

Studies on Phytochemical Analysis, Antibacterial Activity of *Psidium guajava* and *Punica granatum*

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Abstract

Since the beginning of screening of Phyto-chemicals, the use of natural products in medicinal application has reduced. Medicinal plants, since times immemorial, have been used for curing of many diseases as a source of medicine. The presence of study focuses on extraction of *Psidium guajava* and *Punica granatum* plant leaves for treatment against diarrhoeal infection. Different parts of the plants are considered to have potential medicinal properties and are used in different system of medicine. Two plant species *Psidium guajava* and *Punica granatum* were screened for bio-active compound and detection of anti-bacterial activity against isolated pathogenic strains.

Keywords: Antibacterial Activity, Phyto-chemicals, *Psidium guajava*, *Punica granatum*

1. Introduction

Diarrhoea is a serious problem that cause frequent elimination of watery stools with increased bowel movements. Severity causes dehydration and in long run leads to nutritional problems. Treating diarrhea using traditional medicines is in practice in many developing countries.

Diarrhoea accounts for more than 5.8 million deaths each year in under five age group¹. Biologically activity of many plant extracts have validated in the treatment of diarrhea. Leaf, stem and root decoctions of guava plant, *Psidium guajava* Linn (Myrtaceae) have been used in traditional medicine as anti-diarrheal therapy

2. Materials and Methods

2.1 Plant Materials

Plant materials were collected from Erode District. Leaves of *Psidium guajava* and *Punica granatum* were collected and kept for shade drying. The air dried materials was used as a natural plant extract and used for phyto-chemical analysis.

2.2 Preparation of Plant Extract

About 10 g of air dried powder was taken in 100 mL of methanol. Plugged with cotton wool and then kept on a rotary shaker at 220 rpm for 24 h. Then the supernatant was collected and the solvent was evaporated to make the

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final volume one-fourth of the original volume and stored at 4°C in air tight container.

2.3 Qualitative Analysis of Phytochemicals

The methanolic extract *Psidium guajava* and *Punica granatum* was screened for the presence of secondary metabolites using the procedures of (Harborne, Kokate *et al.*)^{2,3}.

2.4 Antibacterial Assay

Bacterial culture: Clinical isolates of microorganisms *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* were obtained from PSG Hospital, Coimbatore.

2.5 Preparation of Inoculums

A loopful of strain was inoculated in 30 mL of nutrient broth bacteria composition (Dextrose, 4 g; Peptone, 1 g; Distilled water, 100 mL) in an Erlenmeyer flask and incubated on a rotary shaker at 37°C for 24 h to activate the strain.

Anti-bacterial activity of *Psidium guajava* and *Punica granatum* leaf extracts was determined by the cup diffusion method on nutrient agar medium was prepared

and dissolved in 15 mL amounts into sterile Petri plates and allowed to solidify. Each medium was uniformly smeared with the cultures of the different strains of bacteria. Independently 5 wells were made in each Petri plates to which leaf extract (20 µl) dissolved in different organic solvents like acetone, ethanol and petroleum ether and added into separate wells in a Plate. The Plates were incubated at 37°C for 24 hours and zone formation was observed and recorded.

3. Results and Discussion

Medicinally important plant species via; *Psidium guajava* and *Punica granatum* were selected for screening of secondary metabolites. During this screening an attempt has been made to decipher the effect of these secondary metabolites regards to its anti-microbial activity. The information about plant species of Ethano-medicinal value of these plant species is furnished and the results are incorporated in (Table 1).

3.1 Phyto-chemical Analysis

The Phyto-chemical analysis of *Psidium guajava* and *Punica granatum* plant extracts of the major Phyto-chemicals consistants including phenolics, flavonoids and glycosides, of the two plant species are depicted in (Table 2).

Table 1. Inventorisation of medicinal plants

Sl. No.	Botanical Name	Name of Family	Plant part Used	Mode of Administration	Diseases known to cure
1.	<i>Psidium guajava</i>	Myrtaceae	Leaves	Infusion of leaf juice	Diarrhoea
2.	<i>Punica granatum</i>	Punicaceae	Bark	Infusion of bark juice	Diarrhoea

Table 2. Data on phyto-chemical screening of the different extract of both plants and different extract of both plants species

Name of the Plant	Acetone Extract	Ethanol Extract	Petroleum Ether Extract	Aqueous Extract	Chloroform Extract
<i>Psidium guajava</i>	G	FL	-	AL, PH	-
<i>Punica granatum</i>	-	FL	-	AL	-

G-Glycosides; FL-Flavonoids; AL-Amino acids; PH-Phenolic compounds

Preliminary Phyto-chemicals screening of this experiment showed the major compounds as flavonoids in the crude extract of natural plant and saponins, tannins and phenolic compounds also present in both extracts were found to be carbohydrates, glycosides, proteins and amino acids.

3.2 Total Protein, Carbohydrates and Sugars Content Analysis

The total protein, carbohydrates and reducing sugars contents of the methanolic extracts of two traditionally used plants were expressed as mg BSA, glucose of equivalent/gram dry weight. The total protein compounds of the methanolic extracts of two traditionally used medicinal plants were expressed as mg catechol equivalent/gram dry weight. The determinants of the amount of proteins, carbohydrate and reducing sugars in the plants showed a broad range of values ranging from 35.2 to 39.5 mg/g dry weight (Table 3).

Table 3. Total soluble protein, carbohydrate and reducing sugar content of both leaf materials

Plant Materials	Total Soluble Proteins (mg/gfw)	Total Soluble Carbohydrates (mg/gfw)	Total Amount of Reducing Sugar (mg/gfw)
<i>Psidium guajava</i>	35.2	29	5
<i>Punica granatum</i>	39.5	41	9

3.3 Total Phenolic Compound Analysis

Determination of the amount of phenolics in the plants showed a broad range of values ranging from 4 to 6 mg/g dry weight (Table 4).

Table 4. Total phenolic content of both leaf materials

Plant Materials	Total Phenol (mg/gfw)
<i>Psidium guajava</i>	4.6
<i>Punica granatum</i>	5.5

3.4 Total Flavonoid Compound Analysis

The total flavonoids compound of the methanolic extracts of these two plant species were expressed as mg/gfw. The determinations of the amount of Quercetin in the plants showed broad ranges of value are 0.36 and 0.40 mg/g dry weight (Table 5).

Table 5. Total quercetin compounds of both leaf materials

Plant Materials	Total Quercetin (mg/gfw)
<i>Psidium guajava</i>	0.36
<i>Punica granatum</i>	0.40

Tannins, Phenolic compounds and flavonoids were all detected in a preliminary Phyto-chemical screening of the dried extract of guava leaves. These results are confirmed in the literature. Goncalves *et al.*,⁴ also described flavonoids and tannins. Interestingly, Lozoya *et al.*,⁵ demonstrated that the flavonoids, Quercetin and its glycosides may be the active compounds.

3.5 Anti-bacterial Activity

Ethanol extract exhibited increased anti-bacterial activity against the some of the isolated pathogenic strains of *E. coli*, *salmonella typhi*, *shigella dysenteriae*. Plant leaf extract showed anti-bacterial activity against the pathogenic strains (Plate 1a, 1b and 1c).

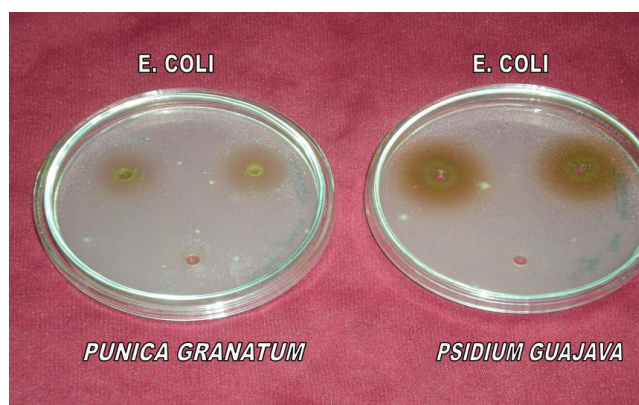


Plate 1a. Anti-bacterial activity of plant material with extraction solvents.



Plate 1b. Anti-bacterial activity of plant material with extraction solvents.

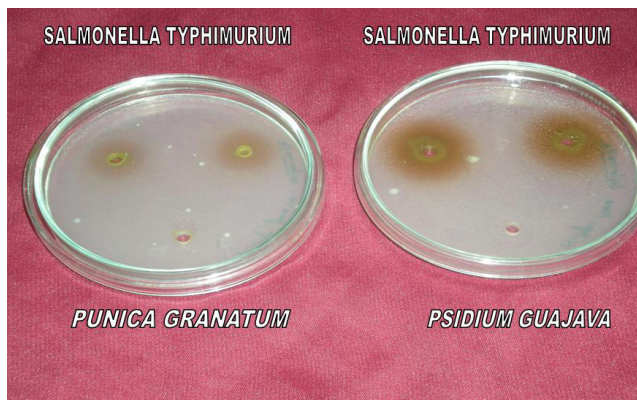


Plate 1c. Anti-bacterial activity of plant material with extraction solvents

Methanol extracts exhibit anti-microbial activity as compared with acetone extracts. Both Methanol and acetone extracts of *Punica granatum* has high degree of anti-bacterial activity tested against GIT infection causing bacterial species which may have novel secondary metabolites. Prasanth *et al.*,⁶ reported that, different extracts of *Punica granatum* has anti-bacterial activity against *Proteus vulgaris* and *Bacillus subtilis*. In present study, ethanol extracts exhibit anti-microbial activity as compared with acetone extracts. Both ethanol and acetone extracts of *Punica granatum* and *Psidium*

guajava has high degree of anti-bacterial activity against *Salmonella spp*, *Shigella spp* and *E. coli*.

4. Conclusion

The compounds isolated from *Psidium guajava* and *Punica granatum* have found to possess anti-diarrheal effects. These plant sources are wide spread, cheap, readily available throughout the year. The rind of pomegranate can be used effectively and further the tannins, phenolic compounds and flavonoids identified in the extracts have proven beneficial for anti - diarrheal therapy with less side effects.

5. References

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