



## INVITRO ANTIMICROBIAL ACTIVITY OF *PSIDIUM GUAJAVA* L. LEAF ESSENTIAL OIL AND EXTRACTS USING AGAR WELL DIFFUSION METHOD

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### ABSTRACT

The present investigation focuses on the antimicrobial potential of essential oil and solvent based extracts extracted from *Psidium guajava* L. leaf were screened against the selected Gram positive and Gram Negative bacterial strains as well as fungal strains by Agar Well Diffusion method. The essential oil, acetone, methanol and hexane fractions of leaves were evaluated for this study. The antibacterial activity was more effect in acetone and methanol extracts against both Gram positive and Gram negative bacterial strains and fungal strains. Moderate activity was shown against hexane and essential oil. The present study suggests that the essential oil containing compounds that can form the basis for the development of novel broad spectrum antimicrobial formulations. These results support the notion that plant essential oil and extracts may have a many roles as pharmaceuticals.

**Keywords:** Antibacterial, Antifungal, *Psidium guajava* extract, Essential oil.

### INTRODUCTION

For a long period of time, plants have been valuable sources of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs<sup>1</sup>. 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.

Infectious diseases accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance too many antibiotics and as results, immense clinical problem in the treatment of infectious disease has been created<sup>2</sup>. In particular, the antimicrobial activity of plant oils (essential oil) and extracts has formed the basis of many applications, including raw and pharmaceuticals, alternative medicine and natural therapies<sup>3,4</sup>.

The essential oils and their components are known to be active against a wide variety of microorganisms, including Gram negative and Gram positive bacteria. Some times Gram negative bacteria were shown to be generally more resistance than Gram positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane, but this was not always true<sup>5</sup>.

*Psidium guajava* L. is a tropical crop that fruit and leaves have been consumed as herbal medicine and as a nourishing food. It is widely used as antispasmodic, antidiarrhoeal, antidepressant, anti-inflammatory, anticough and sedative effects<sup>6</sup>. The leaves have been used in folk medicine for many years to treat diarrhea, stomach ache and hepatic problems. To date, phytochemical investigations have been reported on the tannins, flavanoids<sup>7, 8, 9 & 10</sup>, essential oils<sup>11, 12</sup>, proteins<sup>13</sup>, Sesquiterpenoids alcohols and triterpenoid acids<sup>14, 15, 16</sup>. The bark, leaves, fruit and root have also been evaluated pharmacologically for the treatment of gastrointestinal diseases<sup>17,18,19,20,21,22,23</sup>. This plant possesses antimutagenic<sup>24</sup> and hypoglycaemic<sup>25,26</sup> antimicrobial<sup>27,28,29</sup> properties.

In this present study was carried out to test the antimicrobial efficacy of the essential oil and extracts of *Psidium guajava* L. leaf with reference to Gram positive, Gram negative bacterial strains and fungal strains.

### MATERIALS AND METHODS

#### Plant material

*Psidium guajava* L. was identified by their taxonomical characters and collected under the supervision of botanist from Kaliyakkavilai (Kanyakumari District) in December 2009. The plant material was authenticated and a voucher specimen of the plant was kept at the Department of Botany (BVAC1002), Nesamony Memorial Christian College, Marthandam, Kanyakumari District, Tamil Nadu, South India.

#### Extraction of essential oil

The essential oil was obtained by hydro distillation using a Clevenger type apparatus for 5 h, from the fresh leaves of *P.guajava*. The oil obtained was dried over anhydrous sodium sulfate overnight and kept in sterile sample tubes in refrigerator. The oil yields were calculated as 0.13%.

#### Preparation of extracts

Leaves of *Psidium guajava* L. were collected air dried and the powered by using homogenizer and 20 grams were used for different solvent extraction such as acetone, methanol, hexane. In solvent extraction, the sample was extracted in a solvent kept on a rotary shaker in one day and night and then filtrate and it collected for centrifugation. The collected extracts centrifuged at 5000 rpm. The solvent was evaporated to dryness under reduced pressure and the extracted compounds were used for antimicrobial screening. The percentage yield of acetone, methanol and hexane extracts 11.6 %, 13.6 %, 6.7 % respectively.

#### Test microorganisms

The bacterial and fungal strains used for the test were *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-121), *Lactobacillus lactis* (MTCC-440), and *Staphylococcus aureus* (MTCC-87), *Lactobacillus acidophilus* (MTCC-447). The Gram negative strains are *Azotobacter species* (MTCC-2903), *Agrobacterium rhizogenes* (MTCC-532), *Enterobacter aerogenes* (MTCC-2822), *Gluconobacter oxydans* (MTCC-904), *Pseudomonas fluorescens* (MTCC-103) and the fungal strains were *Candida tropicalis* (MTCC - 1000), *Aspergillus niger* (MTCC - 281), *Aspergillus aculeatus* (MTCC - 1331). All stock cultures were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Sector 39-A, Chandigarh, U.T., 160 - 036, India.

## Culture media and inoculums preparation

Muller Hinton Agar / Nutrient Broth and Potato Dextrose Agar (Himedia, India) were used as the media for the culturing of bacterial and fungal strains. Loop full of all bacterial cultures was inoculated in the Nutrient Broth (NB) at 37°C for 72 hours and fungal cultures were inoculated in the Rose Bengal Agar (RBA) at 20°C for 220 hrs.

## Antimicrobial activity study

### Agar well diffusion method

The essential oil and extracts obtained from the leaf were used for studying their antibacterial and antifungal activity. A loop full of bacterial strains and fungal strains was inoculated in 30ml of Nutrient Broth and Rose Bengal Agar separately in a conical flask and incubated for 72hrs and 220hrs respectively to get active strain by using agar well diffusion method<sup>30</sup>. Muller Hinton Agar was pored into Petri dishes. After solidification 0.25 ml of test strains were inoculated in the media separately. Care was taken to ensure proper homogenization.

The experiment was performed under strict aseptic conditions. After the medium solidified, as well as made in the plates with sterile borer (5mm). The extract compound (50ml) was introduced in to the well plates were incubated at 37°C and 20°C for 72 and 220hrs respectively. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition<sup>31</sup>. A control in which Chloromphenical was kept for all test strains and the control activity was deducted from the test and results were recorded.

### Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The antibacterial and antifungal activity was measured using a dilution techniques<sup>32</sup>. The plant extracts (100mg) was solubilized in 1ml of dimethyl sulfoxide (DMSO) and serially two fold diluted Nutrient Broth to obtain a concentration range 15.6-1000mg/ml. That is also same for antifungal study but only instead of Rose Bengal Agar. Nutrient broth and Rose Bengal Agar containing only

DMSO diluted in the same way which did not influence bacterial and fungal growth were included as controls. The bacterial strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05M), homogenized and adjusted to an optical density of 0.05 at 530nm (equivalent to 1X 10<sup>6</sup> CFU/ml). This suspension was used as the inoculums for the test in the agar plates.

Bacterial suspensions (100ml) were inoculated using a micropipette. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of the plant extract which completely inhibited the visible growth (turbidity) of the bacteria in tubes. The Minimal Bactericidal Concentration (MBC) was defined as the minimal concentration of the extract which completely inhibited the visible growth of the bacteria on solid media in Petri plates that were incubated at 37°C for 72hrs. The Minimal Fungicidal Concentration (MFC) was defined as the minimal concentration of the extract which completely inhibited the visible growth of the fungi on solid media in Petri plates that were incubated at 20°C for 220hrs.

### Statistical analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed with SPSS (8<sup>th</sup> version). Difference on statistical analysis of data were considered significant at P<0.05.

## RESULTS AND DISCUSSION

In the present study, *P.guajava* leaf essential oil (fresh leaves) was extracted by the hydrodistillation and the leaf extracts were extracted by Clevenger type of distillation apparatus of the dried guava leaves. The yields of the oil and extracts were calculated on an amount of oil obtained (in ml) / total weight of a sample × 100. The obtained yield was 0.13 % of essential oil from *P.guajava* leaf. Average yield of the different extracts were found out from, amount of extract obtained (in gm) / total weight of the dried leaves × 100. In this sample, 11.6 % of yield from acetone, 13.6 % of ethanol and 6.7 % yield of hexane.

These extracts and essential oil were investigated against 5 Gram positive 5 Gram negative bacteria and 3 fungal microbes (Table 1 and Fig. 1 and 2). In the first stage, essential oil, acetone, methanol and hexane leaf extracts of *Psidium guajava* L. were applied on each bacterial and fungal species.

Table.1: Antimicrobial activity of *Psidium guajava* L. by Agar Well Diffusion Method.

Microorganisms	Mean zone of inhibition (in cm)				
	EO	AE	ME	HE	HE
Gram (+)					
<i>Bacillus cereus</i>	1.2	0.8	1.3	0.7	2.1
<i>Bacillus subtilis</i>	0.8	1.3	1.6	1.5	2.4
<i>Lactobacillus lactis</i>	1.4	1.7	1.5	0.6	2.9
<i>Staphylococcus aureus</i>	0.9	1.8	1.5	0.8	1.9
Gram (-)					
<i>Azotobacter species</i>	1.2	0.8	1.6	1.0	2.3
<i>Agrobacterium rhizogenes</i>	1.1	1.0	1.5	1.0	2.8
<i>Enterobacter aerogenes</i>	1.8	1.7	1.4	1.0	3.1
<i>Glucanobacter oxydans</i>	1.2	1.1	1.4	0.7	3.4
<i>Pseudomonas fluorescens</i>	1.5	1.6	1.5	1.5	3.8
Fungi					
<i>Candida tropicalis</i>	0.7	1.4	1.5	0.6	2.3
<i>Aspergillus niger</i>	0.6	0.6	0.6	0.6	1.9
<i>Aspergillus aculeatus</i>	0.6	0.6	0.8	0.8	2.8

EO-essential oil, AE-Acetone extract, ME-Methanol extract, HE-Hexane extract, Ref drug-Ampicillin.

The oil has antibacterial activity (table-1) particularly, *Bacillus subtilis*, *Staphylococcus aureus*, While the overall inhibitory effect of the essential oils in this experiment was less than for acetone, methanol and hexane its individual effect on *S. aureus* was greater and effect of volatile terpenoids on bacteria<sup>33</sup> that is *Bacillus subtilis*, *Staphylococcus aureus*. Olajide *et al.*,<sup>34</sup> reported that leaves of *P. guajava* contain an essential oil rich in triterpenes.

The inhibitory activities of flavanoids against bacteria and yeast have been investigated by a number of researchers, especially in Latin America<sup>35, 36, 37, 38, 39 & 40</sup>. When compared to already proved results in antibacterial activity and present result of *P.guajava*

essential oil that the following bacteria's are newly identified such as *Lactobacillus lactis*, *Lactobacillus acidophilus*, *Enterobacter aerogenes*, *Pseudomonas fluorescens*. A higher concentration of active chemical compounds in essential oils explains their stronger inhibitory action<sup>28 & 41</sup>.

Gnan and Demello<sup>27</sup> reported a complete inhibition of growth of *Staphylococcus aureus*; aqueous guava leaf extract Vieira *et al.*,<sup>42</sup> reported the microbiocidal effect of guava sprout extract (acetone). Abdelrahim *et al.*,<sup>29</sup> also reported a complete inhibition of *Bacillus subtilis*, *Staphylococcus aureus* with extract of the guava leaf. In present results also very much confirmed

with previously published by Rathish and Sumitra<sup>43</sup>. They stated that the acetone extract was highly active against gram positive and fungal strains. In the previous result also confirmed the present result that is *Bacillus subtilis*, *S. aureus*. And in addition

to that present result also proved more bacteria's has antibacterial activity of acetone extract (*P.guajava*) such as *Lactobacillus lactis*, *Lactobacillus acidophilus*, *Enterobacter aerogenes* and *Pseudomonas fluorescens*.

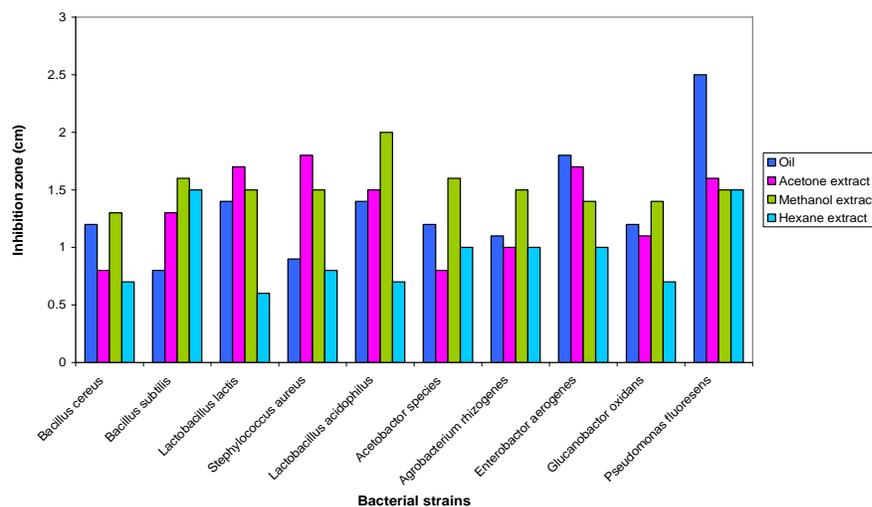


Fig.1: In-vitro antibacterial activities of *Psidium guajava* L. extract using agar well diffusion method.

The acetone extract of *P.guajava* leaf should have flavones, terpenes, etc. so acetone extract have constituents in order to elucidate the active principle within the extract which can turn to be a novel antimicrobial agent of the future. Through this results have been showed contradictory results because the oil treated microbes has been revealed poor activity against corresponding bacteria. While all of the remaining experimentally used extracts were poor response against the microbes. The present result that shows acetone extract of *P.guajava* is highly active against these total 10 microbial strains studied (table1).

The methanolic extract of *P.guajava* leaf was efficient against both Gram positive and Gram-negative bacteria, indicating the broad-spectrum antibiotic activity. Tonia<sup>44</sup> reported Gram-positive bacteria activity against methanol extract of *P.guajava* is more, particularly, the *Bacillus subtilis*. The previous results also confirmed present results because the methanol extract of *P.guajava* leaf. And these extract also inhibit the bacterial growth such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Acetobacter species*, *Agrobacterium rhizogenes*, and *Enterobacter aerogenes* respectively.

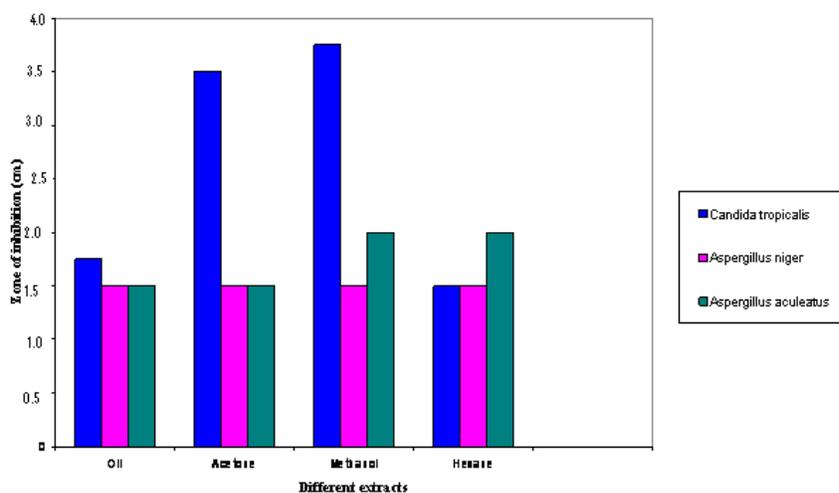


Fig. 2: In-vitro antifungal activity of *Psidium guajava* L. extracts using agar well diffusion method.

In previous work, aqueous extracts of Guava leaves had the highest activity exhibited against *Bacillus cereus*<sup>29</sup>, in the same view of this work also proved antibacterial study of acetone methanol, hexane based leaf extracts against *Bacillus cereus*. In our present study confirmed another one researcher Adeyemi *et al*,<sup>45</sup> and study have indicated flavanoids as the main constituent responsible for the antimicrobial activities of *P.guajava* leaf extracts.

Gram positive bacteria are known to be more susceptible to essential oils than Gram negative bacteria<sup>46, 47</sup>. The weak antibacterial activity against Gram negative bacteria was ascribed to the presence of an outer membrane so depicted this similar observations, such as the flavanoids could also have contributed to the overall effect of the extract as it were in this study, several other studies have indicated flavanoids as the main

constituents responsible for the antimicrobial activities of *P.guajava* leaf extract.

All the three solvent based extracts of *Psidium guajava* (L.) showed good activity. Acetone extract was highly active against gram positive and fungal strains while all of the extracts were equally active against gram-negative strains<sup>43</sup>. In previous report proved, acetone, methanol hexane extracts against fungi i.e., *Aspergillus species*<sup>43</sup>. But this present results find out no activity of acetone, methanol, hexane extracts and essential oil. At the same time the acetone, methanol extracts have antifungal activity against *Candida tropicalis*.

The complex composition of essential oils offers a variety of pharmacological resources and great potential for the development of novel drugs. Plant oils and extracts have been used for a wide variety of purposes for many thousands of years<sup>48, 49</sup>. The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies<sup>3,4</sup>.

The acetone, methanol, and hexane leaf extracts of *P.guajava* should further be studied for its phytochemical constituents in order to elucidate the active principle within the extract which can turn out to be a novel antimicrobial agent of the future.

## CONCLUSION

The results of this study support the traditional usage of the studied plant and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials to carry out further pharmacological evaluation.

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