medicinal plants play a central role not only as traditional medicines, but also as commercial commodities meeting the demand of distant markets. To compete with the growing market, there is a need to expeditiously utilize and scientifically validate more medicinally useful plants. Because of the appearance of drug resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are a preferable option to synthetic ones. Literature indicates that medicinal plants are the backbone of traditional medicine and the antimicrobial activity of plant extract is due to different chemical agent in the extract with antimicrobial compounds (Ogu *et al*., 2012).

Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant (Akinpelu and Onakoya, 2006; Chopra, 2007; Ogu *et al*., 2010). The ethno-medicinal value of *P. guajava* has been reported by many literatures, but there is little scientific proof for further using this plant commercially or in a more effective form. For this, the yield of extraction was calculated because the crude plant extracts are generally a mixture of active and non-active compounds. A number of medicinal plants described in Ayurveda still need to be testified, according to the modern parameters to ensure their activity and efficacy. Drugs used in Ayurveda are mostly prepared by extraction with water. Therefore healers may not be able to extract all the active compound(s) (Khond *et al*., 2009). The yield percentage of medicinal plant extracts which contain the bioactive metabolites vary considerably with plant species and the method or solvent used for extraction. Also, factors like age of the plant and the polarity of the solvent used may have affected the percentage yield (Yahaya *et al*., 2012). In the present study, an aqueous solvent extract gave the highest yield of extraction followed by methanol and ethanol, and the various solvent extracts produced inhibitory activity against all the tested seven different bacterial strains including both gram-positive and gram-negative with varying degrees. Mohamed *et al*. (2012) mentioned that the methanolic extracts of *P. guajava* leaves showed significant antibacterial activity against *S. aureus* and *E. coli*. However, Biswas *et al*. (2013) reported that the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria (*S. aureus* and *Bacillus cereus*), whereas the gram-negative bacteria (*E. coli* and *Salmonella enteritidis*) were resistant to all the solvent extracts.

According to Egharevba *et al*. (2010) the sensitive organisms exhibited the range of MIC from 1.25 - 10mg/ml, and MBC between 2.5-20mg/ml concentrations. In another study, the methanol and ethylacetate extracts exhibited broader spectrum activity, although the methanol extract was generally more active and had a lower MIC values against most of the organisms, which also support previous works (Rabe and Van Staden, 1997; Goncalves *et al*., 2008). However, in the present study, samples of methanol, ethanol and aqueous extracts produced the MIC as well as MBC values a range between 12.5 to 100 mg/ml against the tested organisms.

## CONCLUSION

There is growing focus on the importance of medicinal plants and traditional health systems in solving the health care problems of the world. In the present study *P. guajava* plant leaves extracts with various solvents possesses significant inhibitory activity against tested gram-positive (*M. luteus*, *B. subtilis*, *S. aureus* and *Streptococcus* sp.) as well as gram-negative bacteria (*E. coli*, *P. aeruginosa* and *S. typhimurium*) and the results in agreement to a certain degree with the traditional uses of this plant. The methanol and ethanol extracts possessed strong antibacterial activity while aqueous extract was not as effective against bacteria which showed the compounds extracted in the alcoholic solutions were more effective than aqueous extraction. Based on the result of this study it can be said that *P. guajava* leaves extracts are an effective antibacterial agent that can be used in folk medicine and will be a good source to treat and control many diseases. These findings could also be of commercial interest to both pharmaceutical companies and research institutes in designing and developing new drugs.

**COMPETING INTERESTS**
The authors declare that they have no competing interests.

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