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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 ethanolic, methanolic, 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 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 ethanol and hot water extracts showed least antibacterial activity as compared to methanolic and ethyl acetate extracts. The least concentration were obtained 0.33mg/ml in ethanolic extract of stems, 1.98 mg/ml in ethyl acetate extract of stems and 0.05 mg/ml in methanolic extract of stems against *P. aeruginosa*. The antibacterial compound mainly found in *Psidium guajava* were reducing sugar, tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

Keywords: Antibacterial activities, ethanolic, methanolic & ethyl acetate plant extract, MIC, zone of inhibition.

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INTRODUCTION

For a long period of times plant 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 (WHO) medicinal plants would be the best source to obtain a variety of drugs [1]. Development of microbial resistance to the available antibiotics have led scientists to introduce the antibacterial activity of medicinal plants [2,3]. *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[4,5]. There are over 20 compounds have been reported present in leaves, stem, barks and roots of *P. guajava*[6,7,8,9]. Guava leaves were used to treat diarrhea 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 vitamin, iron calcium and phosphorus. Guava

plants contain some secondary metabolites. The roots were also rich in tannin. The leaves of guava were rich in flavonoids in particular quercetin, saponins, tannins, alkaloids, anthraquinones, phlobatannins and cardiac glycosides. Much of guava therapeutic activity was attributed to these flavonoids. The flavonoids had demonstrated antibacterial 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 diarrhea on many pacific islands. In many of the developing countries the used of the plant drugs was increasing because modern life saving drugs and people spend 40-50% income in drugs for health care. Among ancient civilization, India had been known to be rich repository of medicinal plants. A wide spectrum of activities against a variety of human ailments found in guava leaf extract.

The present study is carried out by evaluation of antibacterial properties of *Psidium guajava* against bacterial pathogens. 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 (1:10); 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 MRD LifeSciences, 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, methanolic, ethyl acetate and hot water extracts by using agar well diffusion method [10]. 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, methanolic, 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 37^0C in shaker incubator [11,12]. MIC of all samples were determined by broth dilution method. A two-fold serial dilution of the methanolic, ethanolic, ethyl acetate and hot water extracts were prepared and optical density was measured at 600 nm [13].

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 1ml or 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 100^0C 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 color gives positive result.

Test for phlobatannins:

Take 2ml plant sample in a test tube and add 10 ml deionized water and boil at 100^0C 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:

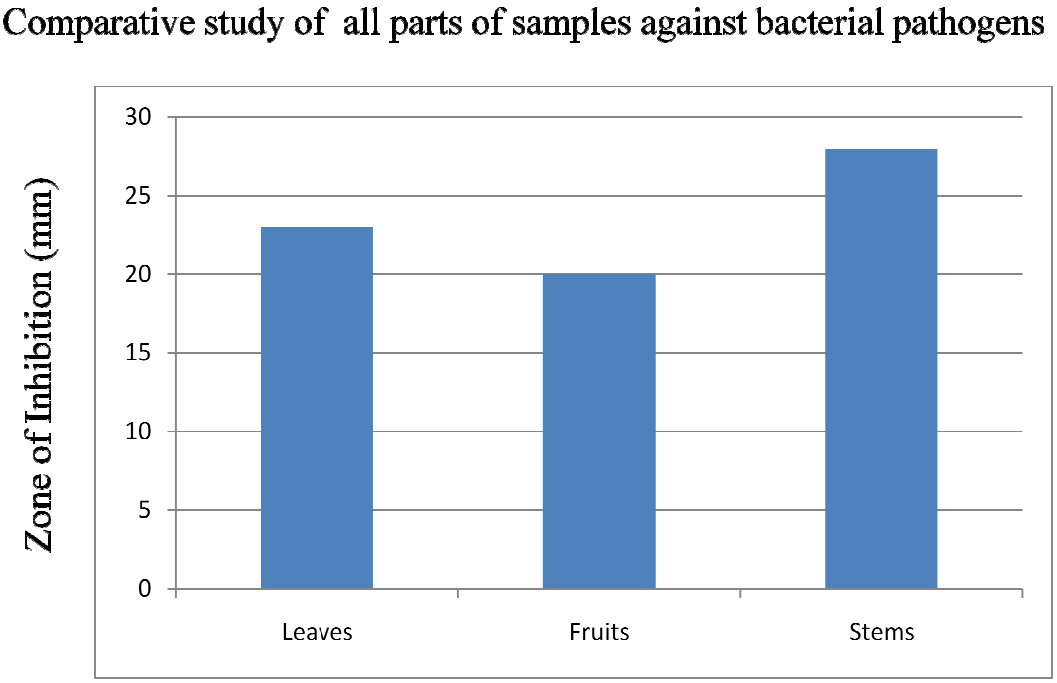
Take 1ml of aqueous extract in test tubes and add 2-3 drops of wagners reagent it gives orange red precipitation.

Test for flavonoids:

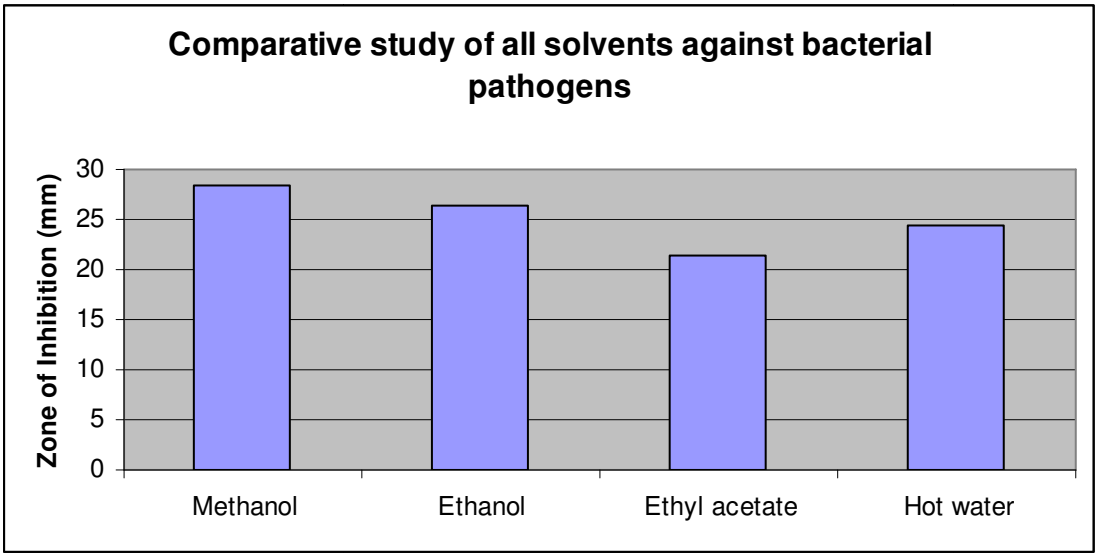
Take 1 ml of sample and add 1% NH_3 solution if yellow color observed, showed presence of flavonoids then after this take ethanolic or aqueous extract and add 10 ml DMSO then heat it followed by adding Mg (magnesium chloride), add conc. HCl gives red color to confirmed flavonoids.

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.



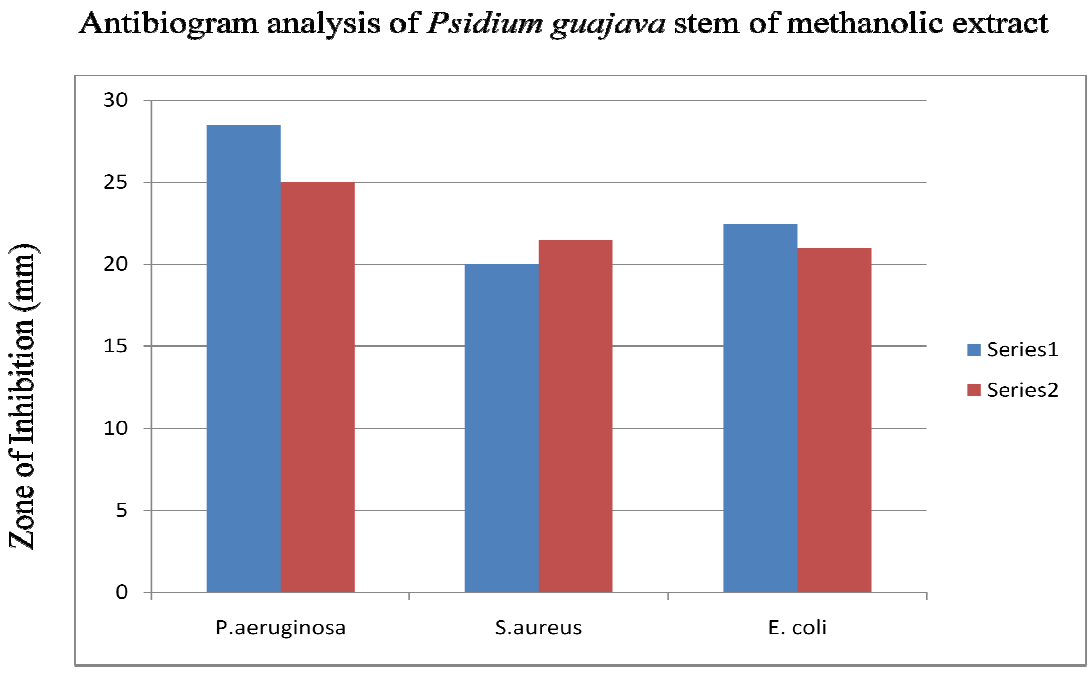
Graph 1: Graph showed that *P. guajava* stems were having maximum antibacterial activity, compare to leaves and fruits.



Graph 2: Graph showed that compare to all solvents, methanol, ethanol and hot water extracts were having maximum antibacterial activity.

Table 1: Antibacterial activity in stems (methanolic extract)

Pathogens	ZOI of Sample (mm)	ZOI of Tetracycline (mm)
P. aeruginosa	28.5	25
S. aureus	20	21.5
E. coli	22.5	21

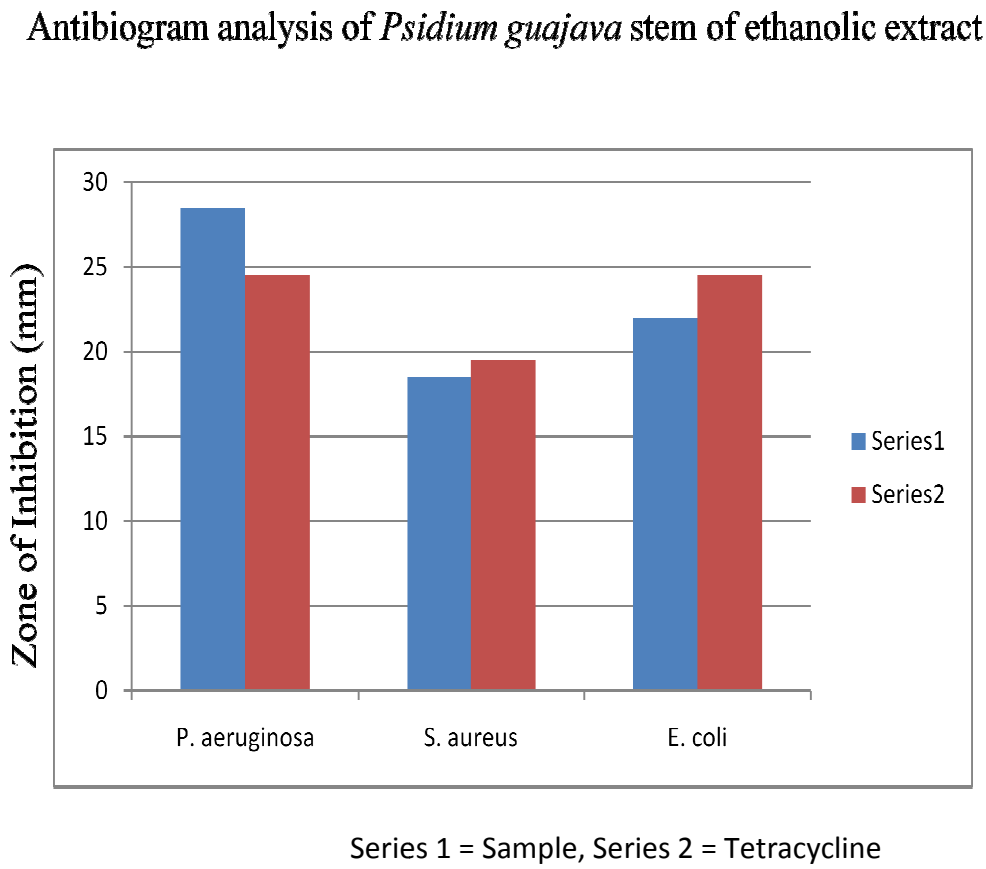


Series 1 = Sample, Series 2 = Tetracycline

Graph 3: Graph showed that maximum antibacterial activity was obtained in *P. aeruginosa* compare to *S. aureus* and *E. coli*.

Table 2: Antibacterial activity in stems (ethanolic extract)

Pathogens	ZOI of Sample (mm)	ZOI of Tetracycline (mm)
P. aeruginosa	28.5	24.5
S. aureus	18.5	19.5
E. coli	22	24.5

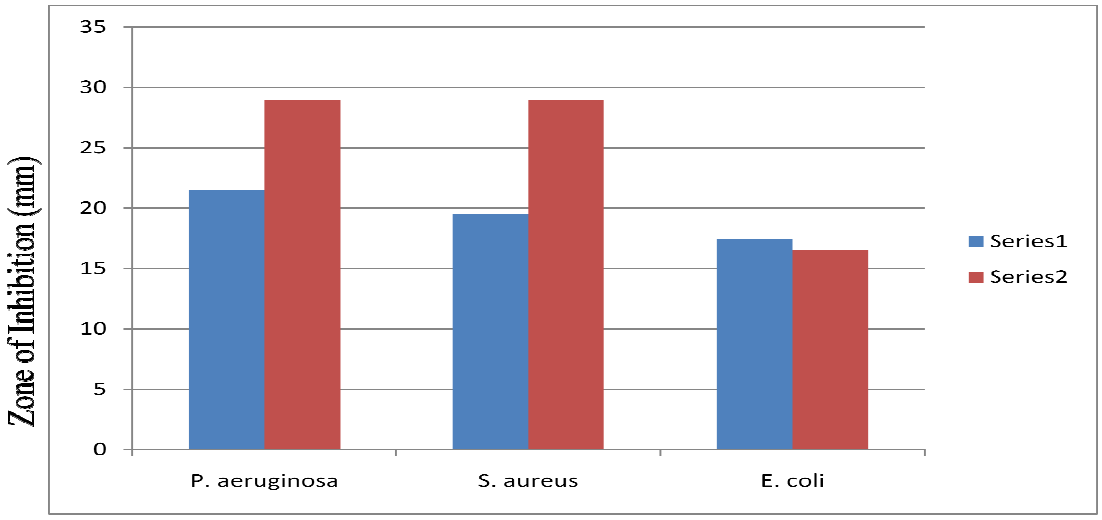


Graph 4: Graph showed that maximum antibacterial activity was obtained in *P. aeruginosa* compare to *S. aureus* and *E. coli*.

Table 3: Antibacterial activity in stems (ethyl acetate)

Pathogens	ZOI of Sample (mm)	ZOI of Tetracycline (mm)
P. aeruginosa	21.5	29
S. aureus	19.5	29
E. coli	17.5	16.5

Antibiogram analysis of *Psidium guajava* stem of ethyl acetate extract



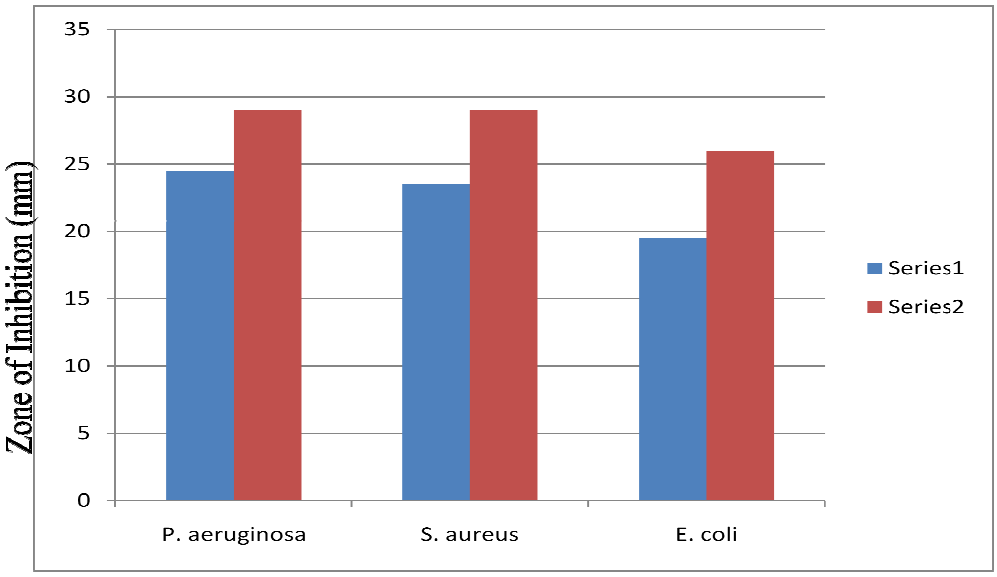
Series 1 = Sample, Series 2 = Tetracycline

Graph 5: Graph showed that maximum antibacterial activity was obtained in *P. aeruginosa* compare to *S. aureus* and *E. coli*.

Table 4: Antibacterial activity in stems (hot water extract)

Pathogens	ZOI of Sample (mm)	ZOI of Tetracycline (mm)
P. aeruginosa	24.5	29
S. aureus	23.5	29
E. coli	19.5	26

Antibiogram analysis of *Psidium guajava* stem of hot water extract



Series 1 = Sample, Series 2 = Tetracycline

Graph 6: Graph showed that maximum antibacterial activity was obtained in *P. aeruginosa* compare to *S. aureus* and *E. coli*.

Table 5: 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)	Ethyl acetate extract of stems O.D against *Pseudomonas aeruginosa* (600nm)
1	71.92	0.06	1.58	0.13
2	11.90	0.09	0.41	0.29
3	**1.98**	0.35	0.35	**0.24**
4	**0.33**	**0.25**	0.49	0.47
5	**0.05**	0.27	**0.50**	0.53
6	0.009	0.36	0.47	0.49

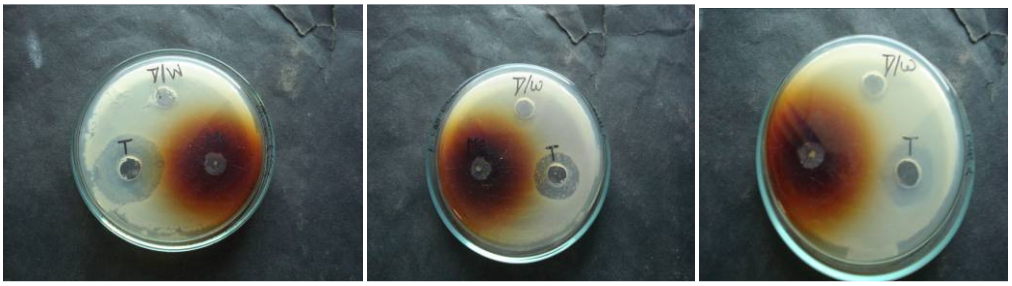
Table 5 showed that the least concentration were obtained 0.33mg/ml in ethanolic extract of stems, 1.98 mg/ml in ethyl acetate extract and 0.05 mg/ml in methanolic extract against *P. aeruginosa.*

Table 6: Phytochemical Analysis

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

Table 6 showed that the antibacterial compound mainly found in *Psidium guajava* were reducing sugar, tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

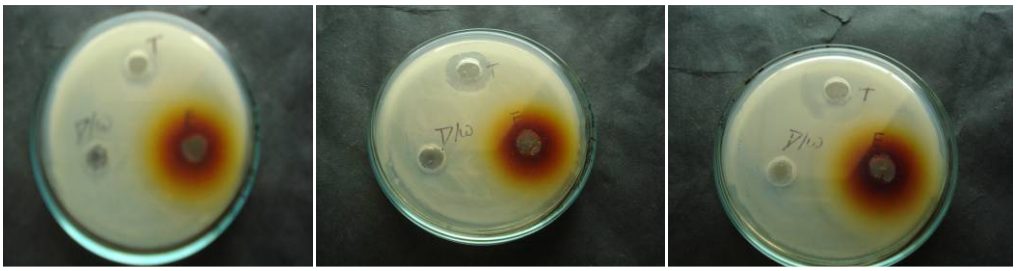
Figure 1: Antibacterial activity of methanolic extract of stems



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

Figure 1showed that maximum antibacterial activity was obtained in *P. aeruginosa* (28.5mm) compare to *S. aureus* and *E. coli*.

Figure 2: Antibacterial activity of ethanolic extract of stems



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

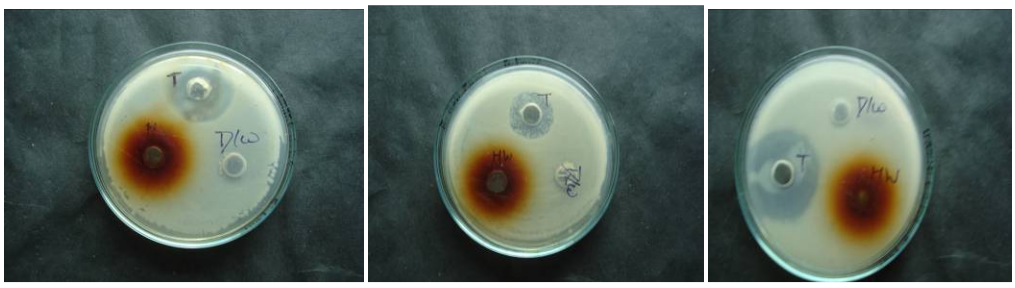
Figure 2 showed that maximum antibacterial activity was obtained in *P. aeruginosa* (28.5mm) compare to *S. aureus* and *E. coli*.

Figure 3: Antibacterial activity of ethyl acetate extract of stems

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

Figure 3 showed that maximum antibacterial activity was obtained in *P. aeruginosa* (21.5mm) compare to *S. aureus* and *E. coli*.

Figure 4: Antibacterial activity of hot water extract of stems



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

Figure 4 showed that maximum antibacterial activity was obtained in *P. aeruginosa* (24.5mm) compare to *S. aureus* and *E. coli*.

DISCUSSION

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 [15]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [16,17,18]. 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 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 ethanolic, methanolic and hot water extract were showing best result while the ethyl acetate extracts 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, 0.33mg/ml in ethyl acetate extract of stems and 0.05 mg/ml in methanolic extract of stems against *P. aeruginosa*. The antibacterial compound mainly found in *Psidium guajava* were were reducing sugar, tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

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