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Antibacterial properties of *Psidium guajava* leaves, fruits and stems against various pathogens in methanolic and ethanolic extracts

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Abstract
To determine the antimicrobial potential of guava (*Psidium guajava*) leaves, Stems and Fruits extracts against positive culture- *Staphylococcus aureus* and negative cultures- *Pseudomonas aeruginosa* and *E. coli* which are some of food borne and spoilage bacteria. The guava leaves, Stems and fruits were extracted in two different solvents methanol and ethanol. Compare to all parts, the stems were showing best result and the zone of inhibition was obtained 28.5 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 ethanolic extract showed least antibacterial activity as compared to methanolic extract. The least concentration was obtained 0.33 mg/ml in ethanolic extract of stems and 0.05 mg/ml in methanolic extract of stems against *P. aeruginosa*.
This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties. The antibacterial compound mainly found in *Psidium guajava* were tannins, phlobatannins, Saponins, terpenoids, alkaloids and poly phenols.

Keywords: Antibacterial activities, ethanolic and methanolic plant extract, MIC, zone of inhibition

Introduction
Recently there has been a lot of attention focused on producing medicines and products that are natural. Several fruits and fruit extracts, as well as arrowroot tea extract [1] and caffeine [2], have been found to exhibit antimicrobial activity against *E. coli*. This suggests that plants which manifest relatively high levels of antimicrobial action may be sources of compounds that can be used to inhibit the growth of food borne pathogens. Bacterial cells could be killed by the rupture of cell walls and membranes and by the irregular disruption of the intracellular matrix when treated with plant extracts [1].
The guava (*Psidium guajava*) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. Many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [3-5]. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity [3, 6-10]. In this study, we aim to evaluate the total extracts of *P. guajava* leaves, Stems and Fruits growing at, Gomti Nagar, Lucknow, Uttar Pradesh.
The genus Psidium belongs to the family Myrtaceae, which is considered to have originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world like Asia, Egypt, Hawaii, Florida, Palestine and others. The genus Psidium comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits [11]. The most commonly cultivated species of Psidium is P. guajava L. which is the common guava. Other species are utilized for regulation of vigor, fruit quality improvement and resistance to pest and disease [11]. Guava fruit today is considered minor in terms of commercial world trade, but it is widely grown in the tropics, enriching the diet of hundreds of millions of people in those areas of the world. The fruits also contain vitamin C, vitamin A, iron, calcium and phosphorus. 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. Morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside and flavonoids, guaijavarin.

The guava tree is an evergreen small tree. The guava leaves are 2 to 6 inches long and 1 to 2 inches wide, aromatic when crushed, and appear dull-green with stiff but curvaceous with pronounced veins [12]. There are bioactive components in the guava leaf that can fight against pathogens, regulate blood glucose levels, and can even aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances [13-15].

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction, supercritical fluid extraction, and phytonic extraction. Maceration extraction is crude extraction; solvents diffuse into solid plant material and solubilize compounds with similar polarity [16]. Effect of plant material depends on its origin, variations in the extraction technique, the time, temperature of extraction, solvent concentration and polarity, quantity, and secondary metabolite composition of an extract [17]. Variations in extraction methods are usually found in the length of the extraction period, the solvent used pH, temperature, particle size, and the solvent-to-sample ratio [15].

Gonçalves *et al*. [18] conducted a study where they screened the antimicrobial effect of essential oils and methanol, hexane, and ethyl acetate extracts from guava leaves. The extracts were screened against bacteria strains isolated from sea bob shrimp and laboratory culture strains. The guava leaves were extracted using a Soxhlet extractor and solvents in order of polarity and then concentrated in a rotary evaporator.

The fresh Leaves, Stem and Fruits were submerged in distilled water in a 5 L glass bowl and submitted to the hydro distillation technique for 24 h. The extracts were evaluated by the disc diffusion method with these extracts being tested at different concentrations. They found that the methanol extract showed greatest bacterial inhibition. No statistically significant differences were observed between the tested extract concentrations and their effect. The researchers concluded that guava leaves, Stems and Fruits are very active against *S. aureus*, thus making up important potential sources of new antimicrobial compounds.

Antibacterial screening has been done selectively by many researchers in guava solvent extract [1, 4, 19, 21]. The mechanism by which they can inhibit the microorganisms can involve different modes of action. It has been reported that these extracts penetrate the lipid bilayer of the cell membrane, rendering it more permeable, leading to the leakage of vital cell contents [21, 22]. Sanches *et al*. [23] evaluated the antibacterial activities of *guava* against gram-positive and gram-negative bacteria testing ethanol and water extract of *P. guajava* leaves, stem, bark and root, and aqueous extract against *Staphylococcus aureus* were found to be more active by using ethanol and water extract than with just aqueous extract [1, 7]. Gnan and Demello [24] testing guava leaf extract found good antimicrobial activity against nine different strains of *Staphylococcus aureus*. The antibacterial activity of guava leaf extract was tested against acne developing organisms by Qa'dan *et al*. [25] concluding that the leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities.

Phytochemicals are non-nutritive chemicals produced by plants for their own protection, but they have been found to protect humans against diseases through recent research. Scientists have identified thousands of phytochemicals, although only small fractions have been studied closely and each one works differently [26]. Begum *et al*. [27] reported the isolation of two triterpenoids: guavanoic acid and guavacoumaric acid from the leaves of guava. Four flavonoids were isolated and identified by Arima and Danno [28] which were found to inhibit the growth of *Salmonella enteritidis* and *Bacillus cereus*. A study was done to evaluate the spasmolytic activity of guava leaf and was found that a compound called “aglycone quercetin” is responsible for spasmolytic activities, which is formed when flavonoids of guava leaves are hydrolyzed by the gastrointestinal fluids.

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 photochemicals 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, 1g 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) [29].

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

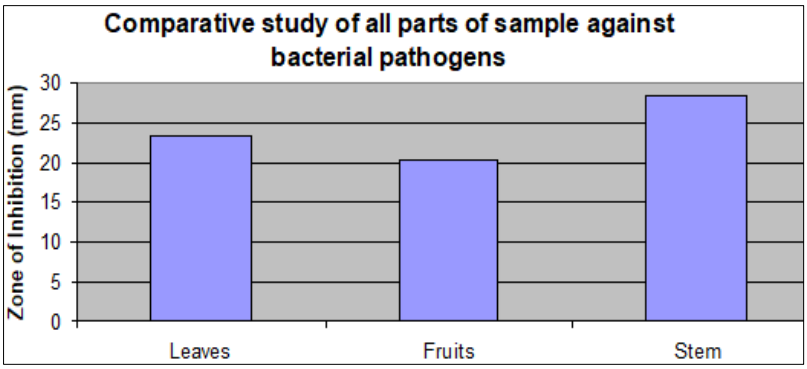
The antimicrobial activity of *Psidium guajava* was evaluated against bacterial strains in ethanolic and methanolic extracts by using agar well diffusion method (Ahmad *et al*.; 2001) [30]. Nutrient agar plates were prepared for all extracts, 50µl inoculum of each selected bacterium was uniformly spreaded 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 ethanolic and methanolic extracts

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.*, 2001) [31, 32]. MIC of all samples was

determined by broth dilution method. A two-fold serial dilution of the methanolic and ethanolic extracts was prepared and optical density was measured at 600 nm (Bauer *et al.* 1966) [33].

Results and Discussions

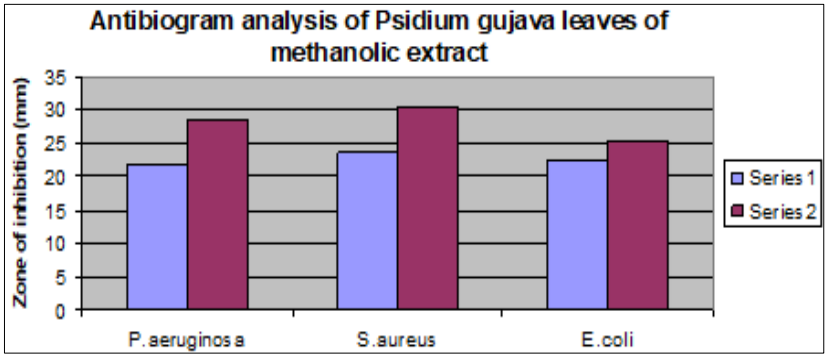


Graph 1: Graph showed that *P. guajava* stems were having maximum antibacterial activity, compare to leaves and fruits

Table 1: Antibacterial activity in leaves (Methanolic extract)

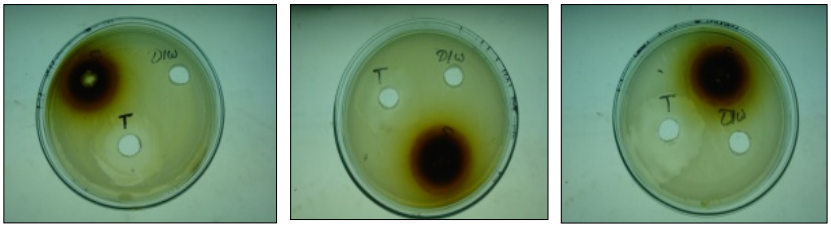
Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	22.0	28.5
S. aureus	23.5	30.5
E. coli	22.5	25.5

Table showed that the zone of inhibition was observed maximum against *S. aureus* in methanolic extract of *P. guajava* leaves.



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

Series 1 = Sample, Series 2 = Tetracycline



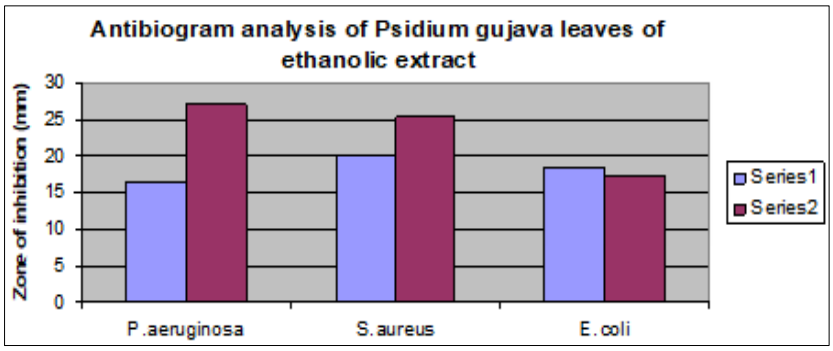
E. coli *P. aeruginosa* *S. aureus*

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

Table 2: Antibacterial activity in leaves (Ethanolic extract)

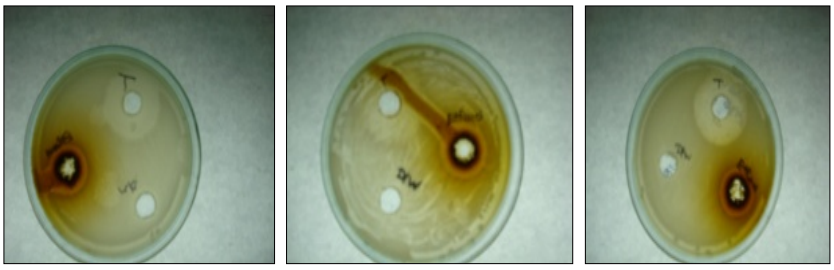
Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	16.5	27.0
S. aureus	20.0	25.5
E. coli	18.5	17.5

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



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

Series 1 = Sample, Series 2 = Tetracycline



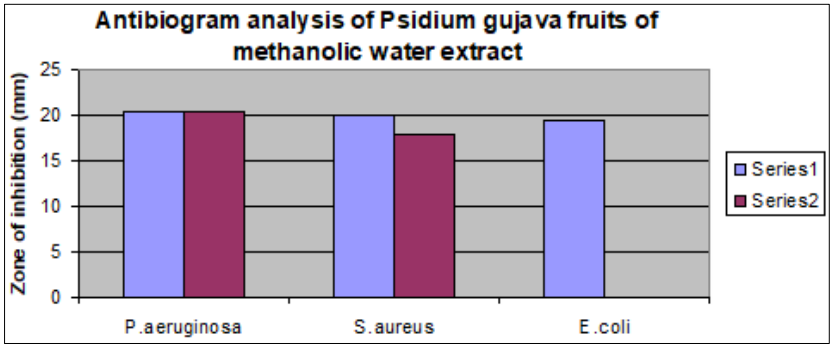
E. coli *P. aeruginosa* *S. aureus*

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 (Methanolic extract)

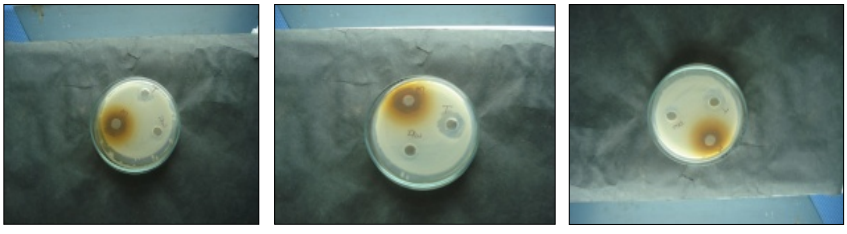
Pathogens	Z.O.I(mm)	Tetracycline (mm)
P. aeruginosa	20.5	20.5
S. aureus	20.0	18.0
E. coli	19.5	0.0

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



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

Series 1 = Sample, Series 2 = Tetracycline



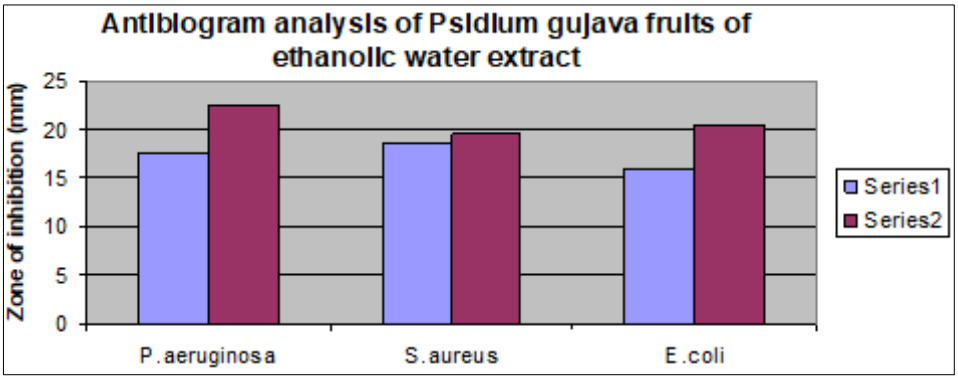
E. coli *P. aeruginosa* *S. aureus*

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

Table 4: Antibacterial activity in Fruits (Ethanolic extract)

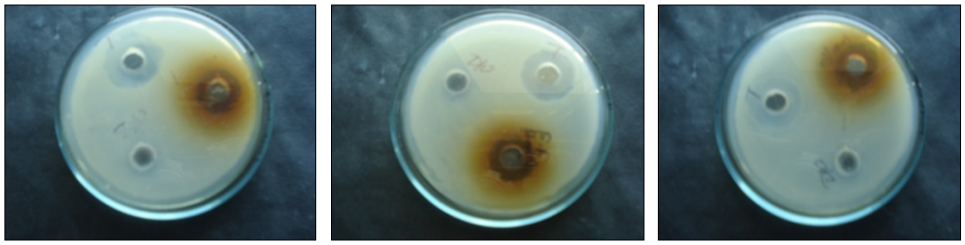
Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	17.5	22.5
S. aureus	18.5	19.5
E. coli	16.0	20.5

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



Graph 5: 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



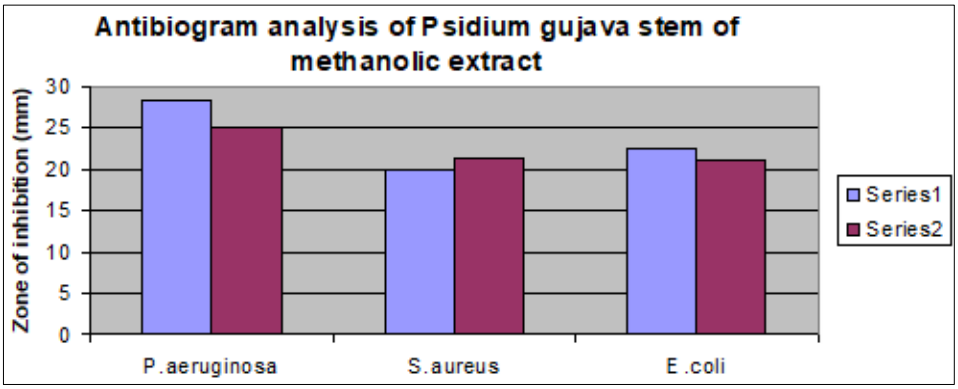
E. coli *P. aeruginosa* *S. aureus*

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

Table 5: Antibacterial activity in Stems (Methanolic extract)

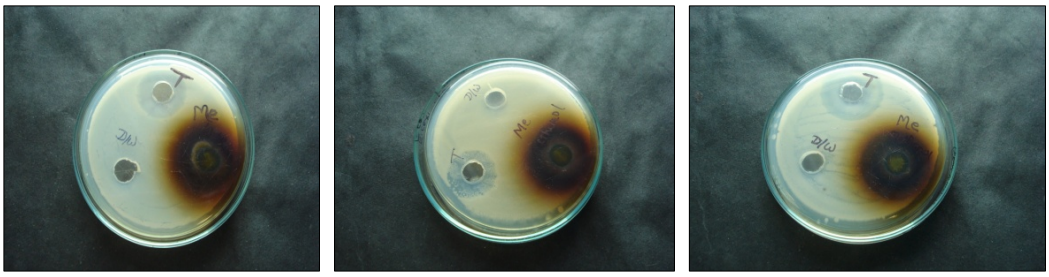
Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	28.5	25.0
S. aureus	20.0	21.5
E. coli	22.5	21.0

Table showed that the zone of inhibition were observed maximum against *P. aeruginosa* in Methanolic extract of *P. guajava* stems.



Graph 6: 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



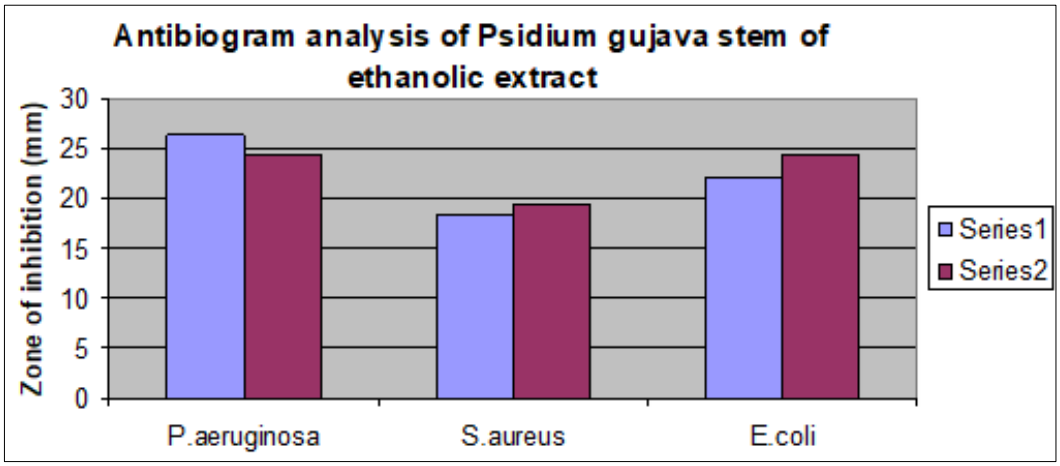
E. coli *P. aeruginosa* *S. aureus*

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

Table 6: Antibacterial activity in Stems (Ethanolic extract)

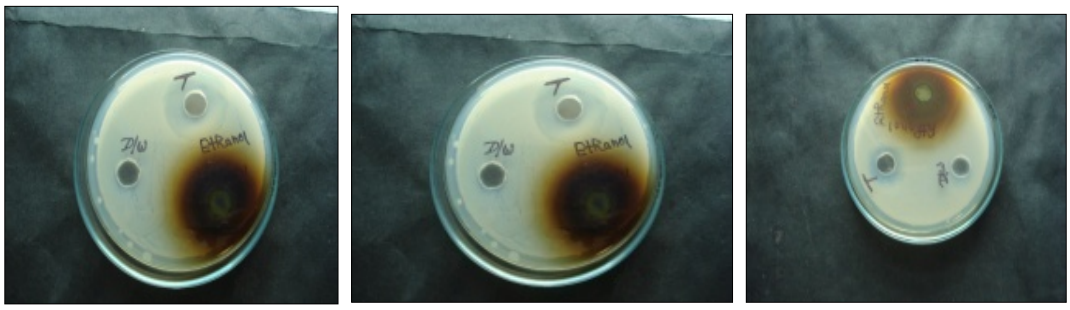
Pathogens	Z.O.I (mm)	Tetracycline (mm)
P. aeruginosa	26.5	24.5
S. aureus	18.5	19.5
E. coli	22.0	24.5

Table showed that the zone of inhibition were observed maximum against *P. aeruginosa* in ethanolic extract of *P. guajava* stems.



Graph 7: Graph showed that the maximum zone of inhibition in *P. aeruginosa* as compare to *S. aureus* and *E. coli*

Series 1 = Sample, Series 2 = Tetracycline



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

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

Table 7: MIC value of stems against bacterial pathogen for solvents

Test tube	Conc. of extracts (mg/ml)	Ethanolic extract of stems O.D against *Pseudomonas aeruginosa* (600nm)	Methanolic extract of stems O.D against *Pseudomonas aeruginosa* (600nm)
1	71.92	0.06	1.58
2	11.90	0.09	0.41
3	1.98	0.35	0.35
4	0.33	0.25	0.49
5	0.05	0.27	0.50
6	0.009	0.36	0.47

Table showed that the least concentration were obtained 0.33mg/ml in ethanolic extract of stems, and 0.05 mg/ml in methanolic extract against *P. aeruginosa.*

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). 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). 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 methanolic extract was showing best result while the ethanolic extract was showing minimum inhibition. The antibiogram analysis showed that zone of inhibition was observed 28.5 mm against *P. aeruginosa* for methanolic and ethanolic extract. The MIC values were obtained 1.98 mg/ml in ethanolic extract of stems and 0.05 mg/ml in Methanolic extract of Stem against *P. aeruginosa*. The antibacterial compounds mainly found in *Pisdium guajava* were Tanin, Phlobatonin, Saponin, Terpenoids, alkaloids and Poly phenols.

The future prospects of the present research work include isolation and purification of the therapeutic antimicrobials from the active extracts and carry out further pharmacological evaluation by several methods such as NMR, MS,GC-MS, TLC, HPLC to study the effect of different elicitors as heavy metals, cations, anions on antimicrobial property of plant extracts and Screening of several more RAPD markers in order to confirm the genetic diversity among several different varieties selected in our study, showing different antimicrobial activity against same microbe needs further screening with some more unique primers using the same genomic DNA molecules.

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