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# Phytochemical and Antimicrobial Screening of *Psidium guajava* L. Leaf Extracts against Clinically Important Gastrointestinal Pathogens

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**ABSTRACT**

*Psidium guajava L. commonly known as Guava, is a medicinal plant belonging to the family Myrtaceae. P. guajava is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The present study provides phytochemical and antimicrobial details of the methanolic leaf extract of P. guajava against clinically important gastrointestinal pathogens viz. Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Vibrio cholerae. The phytochemical analysis carried out revealed the presence of flavanoids, glycosides, alkaloids and steroids and many other metabolites and absence of tannins and saponins. Minimum inhibitory concentration (MIC) assay was determined for the extract. The methanolic extract showed toxicity against all the bacteria, S.typhi being highly susceptible with a zone of inhibition of 2mm at 4mg/ml. Thus Psidium guajava leaf extract has a potential of providing safe and cheap drugs and drug leads for human use.*

**Key-words**: *Psidium guajava* , extracts, gastrointestinal, pathogens.

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## INTRODUCTION

Guava (*Psidium guajava* Linn.), belonging to the Family Myrtaceae, is originated in the tropical South America [1] and grows wild in Bangladesh, India, Thailand, Brazil, Florida, West Indies, California and also in several other countries [2]. The pharmacological actions and the medicinal uses of methanolic extracts of guava leaves in folk medicine include the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhoea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain [3,4,5,6]. The boiled water extract of guava, plant leaves and bark are used in medicinal preparations which are utilized as remedies for dysentery, diarrhoea and upper respiratory tract infections in Florida, the West Indies and parts of South America [7]. In Malaysia, *Psidium guajava* is used for stomach ache and gastroenteritis [7, 8, 9]; Leaf, root, and bark extracts are used for treatment of diarrhoea, cholera [10]. *Guajava* leaf extract contains guajava polyphenol that has an anti-oxidation action [11, 12] and flower and leaf of the plant have been reported to have antibiotic activity [13].

The leaves contain various constituents such as fixed oil 6%, volatile oil 0.365% 3.15% resin, 8.5% tannin, fat, cellulose, chlorophyll and mineral salts and a number of other fixed substances [14, 15]. In addition, the leaves contain an essential oil rich in cineol and four triterpenic acids as well as three flavonoids; quercetin, its 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside with strong antibacterial action [16]. Quercetin was found to reduce the capillary permeability in the abdominal cavity [17]. The alcoholic extract of the leaves possesses a morphine like inhibition of acetyl choline release in the coaxially stimulated ileum, this morphine-like inhibition was found to be due to quercetin.[18, 19]. Chemicals in guava were shown to bind to *E. coli*, preventing its adhesion

to the intestinal wall and thus preventing infection and resulting diarrhoea [20]. Guava leaf extract has also shown to have tranquilizing effect on intestinal smooth muscle, inhibit chemical processes found in diarrhoea and aid in the re-absorption of water in intestines. A recent study suggested that the antidiarrhoeal activity is through the inhibition of intracellular calcium release [21].The effective use of guava in diarrhoea, dysentery and gastroenteritis can also be related to guava's documented antibacterial properties [21,22].

Hence, the aim of this work is to pursue a study on the phytochemical and antimicrobial potentiality of methanolic extracts of *Psidium guajava* leaves, against multi-drug resistant gastrointestinal pathogens including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Vibrio cholera* as this plant is widely used in indigenous system of medicine due to its easy availability.

## MATERIALS AND METHODS

***Collection of Plant material:*** The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required.

***Extract Preparation*:** 50 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ~350 ml methanol and distilled water separately. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45ºc, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

***Phytochemical Analysis*:** Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto constituents such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins, steroids, proteins, lipids, oils by standard methods [ 23, 24].

***Anti-bacterial analysis***

***Test Microorganisms:*** The organisms namely *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Vibrio cholerae* used during the present experiment were procured from Hi-media which are potential causative pathogen for different diseases.

***Concentrations screened*:** 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 mg for agar diffusion method and for broth dilution method up to 64 mg/ml concentrations were used according to the sensitivity of samples.

***Agar diffusion method**:* Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, $10^4$ cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones were noted.

***Broth dilution method*:** Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The tubes containing above media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 µl, $10^4$ cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm. The % of inhibition was calculated by using the formula below.

$$\% \text{ Inhbition} = 100 - \left[ \frac{\text{OD of culture with sample (Test)}}{\text{OD of culture without sample (Control)}} \times 100 \right]$$

## RESULTS AND DISCUSSION

The medicinal importance of tannins, alkaloids, saponins, phenols, glycosides and flavonoids recorded in the present study as shown in Table-1 is also common in various antibiotics used in treatment of common pathogenic strains, and these phytochemicals are naturally present in the plant extracts which could make the plant useful for treating different ailments and having a potential of providing useful and safe drugs and drug leads for human use [25, 26].

**Table 1: Result of phytochemical screening of the *Psidium guajava* leaves**

| Test | Results |
|---|---|
| Flavonoids, Triterpenoids, Steroids, Carbohydrates, Oils, Lipids, glycosides, Alkaloids | Positive |
| Tannins and Saponins | Negative |

It was revealed from the results that *Psidium guajava* leaf extracts shows different degree of inhibition against different microorganisms. The diameter of zone of inhibition (ZOI) produced depends on several factors broadly classified as extrinsic and intrinsic parameters. The extrinsic parameters like pH of the medium, period and temperature of incubation, volume of the well, concentration of plant extracts and size of inoculums can be fixed and standardized during experiment, hence no error results due to extrinsic factors. However, intrinsic factors such as nature of medicinal plant including its components, solubility and diffusing property are predetermined. Due to variable diffusibility, the antibacterial with very high potency may not demonstrate ZOI commensurate to its efficacy [27]. The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone (in mm) against the test microorganisms and the results are shown in Table 2. The test organisms were also inoculated with pure antibiotics-Gentamycin and the results are shown in Table.3. The range observed for the methanolic extract was from 2mm-9mm at 2mg/ml-4mg/ml and *S.typhi* was found to be highly susceptible as it showed an inhibition zone of 5mm at 2mg/ml concentration whereas *E.coli* and *P.aeruginosa* were sensitive at 4mg/ml exhibiting 2mm and 3mm ZOI. *S.aureus* and *V.cholerae* does not show any zone of inhibition. Therefore broth dilution method was done to find out % inhibition and it was found that 100% inhibition of *E.coli* and *P.aeruginosa* was at 4mg/ml concentration (Fig. 1,2) and S.typhi at 2mg/ml (Fig. 3) which is a confirmation of the results of agar diffusion method. *S.aureus* and *V.cholerae* were 100% inhibited at 32mg/ml so the minimum inhibitory concentration (MIC) for them is 32 mg/ml (Fig.4, 5). In the present study, *S.typhi* was found to be the most sensitive to the extracts of *Psidium guajava* exhibiting the minimum zone of inhibition of 3mm at 2mg/ml while pure antibiotics gentamycin exhibited 2mm at 25µg/ml. The present study revealed that the extracts of *Psidium gvajava* is very effective in inhibiting *S.typhi* hence we suggest use of *Psidium gvajava* leaf extracts in treating various gastrointestinal disturbances.

All the pathogens screened in the present study are potent causative agents of watery diarrhoea (influx of water and ions to the intestinal lumen increase in intestinal motility and watery stools), diarrhoea (usually non-bloody), nausea, vomiting, abdominal pain, pediatric diarrhoea, typical gastroenteritis, and necrotizing enterocolitis [28,29,30,31] therefore it can be conclusively stated the *Psidium gvajava* leaf extract is a potential antibacterial agent for the bacteria causing gastrointestinal problem and can be used for such ailments.

**Table.2 : Zone of inhibition (in mm) of methanolic leaf extract of *Psidium guajava***

| Concentration(mg/ml) | *E.coli* | *S.aureus* | *P.aeruginosa* | *V.cholerae* | *S.typhi* |
|---|---|---|---|---|---|
| **0.125** | - | - | - | - | - |
| **0.25** | - | - | - | - | - |
| **0.50** | - | - | - | - | - |
| **1.0** | - | - | - | - | - |
| **2.0** | - | - | - | - | 5 |
| **4.0** | 2 | - | 3 | - | 8 |
| **MIC(mg/ml)** | 4 | NF* | 4 | NF* | 2 |

**NF-not found*

**Table.3 MIC of Gentamycin against the test organisms**

| Microorganisms | MIC(µg/ml) | ZOI(mm) |
|---|---|---|
| *E.coli* | 25 | 18 |
| *P.aeruginosa* | 100 | 1 |
| *S.aureus* | 25 | 13 |
| *S.typhi* | 25 | 2 |
| *V.cholerae* | 25 | 13 |

**Fig.1: Inhibition (%) of *E.coli* by methanolic extract of *P.guajava* in broth medium**

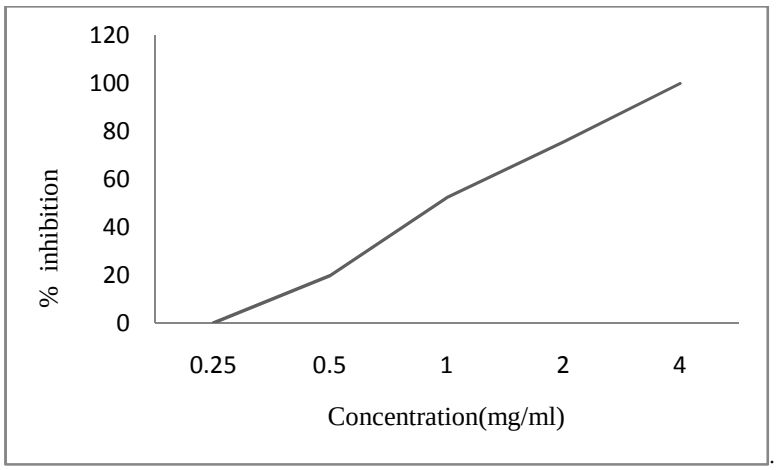



**Fig.2: Inhibition (%) of *P.aeruginosa* by methanolic extract of *P.guajava* in broth medium**

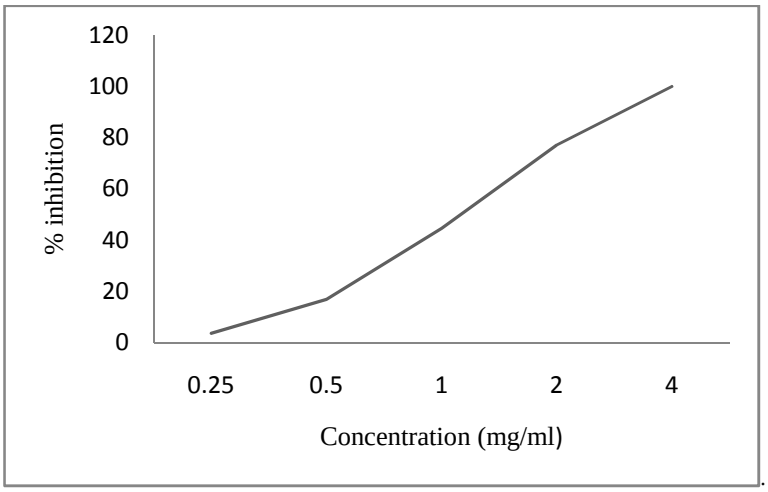



**Fig.3: Inhibition (%) of *S.aureus* by methanolic extract of *P.guajava* in broth medium**

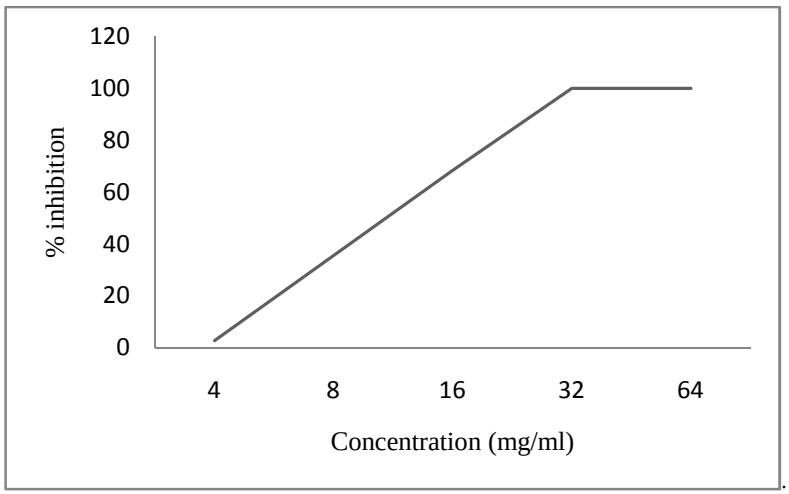


**Fig.4: Inhibition (%) of *S.typhi* by methanolic extract of *P.guajava* in broth medium**

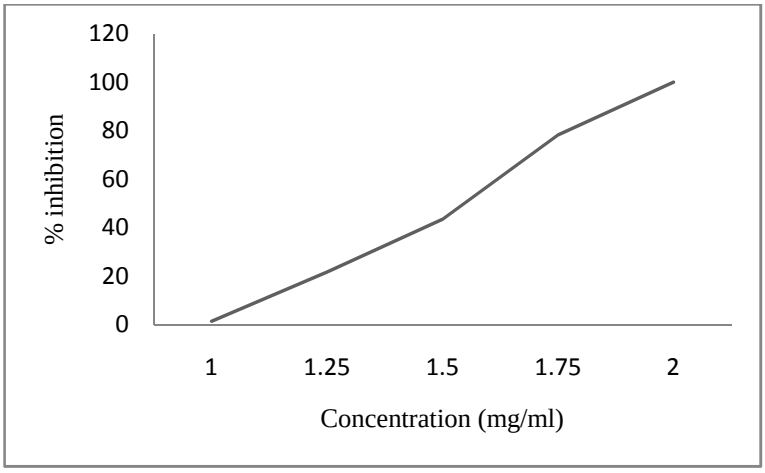
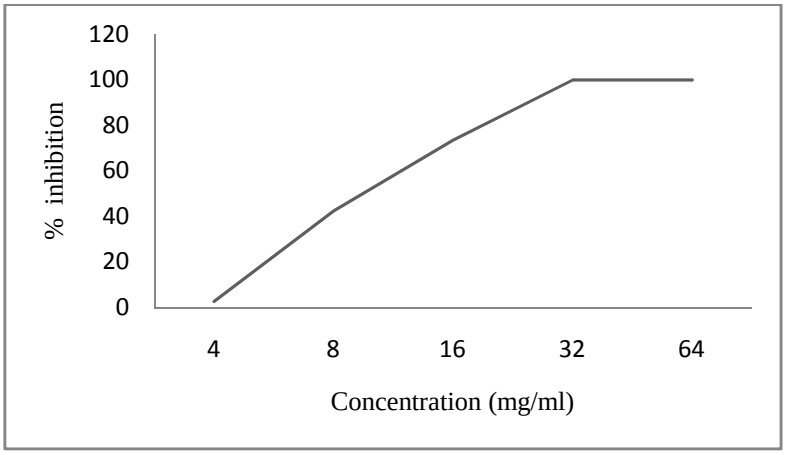



**Fig.5: Inhibition (%) of *V.cholerae* by methanolic extract of *P.guajava* in broth medium**

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