

In Vitro* Evaluation of Antibacterial Properties of *Psidium guajava* Jahir Alam Khan¹, Vivek Chaurasia²¹ R&D division, MRD LifeSciences (P) Ltd., Lucknow, India.² Institute of Biosciences & Biotechnology, CSJM University, Kanpur, India.**Abstract:**

In the present study, antibacterial activity of cold aqueous, hot aqueous, methanolic, ethanolic, ethyl acetate, and acetone extracts of leaf, stem, bark, ripe and unripe fruit of *Psidium guajava* were screened against pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using Agar well diffusion method. All the extracts showed significant antibacterial properties. Cold aqueous extract of ripe fruit was found to be the most effective followed by acetone extract of leaf, hot aqueous extract of stem, ethanolic extract of unripe fruit and acetone extract of bark.

Key words: *Psidium guajava*, herbal antibiotics, pathogenic, antibacterial properties.

Introduction:

Widespread use of drugs is leading to the development of resistance against them in the pathogens^[1] and also the side effects associated with them is urging people not to use them. Therefore this is the need of the time to search for some alternatives which have no side effects associated with them and as they will be new there is no question about the resistance. Since 1980 the introduction of new drugs has declined due to the huge expense of developing and testing them.

The best solution to this problem is the use of traditional methods for the fight against pathogens that is the use of herbal medicines, medicinal plants have been used since time immemorial for the treatment of uncountable diseases, 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents^[2]. Plants have been studied in detail for their antimicrobial properties and some secondary metabolites have been found to be responsible for the antimicrobial properties. So it's needed to screen all the plants in the locality for the presence of these active metabolites.

Psidium guajava of the family *Myrtaceae* which is a tropical crop of which fruit and leaves have been consumed as herbal medicine and as a nourishing food. It is widely used as antispasmodic, anti-diarrhoeal, antidepressant, anti-inflammatory, anticough and sedative effects^[3]. The leaves have been used in folk medicine for many years to treat diarrhea, stomach ache and hepatic problems. The leaves, bark, and root have also been evaluated pharmacologically for the treatment of gastrointestinal diseases^[4-6].

Looking at the importance of *Psidium guajava* the present study has been designed to evaluate the antibacterial

properties of Leaf, stem, Bark and specially fruit of which a little literature is available.

Materials and methods:**Plant material**

Fresh Leaves, Stem, Bark, Ripe and Unripe fruits of *Psidium guajava* were collected after proper identification in the month of August 2011. Plant materials collected were ground into fine powder after complete drying and were used for extract preparation.

Preparation of extracts

Ground plant material was used to prepare a large range of extracts namely cold aqueous, hot aqueous, methanolic (80%), ethanolic (70%), ethyl acetate, and acetone. For preparing hot aqueous extract 5gm of ground plant material was added to 50 ml hot water and kept at 80 °C for 2 hours in water bath, the extract was then filtered by Whatman filter paper No. 1 and kept in hot air oven for drying, the dried extract was dissolved in double volume of DMSO (Di methyl sulfoxide) thus making the final concentration of stock solution to 500mg/ml.

Rest all the extracts were prepared by soaking 5gm plant material in 50ml of respective solvents for extraction of metabolites in dark for 3 days, after that the extract was filtered and dried, the dried extract was dissolved in double volume of DMSO thus making the final concentration of stock solution to 500mg/ml.

Microorganisms Studied

The following microorganisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* available at MRD LifeSciences (P) Ltd., Lucknow were used throughout the study.

Table 1: Antibacterial Activity of Extracts of Leaf of *Psidium guajava*

S.No.	EXTRACTS	Diameter of ZOI Against <i>P. aeruginosa</i> (In mm)		Diameter of ZOI Against <i>S. aureus</i> (In mm)		Diameter of ZOI Against <i>E. coli</i> (In mm)	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1.	Cold aqueous	0	24	0	18.5	16	25.5
2.	Hot aqueous	14	24	0	18.5	17.5	25.5
3.	Methanolic	20.5	24.5	18	22	18	18.5
4.	Ethanolic	19	24.5	19	22	18	18.5
5.	Ethyl acetate	17.5	25	13.5	21.5	0	23.5
6.	Acetone	22	25	18	21.5	16	23.5

Note: well diameter= 8mm

Antibacterial Sensitivity Testing

Extracts were tested for their antibacterial properties by Agar well diffusion method of Kirby Bauer with slight modification in which Nutrient agar plates were prepared and spread with pathogens and after that four wells were dug on each plate by the help of a sterile borer, first two wells were loaded with prepared plant extracts, third with a standard antibiotic Tetracycline (50 µg/ml) and fourth

with sterile distilled water as control. All experiments were performed in triplicates.

Results:

After antibacterial testing nearly all the extracts prepared were found to show some activity but the extracts of leaf, ripe and unripe fruit were found to be the most effective against used pathogens, Table 1-5 below show the results of all the extracts in detail.

Table 2: Antibacterial Activity of Extracts of Stem of *Psidium guajava*.

S.No.	EXTRACTS	Diameter of ZOI Against <i>P. aeruginosa</i> (In mm)		Diameter of ZOI Against <i>S. aureus</i> (In mm)		Diameter of ZOI Against <i>E. coli</i> (In mm)	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1.	Cold aqueous	0	20	0	20	0	16
2.	Hot aqueous	16.5	20	18.5	20	17	16
3.	Methanolic	0	24.5	14.5	25.5	0	25
4.	Ethanolic	0	24.5	14.5	25.5	0	25
5.	Ethyl acetate	0	20	0	24	0	21
6.	Acetone	12	24	15	24	11.5	21

Note: well diameter= 8mm

Table 3: Antimicrobial Activity of Extracts of Bark of *Psidium guajava*.

S.NO.	EXTRACTS	Diameter of ZOI Against <i>P. aeruginosa</i> (In mm)		Diameter of ZOI Against <i>S. aureus</i> (In mm)		Diameter of ZOI Against <i>E. coli</i> (In mm)	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1.	Cold aqueous	0	20.5	0	16.5	0	18
2.	Hot aqueous	0	20.5	0	16.5	0	18
3.	Methanolic	0	27	0	24	0	23.5
4.	Ethanolic	0	27	0	24	0	23.5
5.	Ethyl acetate	0	23	0	23.5	0	21
6.	Acetone	0	23	0	23.5	0	21

Note: well diameter= 8mm

Table 4: Antimicrobial Activity of Extracts of Ripe Fruit of *Psidium guajava*.

S.NO.	EXTRACTS	Diameter of ZOI Against <i>P. aeruginosa</i> (In mm)		Diameter of ZOI Against <i>S. aureus</i> (In mm)		Diameter of ZOI Against <i>E. coli</i> (In mm)	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1.	Cold aqueous	22.5	20	22	25	21.5	24.5
2.	Hot aqueous	0	20	0	25	0	24.5
3.	Methanolic	20	26	17	24.5	18	24
4.	Ethanolic	18.5	26	16.5	24.5	15.5	24
5.	Ethyl acetate	0	25	0	23.5	0	21.5
6.	Acetone	13.5	25	14.5	23.5	18.5	21.5

Note: well diameter= 8mm

Table 5: Antimicrobial Activity of Extracts of Unripe Fruit of *Psidium guajava*

S.NO.	EXTRACTS	Diameter of ZOI Against <i>P. aeruginosa</i> (In mm)		Diameter of ZOI Against <i>S. aureus</i> (In mm)		Diameter of ZOI Against <i>E. coli</i> (In mm)	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1.	Cold aqueous	0	21	16	19.	12.5	27
2.	Hot aqueous	0	21	0	19.5	0	27
3.	Methanolic	11.5	27	11	24.5	12.5	24
4.	Ethanolic	13.5	27	13	24.5	17	24

5.	Ethyl acetate	0	30	0	23.5	0	22
6.	Acetone	14.5	30	0	22	0	23.5

Note: well diameter= 8mm

Discussion:

A drug without side effect is the need now, in the search of such a drug we investigated the plant parts of *Psidium guajava* which is a traditional medicine used since time immemorial in Indian villages^[7].

Plant parts leaf, stem, bark, have been studied a lot by various scientists for their antibacterial properties^[8] ^[9] but there is very few literature on the antibacterial properties of fruits. The present study is one of the few reports on antibacterial properties of fruits of guava.

Cold aqueous, hot aqueous, methanol, ethanol, ethyl acetate and acetone extracts were prepared as done earlier by ^[7] ^[9] ^[10]. All the extracts of leaf showed good antibacterial activity but acetone extract showed a zone of inhibition of 22mm against *Pseudomonas aeruginosa* which was comparable to the zone of inhibition of 25mm shown by standard antibiotic Tetracycline. In case of stem hot aqueous extract was most effective and showed a zone of 18.5mm against *Staphylococcus aureus* which was very close to the 20mm zone shown by the standard antibiotic Tetracycline. Only acetone extract of bark showed little inhibition of 14.5mm against *Staphylococcus aureus*, all the extracts of ripe and unripe fruit were effective but cold aqueous extract of ripe fruit was found to be forming a zone of inhibition of 22.5mm against *Pseudomonas aeruginosa* which was greater than the zone of inhibition of 20mm formed by the standard antibiotic Tetracycline, ethanolic extract of unripe fruit was also most effective among all other extracts of unripe fruit showing a zone of inhibition of 17mm against *Staphylococcus aureus*.

Conclusion:

Based on the above study it can be concluded that *Psidium guajava* which has been used a traditional medicine since time immemorial can be a good source for the commercial production of antibacterial drugs which will be natural and without side effects.

Future work of the current study includes the further pharmacological evaluation of the factors responsible for the antibacterial properties.

Acknowledgement:

We are thankful to the Management and Staff of MRD LifeSciences (P) Limited, Lucknow and Mrs. Nilofar Khan

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for their kind support throughout the research work, we are also thankful to the Almighty without whose consent nothing is possible.

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