STUDIES ON PHYTOCHEMICAL AND INHIBITORY ACTIVITY OF *PSIDIUM GUAJAVA* LEAF EXTRACTS WITH *ESCHERICHIA COLI*, *BACILLUS SUBTILIS* AND *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

The rising threat of antimicrobial resistance in numerous pathogenic organisms has prompted the quest for enduring cure. The aim of our study was to analyse the phytochemical and antimicrobial properties of different extracts of *Psidium guajava* leaves against some bacterial strains. The plant leaves were removed in four solvents in particular; ethyl acetate, ethanol, aqueous and chloroform. The bacterial strains were *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The phytochemical examination showed the presence tannins, flavonoids, alkaloids, saponins, reducing sugar and proteins in various extents. The antibacterial action of the extracts in vitro showed that the crude extract in all bacterial strains show increased antimicrobial activity as the concentration goes on increasing. It can also be seen that E. coli show a bit less inhibitory activity in comparison to other 2 bacterial strains. The inhibition zone of around (16.02±0.04 mm) in diameter at 40µl where as other two strains *Bacillus subtillis* and *Pseudomonas aeruginosa* recorded with 20.05±0.07 mm and 19.00±0.16 mm in diameter in crude extracts of *P. guajava*. The Soxhlet extracts with 40 micro-litre concentration, the inhibition zone's diameter was 19.99±0.12 mm for E. coli where as for other two strains *Pseudomonas aeruginosa* and *Bacillus subtillis* inhibition zone recorded 15.06±012 mm and 17.08±0.11 mm in diameter respectively. This study has shown that the leaves extract of *P. guajava* contains antibacterial and phytochemical substances which can be used in satiation of human journey for healthier and better living.

Keywords: Leaf extract, *Psidium guajava*, Phytochemical, Antibacterial activity

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INTRODUCTION

Nowadays a lot of interest has been centered on making drug treatments and merchandise which are natural. Also, several fruit extracts and fruits, as nicely as tea extract of arrowroot

[1] and caffeine [2] is found to show off anti-microbial recreation in opposition to E. coli (O157:H7), which suggest that plants which occur pretty excessive stages of antimicrobial activity may also be the sources of bio-active component that tends to inhibit or resist the growth and development of food-borne micro-organism. Bacterial cells should be destroyedby rupturing cell membrane and cell walls and by using the irregular intracellular matrix disruption when dealt with various extracts of plants. [1].

Psidium guajava is a plant with phytotherapy benefits widely used in folks' medicinal drug which is considered to have various bioactive components that also help to treatment and management of several diseases. Many components of this plant have been used in typical remedy to control stipulations like gastroenteritis, malaria, vomiting, dysentery, diarrhoea, ulcers, wounds, toothache, sore throat, coughs, infected gums, and various other circumstances [3–5]. Furthermore, this plant has also been promising as it was used as a medicine to control life-changing situations like hypertension, diabetes, and weight problems[3, 6–10].

The genus (Psidium) is of Myrtaceae family, and it is believed to be originated from tropical area of South America. *Psidium guajava* are grown in subtropical and tropical areas of the globe like Egypt, Asia, Florida, Palestine, Hawaii and others. This genus includes about 150 species including small bushes and shrubs out of which only 20 species produce fruits (edible)and other are wild fruit producing species [11]. Usually, the most cultivated Psidium species is *Psidium guajava L*. considered as the frequent guava plant. Other species are utilized for rules of fruit fantastic enhancement, vigour and resistance to insects or pests and ailment [11]. The guava fruits these days are regarded minor in context of business world trade, however they are widely grown in the tropical area, which enhance the weight loss program of lots of thousands of humans in these areas.

Guava is a small tree which is evergreen. Their leaves are around 2-6 inches in length 1-2 inches in width, fragrant when it is crushed, and seems dull green and stiffy however they arecoriaceous having pronounced veins [12]. They have white flower and sweet green fruit which became pale when ripen. Bioactive components are present in the guava leaf that can be promising anti-microbial compound against pathogenic organism, modify glucose levels in blood, and also help in weight-loss. Guava leaves incorporate an important oil rich in tannins, cineol, flavonoids, triterpenes, resin, fat, eugenol, cellulose, malic acid, mineral salts, chlorophyll, and a variety of different bio-active substances [13–15]

Figure 1: Guava tree

Figure 2: Guava leaf

Figure 3: Guava fruit

The frequent methods of extraction of medicinal plant consist of steps such as maceration, percolation, infusion, decoction, and digestion, aqueous-alcoholic extraction with the aid of fermentation, Soxhlet extraction, microwave-assisted extraction, counter-current extraction, ultrasound extraction, Phyto-tonic extraction and supercritical fluid extraction. The maceration extraction method is a crude extraction; solvent diffuse into the thick plant material and solubilize compounds having comparable polarity [16]. The effect of plants fabric relies on its origin, versions of the extraction process, temperature of extraction, the time, solvent attention and quantity, polarity and secondary metabolite composition present in an extract [17]. Differences in extraction technique are normally related to the length of the extraction period, pH, the solvent used, temperature, the solvent-to-sample ratio, and particle size [15].

In this study, three different strains of food-borne pathogens are used to detect and evaluate the anti-microbial property of guava leaf extracts. Three different bacterial strains are

I. *Escherichia coli*: They are facultative anaerobic, gram-negative, rod shaped, coliform bacteria. They are generally found in the lower intestine of poikilothermic animals.
II. *Bacillus subtilis*: also known as the hay bacillus or grass bacillus, it is gram-positive bacteria generally found in soil and the gastrointestinal tract of sponges, humans andruminants.
III. *Pseudomonas aeruginosa*: It is a gram-negative, rod-shaped bacteria. They are responsible for causing infection in lungs, blood and other parts of body after surgicaltreatment.

We, in this study, aim to prepare and use different extracts of *Psidium guajava* leaves, from ITM University, the usage of organic and aqueous solvents to find out if it is wonderful towards destroying as well as inhibiting the growth of food-borne bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* which are responsible for food spoilage and cause illness.

MATERIAL AND METHODS

1. Sample Collection

The sample (leaves) *Psidium guajava* were obtained from the herbarium of ITM UNIVERSITY,

Gwalior.

Chemicals such as ethanol, methanol, chloroform, DMSO, acidic anhydride, ferric chloride, Fehling's solutions I and II, copper sulphate, Mayer's reagent, sodium hydroxide,hydrochloric acid etc. and micro-organism such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* were obtained from laboratories of Department of life science (biotechnology and microbiology)

Figure 4: Fresh guava leaves

Figure 5: Dry guava leaves

2. Methods

❖ Sample Preparation

Around 200-250 leaf of *Psidium guajava* was plucked and washed well. Around 150 leaves were kept in shade for drying for around 10 days.

❖ Extract Preparation

Fresh leaves are well washed and water was removed by drying at room temperature. A gramof leaf was taken and grinded with the help of mortar and pestle. They are then poured into test tube which contains 10 ml DMSO. It was filtered out after 24 hours. It was labelled fresh extract.

The dried leaf was grinded with the help of grinder. The extract was prepared in a test tube using 1 gram of dried powder with 10 ml of DMSO. It is left for 24hrs for optimum extraction.Next day, the solution was filtered and stored for further use. It was labelled as crude extract.

5 gram of leaf powder was packed in filter paper (Whatman No.3) and then subjected to Soxhlet extraction with 250 ml of 70 % ethanol at 80C for 12 hr. The extracts were then dried, scrappedoff and 1 gram of that extract was mixed with 10 ml DMSO to prepare stock solution. It was labelled Soxhlet extract.

Figure 6: Soxhlet extraction

Figure 7: Product of Soxhlet extraction to be dried

❖ Maintenance Of Bacterial Culture

All three strains of bacteria viz. *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* are obtained from and maintained in the lab of biotechnology, ITM University. The bacterial culture is made with the help of respective strains master plates that were available in the lab. The bacteria are first sub cultured on nutrient agar plate and after 24 hours of incubation at 37^{o}C temperature, they are then again sub cultured in nutrient broth media. They are then keptin an incubator for further use. The ideal temperature and nutrient content were maintained andthat was used as sample for antimicrobial activity testing.

Preliminary Phytochemical Screening

Preliminary phytochemical screening or qualitative phytochemical analysis were done with dry leaf extract of *Psidium guajava*. The extract used were of aqueous, chloroform, ethanol, and ethyl acetate. The test was conducted to detect tannins, flavonoids, reducing sugar, protein, alkaloidsand saponins in accordance to the following protocol: : All the extracts were screened for the presence of phytochemicals - Tannins, Saponins, Flavonoids, Alkaloid, Reducing sugar and Proteins using the procedures given by researchers 18,19.

❖ Antimicrobial Activity

Antibacterial test was carried out by Agar well diffusion method against three different microbial strain namely *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. Briefly, 0.4 ml culture was uniformly distributed over Nutrient agar plates. Well of 8 mm diameter were

made on agar plate. To different well numbered I, II, III, IV respectively 10, 20,30 ,40 microliter of plant extract (crude and Soxhlet extract) was added. They are proper labelled and incubated for 24 hrs. After incubation for a day, inhibition zone was measured with the help of scale and it was noted for further evaluation (20).

RESULTS

1. Preliminary phytochemical analysis

In this study, preliminary phytochemical analysis was done in order to check the presence or absence of bioactive constituents such as tannins, flavonoid, reducing sugar, protein, alkaloids and saponin which are present in the leaves of the guava plant. The preliminary Phyto-chemical analysis of different extract such as ethanol, ethyl acetate, aqueous and chloroform of P. guajava gives the following result. The dried leaves of P. guajava were firstpowdered and dissolved in different solvents; ethanol, water, chloroform and ethyl acetate and thus obtained extracts were analyzed for the presence of secondary metabolites.

In *Psidium guajava*, tannins, flavonoid were found to be present in all the extracts except chloroform. Reducing sugars and alkaloids were observed to be absent in all the tested solvent extracts. Among the used solvent extracts, saponin was present in aqueous and ethanol extract only but the protein was present only in aqueous extracts (Table no -1)

SN	Phytochemicals	Ethyl Acetate	Ethanol	Aqueous	Chloroform
1	Tannins	+	+	+	-
2	Flavonoid	+	+	+	-
3	Reducing sugar	-	-	-	-
4	Protein	-	-	+	-
5	Alkaloids	-	-	-	-
6	Saponin	-	+	+	-

Table 1: Preliminary Phytochemical Analysis of *Psidium guajava*

"+" presence "-" absence

This analysis on plant extracts showed that there is presence of bio-active compounds which are acknowledged to show medical and biological activities. For instance, tannins which are poly-phenolic compounds which bind to protein rich in proline and affects protein production [31, 32, 33] and has revealed to have anti-bacterial activity [34, 35]. Flavonoids, polyphenolic compounds (hydroxylated) are known to be as a product of plant which are produce in response to infection caused by microorganism and because of which this feature has been widely studied and it isalso found to exhibit antimicrobial activity against various microorganisms *in vitro* [36]. Their skill has been ascribed to their ability of complex formation with both extracellular and soluble proteins as well as bacterial cell walls too [37].

Figure 8: Ethyl Acetate Extract

Figure 9: Ethanol Extract

Figure 10: Aqueous Extract

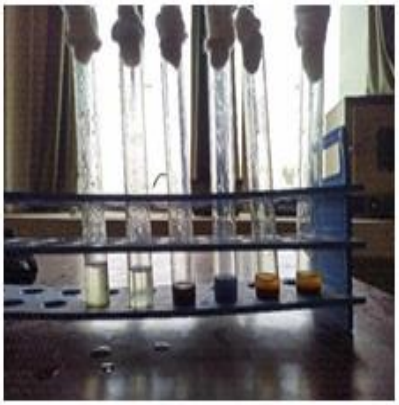

Figure 11: Chloroform Extract

The result showing preliminary phytochemical analysis of different solvent extract of *Psidium guajava* where L → R indicates test result of tannins, flavonoids, reducing sugar, protein, alkaloids and saponin.

2. Antimicrobial activity

Antimicrobial activity was done using well diffusion method [20]. As antimicrobial activity was carried out with 2 different extracts against 3 different bacterial strains and 4 different concentration levels, the results were noted down.

Antibacterial activity with Crude extract

The crude extract was effective against both gram-positive and gram-negative bacteria as it is shown in Figure 13, 14 and 15. The crude extract in all bacteria show increased antimicrobial activity as the concentration goes on increasing. All three bacterial sample were inhibited by the crude guava leaf extract. It can also be seen by the table 3 that *E. coli* show a bit less inhibitory

activity in comparison to other 2 strains. At 40 microliters, the inhibition zone was of around 15.02±0.43 mm in diameter where as other two strains have nearly 19.00±0.16 and 20.05±0.07 mm in diameter. The highest inhibition zone 20.05±0.07 is recorded with *Pseudomonas aeruginosa* compared with Bacillus subtilis and E. coli. The inhibition zone's diameter for 3 distinct bacteria is shown in the table 3:

SN	Bacterial strain	10x	10y	20x	20y	30x	30y	40x	40y
1	*Bacillus subtills*	16.92± 0.07	14.83± 0.16	16.86± 0.32	17.18± 0.25	19.84± 0.18	16.95± 0.14	19.00± 0.16	19.76± 0.13
2	*Pseudomonas aeruginosa*	15.01± 0.09	14.95± 0.14	14.97± 0.14	16.14± 0.18	20.83± 0.13	20.15± 0.20	20.05± 0.07	20.22± 0.15
3	E. coli	10.93± 0.08	10.95± 0.27	11.97± 0.24	13.01± 0.20	13.99± 0.28	14.94± 0.11	16.02± 0.04	15.02± 0.43

Table 3: Antibacterial activity of guava leaf extract (crude) for 3 distinct bacterial strains- *Bacillus subtilis*, *Pseudomonas aeruginosa* and *E. coli* (value in mm)

Figure 15

Figure 16

Figure 17

Pictures for crude extract results. Antimicrobial activity and inhibition zone and its diameter is shown in above Figure where Figure 15, 16 and 17 are for *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively.

Antibacterial activity with Soxhlet extraction

The extract made with the help of Soxhlet seems less effective than crude extract. All 3 strains of bacterial growth were inhibited by this extract as it is shown in Figure 16, 17 and 18. The extract shows greater result in *E. coli* strain, while the concentration did not show any differences in context of *Pseudomonas aeruginosa*. These values are not as greater as that of crude extract data in which nearly at higher concentration the inhibition zone's diameter was nearly 20 mm. At 40 microliters, the inhibition zone was of around 15.06±0.12 mm in diameter in *Pseudomonas aeruginosa* where as other two strains Bacillus subtilis and E. coli

recorded 17.08±0.11 and 19.99±0.12 mm in diameter. The highest inhibition zone 19.99±0.12 is recorded with E. coli compared with Bacillus subtilis and *Pseudomonas aeruginosa*. The inhibition zone's diameter for 3 distinct bacteria is shown in the table 4:

SN	Bacterial strain	10x	10y	20x	20y	30x	30y	40x	40y
1	*Bacillus subtills*	11.01± 0.15	10.03± 0.17	12.13± 0.24	12.02± 0.27	12.99± 0.24	14.94± 0.11	17.08± 0.11	16.02± 0.32
2	*Pseudomonas aeruginosa*	14.96± 0.06	12.05± 0.36	13.91± 0.28	14.04± 0.26	14.02± 0.15	14.94± 0.27	15.06± 0.12	15.01± 0.09
3	*E. coli*	10.96± 0.07	11.13± 0.43	15.14± 0.15	14.07± 0.11	13.86± 0.07	14.99± 0.29	19.99± 0.12	18.16± 0.45

Table 4: Antimicrobial activity of guava leaf extract (Soxhlet) for 3 distinct bacterial strains *Bacillus subtilis*, *Pseudomonas aeruginosa* and *E. coli* (value in mm)

Figure 18

Figure 19

Figure 20

Figures showing for Soxhlet extract of antimicrobial activity and inhibition zone and its diameter is shown in above Figure where Figure 18, 19 and 20 are for *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively.

DISCUSSION

The resistance for inhibition of the Gram-negative bacteria in some cases could be ascribed toits structure of cell wall. Such bacteria possess an effective permeability barrier which consistof an exterior membrane of thin lipopolysaccharide that could be a reason for its restriction towards the penetration of the plant extract. Earlier it has been revealed that these Gram- negative bacteria are more likely to show resistant towards the plant based antimicrobial agents and also did not show any effect, compared to Gram-positive bacteria [21-23]. Gram positive bacteria have peptidoglycan layer (mesh-like) which has more permeability to plant extracts [21, 22, 24, 25,].

Results of this study resembles or show differences in the data reported in research papers. Nascimento et al. [26] performed research which somewhat resembles with the finding of this

study in which the extract of guava was able to exhibit inhibitory effects against *Bacillus* and Staphylococcus whereas Chanda and Kaneria [27] oppose the results related to the Gram-negative bacteria. Biswas et al. [28] has reported that P. guajava has Gram-negative and Gram-positive antibacterial characteristics however, Vieira et al. [29] found *Psidium guajava* shoot extracts were active against inhibiting *E. coli*. Sanches et al. [30] revealed that aqueous extract of guava leaf extract is active against *Staphylococcus* and *Bacillus*.

FUTURE ASPECT

The antimicrobial property of guava leaf extract indicates towards immense possibilities in future. Some of the future aspect of this experiment that guava leaf extract holds are listed below:

i. It can be possible source of essential oil and flavonoids extraction
ii. It can be a source of natural supplement for controlling blood pressure, cholesteroland sugar.
iii. The phenolic compounds present in it holds a possible future of making an excellentantioxidant agent.
iv. This plant will be beneficial in making medicine related to anti-diarrheal andantibacterial usage.
v. The antimicrobial activity it possesses will also help in solving worldwide problemrelated bacterial resistant over antibacterial drugs.
vi. It can be a good source of natural anti-bacterial agent and also help in makingcompounds that prevent food-spoilage.

CONCLUSION

This experiment demonstrates the antimicrobial property of *Psidium guajava* and also gives idea about various phytochemicals present in the leaf of guava plant. It also compares the antimicrobial property of crude and Soxhlet extract of guava leaf. The result indicates that various phytochemicals are present including tannins, flavonoids, reducing sugar, saponin etc. This study also elaborates the future use of such phytochemicals in making anti-bacterial drugs. Upon comparison with other related data from other research papers indicate that different methodologies of studies on antibacterial activity, results in diverse outcomes. This study also provides scientific vision to further research on the antimicrobial ideologies and examine other pharmacological assets of guava. On the foundation of the present conclusion, *Psidium guajava* leaves hold the abilities of being a good source for the search for a natural antimicrobial agent against infections and/or diseases caused by *P. aeruginosa, B. subtilis* and *E. coli.* The phytochemical and antimicrobial studies of P. guajava leaf extract gives scientific evidence for prevention of diseases due to the activity of phytochemicals and in the treatment of various diseases by *E. Coli*, *B. subtilis* and *P. aeruginosa*. Further examination is important to uncover its point-by-point molecular mechanism behind these phytochemical and antibacterial activities.

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