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Antibacterial properties of *Psidium guajava* leaves, fruits and stems against various pathogens

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Abstract
The present study was designated to evaluate the antibacterial activities of ethyl acetate and hot water extract from leaves, fruits and stems of *Psidium guajava.* Compare to all parts, the stems were showing best result and the zone of inhibition was obtained 24.5 mm. The antibacterial activities of the extracts against bacteria were tested by using agar well diffusion assay and the MIC values were determined by broth dilution assay. The hot water extract showed least antibacterial activity as compare to ethyl acetate extract. The least concentration were obtained 1.98 mg/ml in ethyl acetate extract of stems against *P. aeruginosa*. The antibacterial compound mainly found in *Psidium guajava* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

Keywords: Antibacterial activities, ethyl acetate and hot water plant extracts, MIC, zone of inhibition

Introduction
An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. Antibiotics are those substances that are produced by one microorganism that kill or prevent the growth of another micro-organism. Some of these side effects can be life threatening if the drug is not used properly. Several microorganisms derived antibiotics are currently in use to treat a variety of human disease, therefore the action must be taken to control the use of antibiotics, develop new drugs either synthetic or natural. For a long period of time, plants have a valuable source of natural products for maintaining human health. India has a rich tradition in use of medicinal plants to develop drugs. According to world health organization (WHO), any plant which contain substances that can be used for therapeutic purpose or which are precursor of chemo-pharmaceuticals semi synthetic new drugs is referred as medicinal plant (Dash B *et al.,* 2003) [1]. Medicinal plants would be the best source to obtained a variety of drugs as the phytochemicals are more specific.
Psidium guajava is evergreen shrub native to tropical America that has neutralized in South East Asia. The part of Guava has been reported the wide range of activity against the human ailments (Ross *et al.,* 2003; Olajide *et al.,* 1999) [2, 3]. There are over 20 compounds have been reported present in leaves, stem, bark and roots of *P. guajava* (Meckes *et al.*; 1996; Lozoya *et al.,* 1994; Arima *et al.,* 2002; Begum *et al.,* 2004) [4-7]. Guava leaves were used to treat diarrhoea and stomach, the leaves were used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and toothache. Guava fruits also contain vitamin C, iron calcium and phosphorus. Guava plants contain some secondary metabolites. The roots were also rich in tannin. Guava plants contained phytochemicals. The leaves of guava were rich in flavonoids in particular quercetin, saponins, tannins, alkaloids, anthraquinones, phlobatanins and cardiac glycosides. Much of guava therapeutic activity was attributed to these flavonoids. The flavonoids had demonstrated antibcterial activity. Guava also had antioxidant properties which was attributed to the polyphenols found in the leaves. Guava leaves were often boiled into a tea to treat diarrhoea on many pacific islands.
Guavas are up to 5 times richer in vitamin C than oranges [Conway]. Manganese is also present in the plant in combination with phosphoric, oxalic and malic acids. The fruit contains saponin combined with oleanolic acid. The present study is carried out by evaluation of Antibacterial properties of *Psidium guajava* against Bacterial pathogens and Phyochemical analysis. The used microorganisms were 3 bacterial cultures (*P. aeruginosa, S. aureus* and *E. coli*).

Materials and Methods

Collection of plant

The *Psidium guajava* leaves, fruits and stems were collected from the local area in Gomti Nagar, Lucknow.

Preparation of plant extract

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant. *Psidium guajava* leaves, fruits and stems were washed with distilled water and kept in incubator at 37°C for 3-4 days and grinded into fine powder. Now plant material was dissolved in 70% ethanol and 80% methanol, ethyl acetate and hot water, 1 g sample should be dissolved in 10 ml of solvent. Mixtures were kept in the dark for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation and exposure to sunlight was avoided. After 3 days, mixtures were filtered through Whatman no.1 filter paper and kept it in incubator at 37°C till all solvents had completely evaporated from mixtures. Now all mixtures were dissolved in DMSO (Dimethyl sulfoxide).

Tested microorganisms

Bacterial cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by Innovation Life Sciences, Lucknow. One gram positive culture- *Staphylococcus aureus* (MTCC 2940) and two gram negative cultures- *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC 739) were used.

Antibiogram analysis

The antimicrobial activity of *Psidium guajava* was evaluated against bacterial strains in ethyl acetate and hot water extracts by using agar well diffusion method (Ahmad *et al.*; 2001) [8]. Nutrient agar plates were prepared for all extracts, 50µl inoculum of each selected bacterium was uniformly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. The equal volume (50µl) of antibiotic (tetracycline), distilled water and plant extract were poured into the wells. The plates were incubated at 37°C for 24 hrs.

Determination of minimum inhibitory concentration (MIC) of ethyl acetate and hot water extract

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation at 37 ^{0}C in shaker incubator (Andrews *et al.*, 2001; Thongson *et al.*, 2004) [9, 10]. MIC of all samples were determined by broth dilution method. A two-fold serial dilution of the ethyl acetate and hot water extracts were prepared and optical density was measured at 600 nm (Bauer *et al.*, 1966) [11].

Phytochemical tests

The leaves, stems and fruits extracts were screened for some secondary metabolites like-saponins, tannins, alkaloids, anthraquinones, phlobatannins, flavonoids, terpenoids, reducing sugar and poly phenols.

Test for reducing sugar

Take 1gm of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 100^{0}C in a water bath. Brick red precipitate showed positive result.

Test for tannins

Take 2gm of aqueous extract in a test tube and add 2 drops of 5% ferric chloride, brown colour gives positive result.

Test for phlobatannins

Take 2ml plant sample in a test tube and add 10 ml deionized water and boil at 100^{0}C with few drops of 1% HCl. Deposition of red precipitation gives positive result.

Test for Saponins

Saponins content is determined by boiling 1ml plant sample in 10 ml deionized water for 15 min. and after cooling the extract was shaken vigorously to record froth formation.

Test for terpenoids

Take 5ml of aqueous extract and then add 2ml chloroform followed by addition of 3ml conc. sulfuric acid, observe the reddish brown interface for presence of terpenoids.

Test for alkaloids

Take 1ml of aqueous extract in test tubes and add 2-3 drops of Wagners reagent it gives orange red precipitation.

Test for flavonoids

Take 1 ml of sample and add 1% NH_3 solution if yellow colour observed, showed presence of flavonoids then after this take ethanolic or aqueous extract and add 10 ml DMSO then heat it followed by adding Mg (magnesium chloride), add conc. HCl gives red colour to confirmed flavonoids.

Test for poly phenols

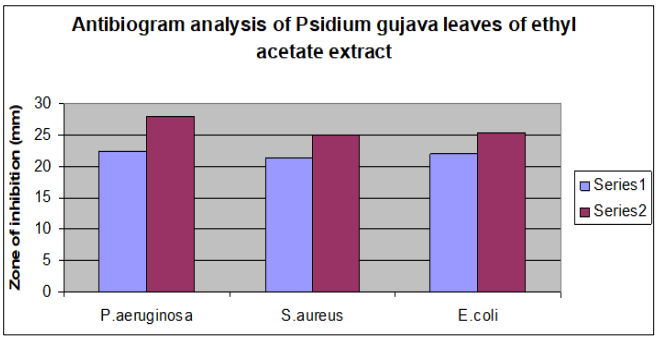
Take 2ml ethanolic extract of plant sample and add 1ml Folin-Ciocalteu reagent and 9ml of d/w. again add sodium carbonate solution (8ml), vortex to mix then kept test tube in dark and take O.D at 760nm.

Results and Discussions

Table 1: Antibacterial activity in leaves (Ethyl acetate extract)

Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	22.5	28.0
S. aureus	21.5	25.0
E. coli	22.0	25.5

Table showed that the zones of inhibition were observed maximum against *P. aeruginosa* in ethyl acetate extract of *P. guajava* leaves.



Graph 1: Graph showed that the highest zone of inhibition observed in *P. aeruginosa* as compare to *S. aureus* and *E. coli*.

Series 1 = Sample, Series 2 = Tetracycline

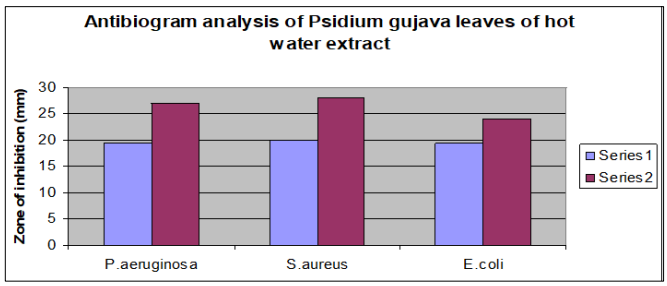
E. coli *P. aeruginosa* *S. aureus*

Fig 1: Fig showed that *P. aeruginosa* was having higher zone of inhibition compare to *E. coli* and *S. aureus*.

Table 2: Antibacterial activity in leaves (Hot water extract)

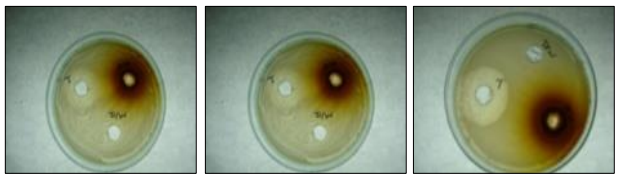
Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	19.5	27.0
S. aureus	20.0	28.0
E.coli	19.5	24.0

Table showed that the zones of inhibition were observed maximum against *S. aureus* in Hot water extract of *P. guajava* leaves.



Graph 2: Graph showed that the maximum zone of inhibition in *S. aureus* as compare to *P. aeruginosa* and *E. coli.*

Series 1 = Sample, Series 2 = Tetracycline



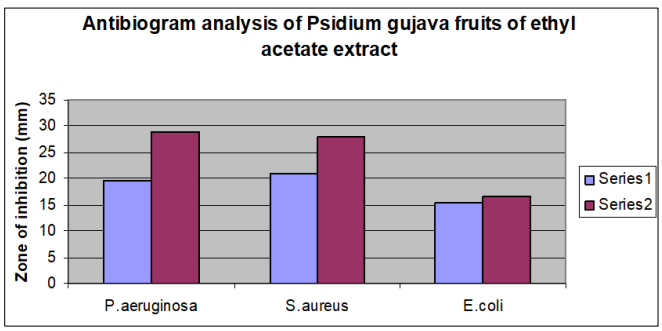
E. coli *P. aeruginosa* *S. aureus*

Fig 2: Fig showed that *S. aureus* was having higher zone of inhibition compare to *P. aeruginosa* and *E. coli*.

Table 3: Antibacterial activity in Fruits (Ethyl acetate extract)

Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	19.5	29.0
S. aureus	21.0	28.0
E.coli	15.5	16.5

Table showed that the zones of inhibition were observed maximum against *S. aureus* in Ethyl acetate extract of *P. guajava* fruits.



Graph 3: Graph showed that the maximum zone of inhibition observed against *S. aureus* as compare to *P. aeruginosa* and *E. coli*.

Series 1 = Sample, Series 2 = Tetracycline

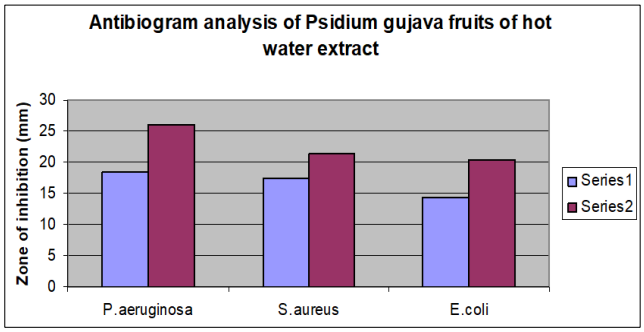
E. coli *P. aeruginosa* *S. aureus*

Fig 3: Fig showed that *S. aureus* was having higher zone of inhibition compare to *P. aeruginosa* and *E. coli*.

Table 4: Antibacterial activity in Fruits (Hot water extract)

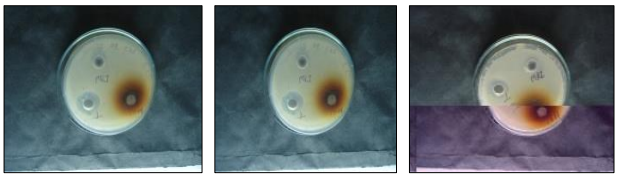
Pathogens	Z.O.I0(mm)	Tetracycline0(mm)
P. aeruginosa	18.5	26.0
S. aureus	17.5	21.5
E.coli	14.5	20.5

Table showed that the zones of inhibition were observed maximum against *P. aeruginosa* in Hot water extract of *P. guajava* fruits



Graph 4: Graph showed that the maximum zone of inhibition observed against *P. aeruginosa* as compare to *S. aureus* and *E. coli.*

Series 1 = Sample, Series 2 = Tetracycline



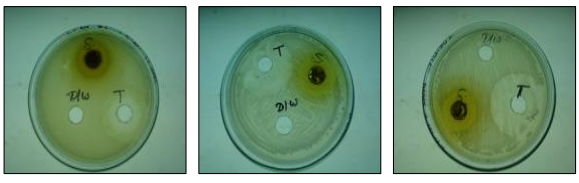
E. coli *P. aeruginosa* *S. aureus*

Fig 4: Fig showed that *P. aeruginosa* was having higher zone of inhibition compare to *E.coli* and *S. aureus*.

Table 5: Antibacterial activity in Stems (Ethyl acetate extract)

Pathogens	Z.O.I0(mm)	Tetracycline0(mm)
P. aeruginosa	21.5	29.0
S. aureus	19.5	29.0
E. coli	17.5	16.5

Table showed that the zones of inhibition were observed maximum against *P. aeruginosa* in Ethyl acetate extract of *P. guajava* stems.



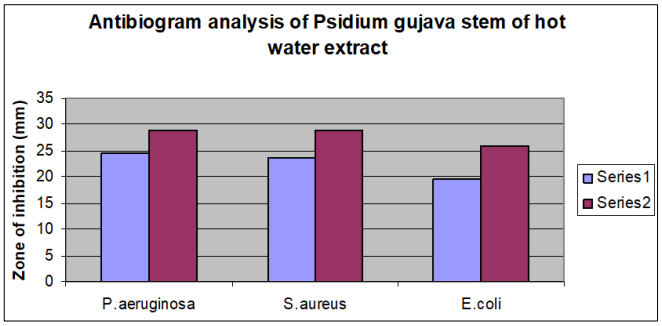
E. coli *P. aeruginosa* *S. aureus*

Fig 5: showed that *P. aeruginosa* was having higher zone of inhibition compare to *E. coli* and *S. aureus.*

Table 6: Antibacterial activity in Stem (Hot water extract)

Pathogens	Z.O.I0(mm)	Tetracycline0(mm)
P. aeruginosa	24.5	29.0
S. aureus	23.5	29.0
E.coli	19.5	26.0

Table showed that the zones of inhibition were observed maximum against *P. aeruginosa* in Hot water extract of *P. guajava* stems.



Graph 5: Graph showed that the maximum zone of inhibition observed against *P. aeruginosa*, compare to *E. coli* and *S. aureus*.

Series 1 = Sample, Series 2 = Tetracycline

E. coli *P. aeruginosa* *S. aureus*

Fig 6: showed that *P. aeruginosa* was having higher zone of inhibition compare to *E. coli* and *S. aureus*

Table 7: Phytochemical Analysis

Tests	Leaves	Fruits	Stem
Reducing sugar	-	+	+
Tannins	+	+	+
Phlobatannins	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+
Alkaloids	+	-	-
Flavonoids	-	-	-
Poly phenols	+	+	+

Conclusion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Rajan *et al.* 2011, Tona *et al.* 1998) [12, 13]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Samy *et al.* 2000 Palambo *et al.* 2001; Stepanovic *et al.*, 2003) [14-16]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the However, not many reports are available on the plants for developing commercial formulations for applications in crop protection.

In this present study the antibacterial properties were found to be best in stem of the *Psidium guajava* and compare to all solvents. The hot water extract were showing best result while the ethyl acetate extract was showing minimum inhibition. The antibiogram analysis showed that zone of inhibition was observed 24.5 mm against *P.aeruginosa* for hot water extract and 22.5 mm for ethyl acetate extract. The MIC values were obtained 1.98 mg/ml in ethyl acetate extract of stems, and 0.33 mg/ml in hot water extract of stem against *P.aeruginosa*. The antibacterial compound mainly found in *Pisdium guajava* were Tanin, Phlobatonin, Saponin, Terpenoids, alkaloids and Poly phenols as showed in phytochemical study.

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