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Antimicrobial Activities, Antioxidant Andphytochemical Analysis Of Leaves And Stems Extracts Of Psidium Guajava

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ABSTRACT: To determine the antioxidant, antimicrobial activity and phytochemical analysis of Psidium guajava leaves and stems extracts against bacteria (Staphylococcus aureus and Pseudomonas aeruginosa) and fungi (Fusarium sp. and Aspergillus niger). The Guava leaves and stems were extracted by aqueous (hot and cold water) as well as solvent (methanol and n-hexane) extraction. The efficacy of these extracts was tested for antimicrobial activity against those bacteria and fungi through a well-diffusion method employing 50 µl leaf and stem extract solution per well. The quantitative analysis of phytochemical was done by using desire solvent; antioxidant activity was determined by 2, 2'-1-picrylhyrazyl (DPPH) radical scavenging method. According to the findings of above assays, all extracts of the guava leaves and stems showed inhibitory activity against such microorganisms. The hot extract of leaves had an antibacterial activity with mean zoon of inhibition of 13 mm. Cold extract of leaves had 4 mm inhibition whereas, absence of inhibition in stems' cold extraction, against Staphylococcus aureus and Pseudomonas aeruginosa. The phytochemical analysis saw the presence of alkaloids, flavonoids, steroids, terpenoids, tannins, phenolics. This plant contains the high amount of phenolics. This study provides scientific understanding to further determine the phytochemicals, antioxidant and antimicrobial values, investigate other pharmacological properties.

Key Word: Guava tree; Antimicrobial activity; Aqueous extracts (hot and cold extraction); Solvent extraction; Rotary vacuum evaporator; Soxhlet.

1. INTRODUCTION

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. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, 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, using various solvents to establish if it is effective against killing or inhibiting the growth of microorganisms. 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 O157:H7. 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 foodborne 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]. Recently there has been a lot of attention focused on producing medicines and products that are natural.

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]. The genus Psidium belongs to the family Myrtaceae, which is considered to have an originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world like Asia, Egypt, Hawaii, Florida (Figure 1), Palestine, and others. 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 guava tree is an evergreen small tree with 2 to 6 inches long and 1 to 2 inches wide, aromatic when crushed, and appear dull-green with stiff but coriaceous 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].

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]. 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].

Sacchetti et al. [16] reported that the oil showed a strong resistance against Yarrowia lipolytica which is a pathogenic yeast. Vieira et al. [17] have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the S. aureus. Gnan and Demello [18] 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. [19] concluding that the leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities.

Phytochemicals are nonnutritive 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 [20]. Begum et al. [21] 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 [22] 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.

1. MATERIALS AND METHODOLODY

Preparation of the plant extract:

The freshly collected the plant parts of Psidium guajava from the Anand Agriculture University. The plant parts were washed with distilled water and air dried at 40ºC by using hot air oven or dried at direct

sunlight for 1 or 2 days. Then, make a powder by using grinder. The powder was collected in zip bag to prevent the moisture.

Plant sample extraction (Aqueous):

The plant sample was extracted by using aqueous method and solvent extraction method.

- **Hot water extraction:**

Take 5 to 10 gm of plant powder sample and add it to in 100 ml of distilled water in a conical flask and incubate to water bath at 80ºC for 2 to 3 hours. After that, the mixture was homogenized and filtered by using muslin cheese cloth and sample was proceed into rotary vacuum evaporator for evaporation of water and sample extraction. The temperature of rotary vaccum evaporator was depends on the boiling point of solvent to be used. After the evaporation, the sample was filtrated (Whatman filter paper) and collected in black screw cap bottle and stored in refrigerator.

- **Cold water extraction:**

Take 5 to 10 gm of the dried powder sample and added to 100 ml distilled water in a conical flak and incubate into orbital shaker incubator at 140 rpm for overnight. Next day, the mixture was homogenized and filtrated with use of muslin cheese cloth and proceed it in to rotary vacuum evaporator and after that the extract must filtrated with (Whatman filter paper) and the final extract collect in to the black screw cap bottle and stored in to refrigerator.

Plant sample extraction (Solvent):

Plant extract is extracted by using Soxhlet extractor with high efficiency to analyze the phytochemicals present in the extract and by using this extract we can perform the different assay. The temperature used in this method is based on the boiling point of solvent. The solvent used for the extraction of plant sample were Methanol (boiling temperature was 64.7ºC or 65ºC) and hexane (69ºC). Different solvent used because some phytochemicals are dissolved in polar and some are dissolved in non-polar solvent.

Phytochemical Analysis:

- **Test for coumarins**:

  Take 2 ml of plant extract and add 10 % NaOH, formation of yellow color indicates the presence of coumarins.

- **Test for Anthocyanin:**

Take 2 ml of extract and add 2N HCL and few drops of ammonia, if the anthocyanin is present in the sample the pink –red color turning to blue-violet color.

- **Test for steroids [Libermann Burchard Test]:** Take 1 ml of extract and add 10 ml chloroform and in that add equal amount of H_2SO_4 positive result gives upper layer red, while lower layer yellow with green fluorescence.
- **Test for saponins:**

  Take 2 ml of extract and add 6 ml of distilled water and then shaken it vigorously, foam was observed when the sample has saponins.
- **Test for terpenoids:**

  2 ml of extract treated with 2 ml of acetic anhydride and then add few drops of H_2SO_4, positive result give blue, green ring formation.
- **Test for tannins [Braymer's Test]:**

  Take 2 ml of extract and allowed it to react with 10% alcoholic ferric chloride solution, positive result gives the formation of blue, green color.
- **Test for phenolics:**

  Add Few drops of extract in to 5% aqueous ferric chloride and when this test is positive deep blue or dark color form.
- **Test for flavonoids [Alkaline reagent test]:**

  2 ml of extract treated with 1N sodium hydroxide solution and give intense yellow color if, sample has flavonoids.
- **Test for alkaloids [Mayer's reagent]:**

  Add 2 ml of extract with few drops of Mayer's reagent and if, sample contain alkaloids than it will give white creamy precipitates.
- **Test for reducing sugar:**

  Take 0.5 ml of sample and add 5 ml of benedict reagent, boil it in boiling water bath for 1 min if the sample has reducing sugar, the solution form brick red color precipitates.

Test microorganisms:

The bacterial (S. aureus, P. aeruginosa)and fungi (A. niger and Fusarium) strain were collected from Shri Alpesh N Patel Post Graduation Institute of Science and Research, Anand.

Culture media and inoculums:

The N-agar media used for the Anti-bacterial activity and PDA media was used for Antifungal activity. The bacterial culture was inoculated in the nutrient broth and incubated overnight to allow the growth.

Antibiotics:

The antibiotic was used as a positive control in antimicrobial activity. In this activity Ampicillin was used as positive control and the concentration was 10mg/ml. In antifungal activity the Fluconazole was used, the concentration was 10 mg/ml.

Antimicrobial screening:

The antimicrobial activity of different parts extract carried out by agar well diffusion method.

- **Antibacterial activity:**

The Antibacterial activity carried out by using agar well diffusion method and test microorganisms. The negative control of antibacterial activity are Methanol, Hexane and distilled water.

- **Antifungal activity:**

The Antifungal activity was carried out by using well diffusion method. In this method the first step is growth of fungus on selective media, then make a suspension of it. If, fungi are sporulated, count the spores by hemocytometer and after that make a suspension which is used for the assay. Take an aliquot of 0.1 ml of suspension and spread it on the appropriate media, then make a well. Wells were filled with different parts extracts, in which for negative control the solvent and distilled water used and for positive control the Fluconazole was used.

Incubate the plates in incubator for 6 to 7 days at 25ºC to 30ºC. If the sample have an antifungal activity, the zone of inhibition was observed after the incubation time.

Antioxidant activity:

The antioxidant activity was determined by 2, 2 – diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging method. The anti-oxidant activity of different extract was measured in terms of H^+ donating or radical scavenging ability, using the stable radical DPPH. The different aliquots of extracts and 2 ml of DPPH in each tube was put in dark for 15 to 20 minutes and then take O.D at 517 nm.

2. RESULTS AND DISCUSSUION:

Phytochemical analysis of plant extracts:

The study of phytochemicals of Psidium guajavashows that, Alkaloids, Flavonoids, Tannins, Saponins, Terpenoids, Steroids, reducing sugar, Coumarins, Phenolics are present [10] [13].

Antimicrobial activity:

- **Antibacterial activity:**

The screening of antimicrobial activity of the Psidium guajavashows the antibacterial activity against the S. aureus, P. aeruginosa.

- **Antifungal activity:**

Antifungal activity was observed against A. niger and fusarium in Psidium guajava, [9]. The extraction of Psidium guajava gives the antifungal activity against A. niger, and some parts give antifungal activity against the Fusarium. The fluconazole antibiotic used as positive control, concentration is 10 mg/ml.

Antioxidant activity:

After screening out the antioxidant activity, the methanol extraction of Psidium guajava gives high antioxidant activity [17] [21]. It also shows the absorbance of ascorbic acid increase with increasing of concentration of ascorbic acid. The radical scavage activity of ascorbic acid increase after 30 min of incubation in dark condition [15] [16].

3. CONCLUSION:

The study it provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, P. guajava leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by P. aeruginosa and S. aureus.

Phytochemicals shows the important part to prevent and protect the plant against the microorganisms. Another importance of phytochemicals of Psidium guajava provide the information about the compound which are responsible for antimicrobial activity like Alkaloids, Phenolics, Flavonoids etc. The antioxidant activity was involved in the prevention of plant cell tissue damage. Antioxidant activity is measured by DPPH. The total phenolics content of this plant were good and there for this has a high antimicrobial activity.

The present work demonstrates the antimicrobial potential of Psidium guajava leaves and stems extract by using various solvents. The results indicate that n-hexane and methanol are better for the extraction

of the antibacterial properties of guava. The results also indicate that the plant extracts have antibacterial and antifungal effect, showing that they contain active ingredients against the organisms. The observed inhibition of bacteria and fungi, Pseudomonas aeruginosa and Staphylococcus aureus and Fusarium sp., Aspergillus niger, respectively, suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol or n-hexane as the solvent. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained.

4. TABLES, PICTURES AND GRAPHS:

NB: '-' stand for negative whereas, '+' stand for positive.

Table 1: Phytochemical analysis of Aqueous extracts

Plant	Parts	extract	Coumarins	Steroids	Terpenoids	Flavonoids	Alkaloids	Saponins	Tannins	Phenolics	Reducing sugar
P. guajava	Leaves	Hot	-	-	-	-	-	+	+	+	+
		Cold	+	-	-	+	-	+	+	-	+
	Stems	Hot	+	-	-	-	-	+	-	+	+
		Cold	+	-	-	+	-	+	-	+	+

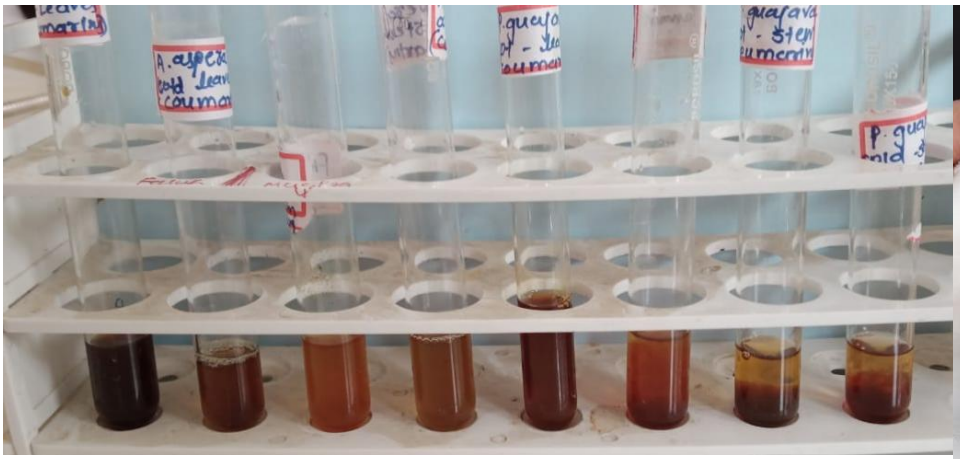


Image 1: coumarins test

Image 2: Terpenoids

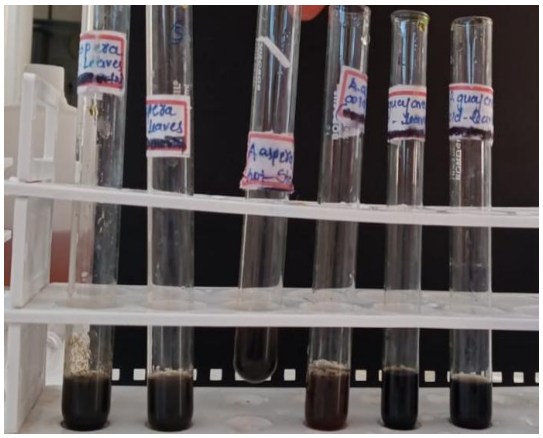


Image 3: Tannins

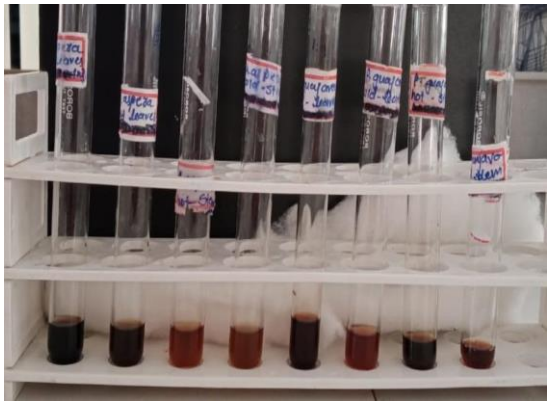


Image 4: Flavonoids

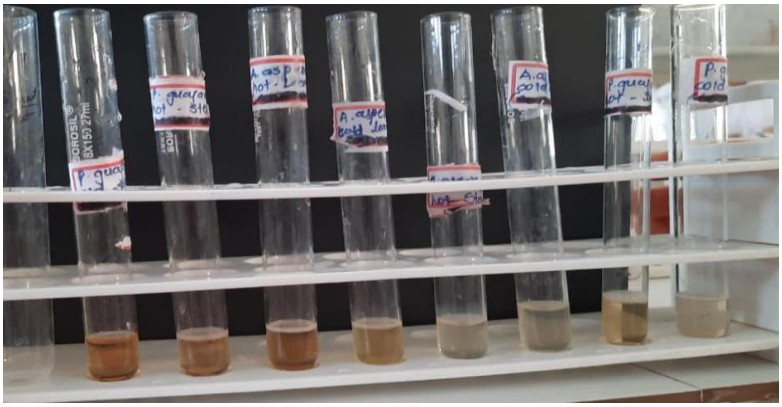


Image 5: Saponins

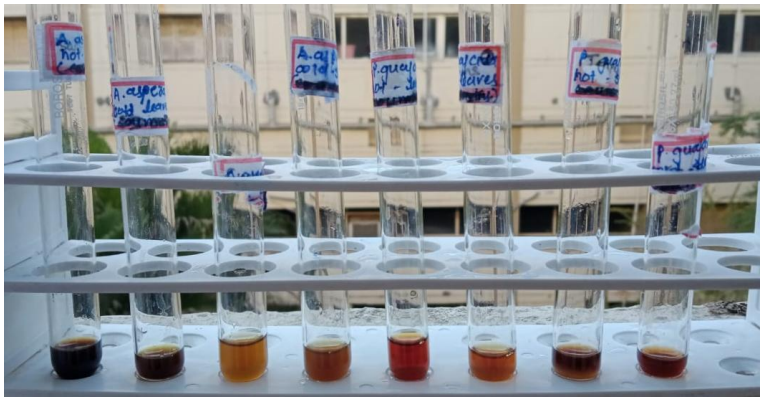


Image 6: Phenolics

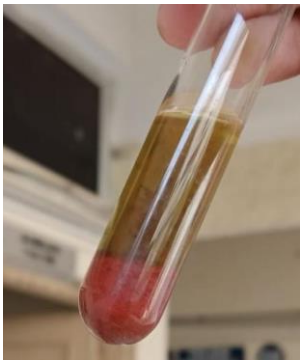


Image 7: Reducing sugar

Table 2: Phytochemical analysis of Methanol extract:

Plant	Parts	Coumarins	Steroids	Terpenoids	Flavonoids	Alkaloids	Saponins	Tannins	Phenolics	Reducing sugar	Gums	Glycosides	Carbohydrates
P. guajava	Leaves	-	+	-	-	+	+	+	+	+	-	-	-
	Stems	-	+	-	-	+	-	+	+	-	-	-	-

Image 8: Coumarins

Image 9: Steroids **Image 10: Saponins**

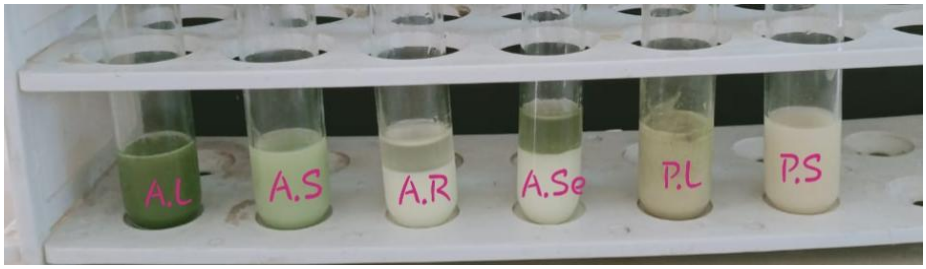


Image 11: Terpenoids

Image 12: Flavonoids

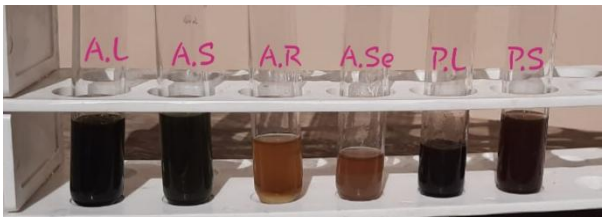


Image 13: Gums

Image 14: Alkaloids

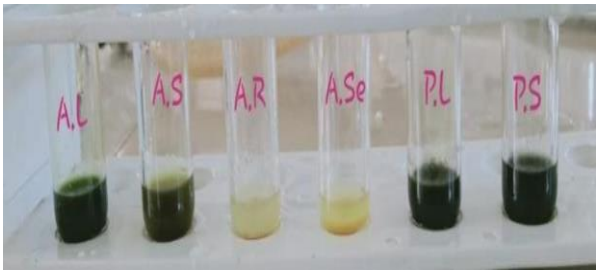


Image15: Tannins

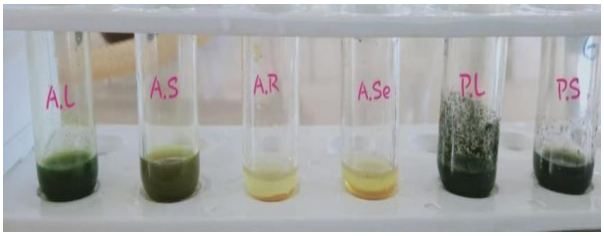


Image 16: Phenolics

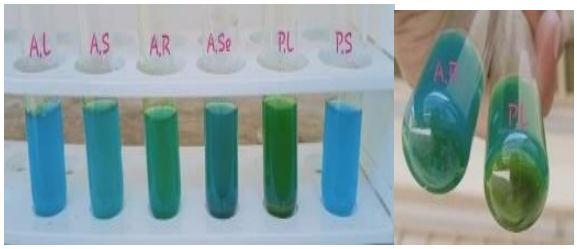


Image17: Reducing Sugar

Table 3: Phytochemical analysis of n- Hexane extracts:

Plants	Parts	Coumarins	Steroids	Terpenoids	Flavonoids	Alkaloids	Saponins	Tannins	Phenolics	Reducing sugar	Gums	Glycosides	Carbohydrates
P.guajava	Leaves	+	+	+	+	+	-	-	-	+	+	+	+
	Stems	+	+	+	+	+	-	-	-	+	+	+	+

Table 4: Antimicrobial activity of Aqueous extracts:

Plants	Parts	Extraction method	S. aureus (mm)	P. aeruginosa (mm)

P.guajava	Leaves	Hot	13	15
		Cold	4	9
	Stems	Hot	-	-
		Cold	-	-
	Positive Control	Antibiotic	44	30

Table 5: Antimicrobial activity of different extracts:

Plants	Parts	Solvent	S. aureus (mm)	P. aeruginosa (mm)
P. guajava	Leaves	Methanol	21	14
		N-hexane	19	13
	Stems	Methanol	26	15
		N-hexane	15	21
control		Antibiotic	44	30

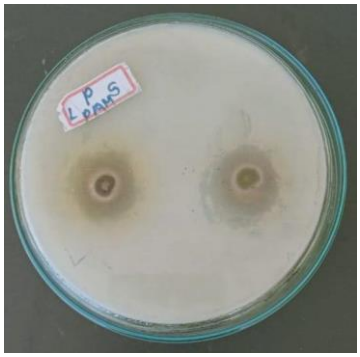


Image 18:P. guajava leaves and stems antimicrobial activity againstS.aureus

Image 20:P. guajava leaves and stemsantimicrobial activity of n-hexane against S. aureus

Image19: P. guajava leaves and stems antimicrobial activity against P. aeruginosa

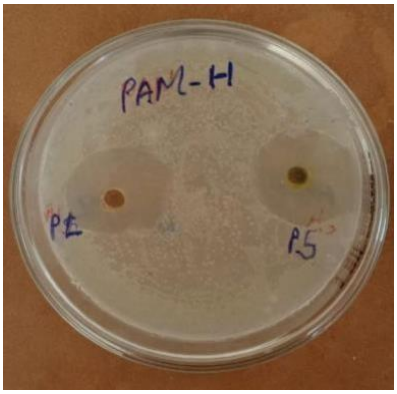


Image 21:P. guajava leaves and stems antimicrobial activity of n-hexane against Pseudomonas aeruginosa

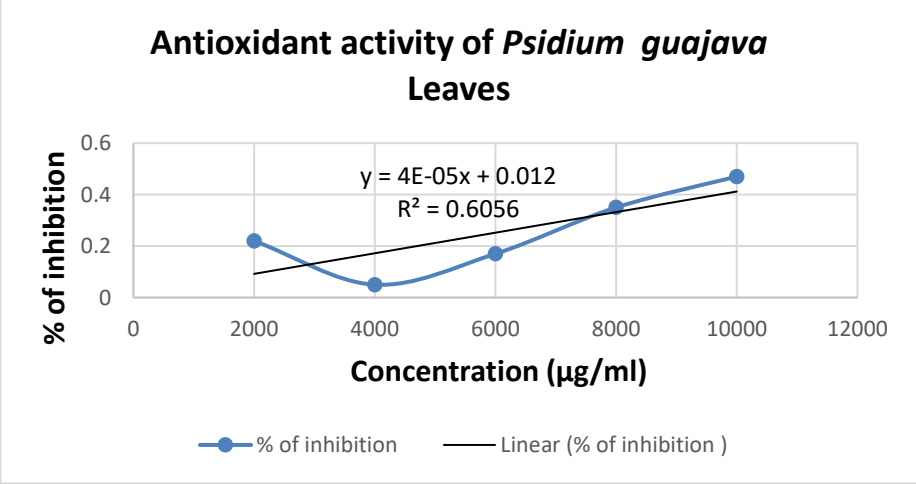
Table 6: Antifungal activity against A. niger:

Plants	Parts	Solvent	Aspergillusniger (mm)
P. guajava	Leaves	Aqueous	8
		Methanol	9
		N-hexane	7
	Stems	Aqueous	6
		Methanol	0
		N-hexane	0
	Control	Antibiotic	20

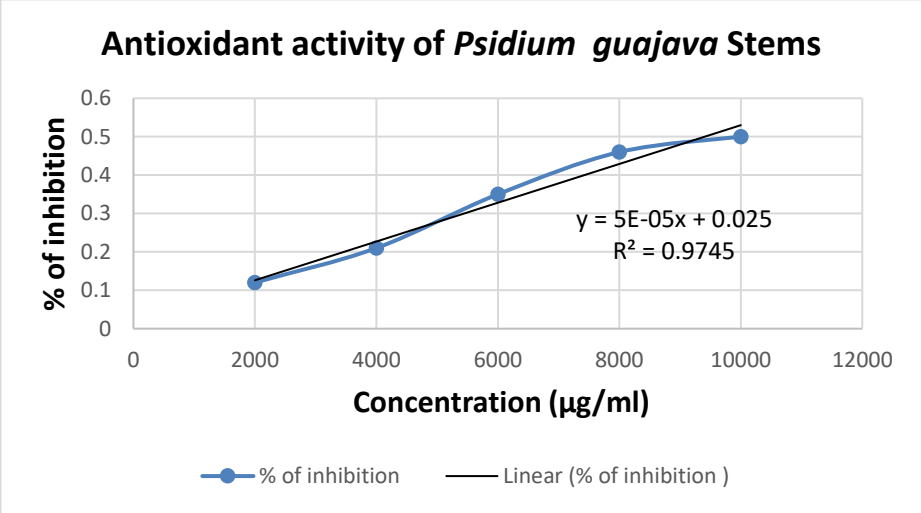
Table 7: Antifungal activity against Fusarium sp.:

Plants	Parts	Solvent	Fusarium sp. (mm)
P. guajava	Leaves	Aqueous (hot)	0
		Aqueous (cold)	0
		Methanol	14
		N-hexane	0
	Stems	Aqueous (hot)	8
		Aqueous (cold)	9
		Methanol	7
		N-Hexane	0
	Control	Antibiotic	47

Graph 1: Antioxidant activity of P.guajava leaves:



Graph 2: Antioxidant activity of P. guajava stems:



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