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Phytochemical investigation and antimicrobial evaluation of *Psidium guajava*

Shubham Tyagi[1], Radhika Tyagi[2], Sourabh Rathour[3], Priya Chamoli[4], Amit Gupta[5]
[1- 4, 5] Department of Life Sciences, Graphic Era Deemed to be University, Dehradun, Uttarakhand, India
[5] Department of Biotechnology, Graphic Era Deemed to be University, Dehradun, Uttarakhand, India

Abstract
Drug resistance due to the extensive mishandling and over exploitation of these antibiotics has become a serious problem. In order to reduce the burden of these antibiotics, we worked on extracts from medicinal plant products and determined its antimicrobial activity against specific bacterial strains. In this study, our objective is to determine the antimicrobial potential of leaf extracts of guava (*Psidium guajava*) against three bacterial strains i.e. *Bacillus subtilis*, *Salmonella enteritidis* and *Pseudomonas aeruginosa*. For these studies four different solvents of increasing polarities (petroleum ether, hexane, methanol, and aqueous i.e. distilled water) were used for extraction and determined its phytochemical analysis and also measuring its antimicrobial activity using disc-diffusion method. The results of these studies showed that methanol and aqueous extract of *Psidium guajava* leaves showed inhibitory activity against *Bacillus subtilis*, *Salmonella enteritidis* and *Pseudomonas aeruginosa*. Overall, guava leaf-extract considered as one of the best candidate for natural antimicrobial agent.

Keywords: *Psidium guajava*; leaves; phytochemical; antimicrobial; bacteria

Introduction
The genus *Psidium* (family *Myrtaceae*), considered to be originated as well as reported in tropical South America. These guava crops are grown especially in tropical along with subtropical areas e.g. Asia, Egypt and others [1, 2]. In literature, genus *Psidium* comprises of 150 species which includes small trees along with shrubs. Out of which, only 20 species of genus *Psidium* produce edible fruits and rest of them are wild type with inferior type of fruit quality. One of the most commonly type of cultivated species of *Psidium* i.e. *P. guajava* (also called as common guava) are mainly considered them as one of the dietary source for human [2-5]. Other *Psidium* species are mainly utilized for proper regulation of vigor along with fruit quality improvement and resistance to pest and disease. One of the fruits i.e. *P. guajava* (evergreen small tree) is of wide commercial value and grown widely reported in the tropics. In literature, bioactive components are reported in the guava leaf (rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances) and showed various immunopharmacological activities e.g. regulate blood glucose levels [6-8]. In addition, *Psidium guajava*), phytotherapic plant which is mainly used in folk medicine and reported some active components which may help to treat and manage various diseases [7-10]. 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 arrow root tea extract and caffeine, have been found to exhibit antimicrobial activity. This type of studies mainly suggests that medicinal plants which showed high levels of antimicrobial action due to existence of secondary metabolites which may be finally used to inhibit the growth of foodborne pathogens. Overall this study aims to evaluate the extracts of *P. guajava* leaves, using different solvent system to establish if it is effective against killing or inhibiting the growth of bacterium i.e. *Bacillus subtilis*, *Salmonella enteritidis* and *Pseudomonas aeruginosa* which can cause illness.

Material and Methods
Phytochemical studies
In *P. guajava*, phytochemical studies were conducted in leaves using different solvent system for determination of metabolites. The plant material of *P. guajava* especially leaves were washed (using tap water) and dried under the open shade area and then macerated in mortar and pestle to prepare fine powder. Finally, powder bags were stored at room temperature. So, these plant samples were dissolved in different solvent system for detecting different phytochemicals. For these studies, take 2 ml of each extract was measured into a test tube for each of the tests and concentrated by evaporating the extractant in a trough. Standard protocols are available for testing different phytochemicals (flavonoids, glycosides, terpenoids, tannins, phenols and saponins) in plant extract.

Extraction of plant material
Plant extracts were prepared by cold extraction method. Cold extraction was done in organic solvents respectively to get the crude extract. Take 10 g dried and fine powdered material was suspended in 100 ml of distilled water/ hexane / methanol for 24-48 hours. The extracts were decanted and filtered with Whatman No.1 filter paper.

Test Microorganisms
To evaluate the potential antimicrobial activity of *Psidium guajava*, 3 bacterial strains were selected namely *Bacillus subtilis*, *Salmonella enteritidis* and *Pseudomonas aeruginosa*. The Nutrient agar medium (NAM) was used to culture microbes required for antimicrobial susceptibility test. Microbial strains were cultured on to the agar plate and the broth, followed by overnight incubation at 37° C.

Selected bacterial strains mentioned above were suspended in normal saline and inoculated in Mueller Hinton agar.

Antibacterial Activity

The antibacterial activity of the *Psidium guajava* extracts was tested using disc diffusion method. A 100 µl of microbial culture of the respective strains is to be spread with the help of an L- shaped spreader containing MHA (20 ml/ plate). The sterile discs (6 mm in diameter) containing residues of the extracts were independently impregnated on the agar plates which have been previously been inoculated with the selected microbial strain. Erythromycin was used as a positive control for bacteria. Discs without samples were used as a negative control. The plates were then incubated at 37° C for 24 hours. Antimicrobial activity was then determined by measuring the diameter of the growth - inhibition zone in millimeters.

Results and Discussion

Due to emergence of antibiotic resistance is one of the major concern especially for treatment of various infectious (intracellular or extracellular) diseases. Lot of research work was conducted for alternative type of treatment including providing some solution for antibiotic resistance against infectious disease. Various findings were reported as mentioned in the literature which indicated that medicinal plants contained several metabolites with antimicrobial function. The results of phytochemicals present in distilled water, hexane and methanol extract of *Psidium guajava* are given in Table.1 As shown in table flavonoids, terpenoids, phenols, tannins and saponins are absent in distilled water extract. Methanol extract shows the presence of alkaloids, flavonoids, glycosides, Terpenoids, Tannins, phenols and absence of saponins whereas hexane extract shows the absence of tannins, phenols, saponins, terpenoids, glycosides and flavonoids. From these studies, we found that methanolic leaves extracts of *Psidium guajava* showed greater amount of phytochemicals as compared to aqueous extracts. The results of these metabolites which may indicate that guava may be considered as one of the better candidate for screening as well as development of nutraceuticals especially for the treatment of infectious diseases including cardiovascular i.e. diabetes. Phytochemicals are nonnutritive type of chemicals which may be produced by various medicinal plants especially for their own protection against different pathogens. In addition, these secondary metabolites may have been found to protect humans against several diseases through recent type of research. Various studies were conducted and identified thousands of phytochemicals were reported and only small fractions of these metabolites have been studied but works totally differently. In literature, two triterpenoids (i.e. guavanoic acid along with guavacoumaric acid) [11-13], four flavonoids [14]. were identified and showed various immunopharmacological properties [11-14]. In this study, we applied disk diffusion method was used pertaining to determine the antibacterial activity of *Psidium guajava* against three bacterial strains. This technique also called as moist disc Absorption Kirby Bauer (agar-based) technique [15]. and applied extensively in all microbiological based laboratories because this technique is more simple and easy to perform simultaneously on large numbers of micro-organisms. In other words, conventional method is a convenient technique to perform and validate the antibacterial effect of *Psidium guajava*. In this study, methanol extract of the *Psidium guajava* had high inhibition zone on *Pseudomonas aeruginosa* that is 15mm. The hexane extract of the *Psidium guajava* had maximum zone of inhibition on *Bacillus subtilis* that is 10 mm, while minimum zone of inhibition found in aqueous extract that is 6 mm on *Salmonella enteritidis*. There was little or no zone inhibition of the hexane and petroleum ether extract on *Pseudomonas aeruginosa and Salmonella enteritidis* whose zone of inhibition was not greater than 0-3.

Overall, antibacterial activity was observed in these extracts which was very significant on the basis of inhibition zone. The results were achieved from these studies after further processing and showed greater potential in medical application especially for the treatment of various microbial based infections.

Conclusion

Qualitative phytochemical analysis conducted on the crude *Psidium guajava* revealed the presence of phytochemicals which are known to exhibit medicinal as well as physiological activities. Thus it can be scientifically conclude that all these plants extracts could be seen as an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Table 1: Phytochemical analysis of *Psidium guajava*

Phytochemicals	Distilled water	Hexane	Methanol
Flavonoids	+ ve	- ve	+ ve
Glycosides	- ve	- ve	+ ve
Terpenoids	+ ve	- ve	+ ve
Tannins	+ ve	- ve	+ ve
Phenols	+ ve	- ve	+ ve
Saponins	+ ve	- ve	- ve

*-ve: Negative, +ve: Positive

Table 2: Evaluation of antibacterial activity of *Psidium guajava*

S. No	Test Organism/s	Inhibition zone (mm)					
		Positive control (Erythromycin)	Negative control	Petroleum ether	Hexane	Methanol	Aqueous
1.	Bacillus subtilis	22	NI	8	10	9	NI
2.	Salmonella enteritidis	20	NI	NI	NI	12	6
3.	Pseudomonas aeruginosa	23	NI	NI	8	15	12

(*Ni= No Inhibition)

References

1. Salazar DM, Melgarejo P, Martínez R, Martínez JJ, Hernández F, Burguera M, *et al*. Phenological stages of the guava tree (*Psidium guajava* L.) Sci. Hortic. 2006; 108:157-161.
2. Gutiérrez RMP, Mitchell S, Solis RV. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 2008; 117:1-27
3. Nair R, Chanda S. *In Vitro* antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically

important pathogenic microbial strains. Braz J Microbiol. 2007; 38:452-458
4. Nisha K, Darshana M, Madhu G, Bhupendra MK. GC-MS analysis and anti-microbial activity of *Psidium guajava* (leaves) grown in Malva region of India. Int. J Drug Dev Res. 2011; 3:237-245
5. Chanu TR, Pai V, Chakraborty R, Raju B, Lobo R, Ballal M. Screening for antidiarrheal activity of *Psidium guajava*: A possible alternative in the treatment against diarrhoea causing enteric pathogens. J Chem Pharm Res. 2011; 3:961-967
6. Birdi T, Daswani P, Brijesh S, Tetali P, Natu A, Antia N, *et al*. Newer insights into the mechanism of action of *Psidium guajava* L. leaves in infectious diarrhoea. BMC Complement. Altern. Med. 2010; 10:33.
7. Laily N, Kusumaningtyas RW, Sukarti I, Rini MRDK. The potency of guava *Psidium guajava* (L.) leaves as a functional immunostimulatory ingredient. Procedia Chem. 2015; 14:301-307.
8. Soman S, Rauf AA, Indira M, Rajamanickam C. Antioxidant and antiglycative potential of ethyl acetate fraction of *Psidium guajava* leaf extract in streptozotocin-induced diabetic rats. Plant Foods Hum Nutr. 2010; 65:386-391
9. Wu JW, Hsieh CL, Wang HY, Chen HY. Inhibitory effects of guava (*Psidium guajava* L.) leaf extracts and its active compounds on the glycation process of protein. Food Chem. 2009; 113:78-84.
10. Anand S, Arasakumari M, Prabu P, Amalraj AJ. Anti-diabetic and aldose reductase inhibitory potential of *Psidium guajava* by *in vitro* analysis. Int J Pharm Pharm Sci. 2016; 8:271-276.
11. Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, Gilani AH, *et al*. Triterpenoids from the leaves of Psidium guajava. Phytochemistry. 2002; 61(4):399-403.
12. Begum S, Siddiui BS and Hassan SI. Triterpenoids from *Psidium guajava* leaves. Nat Prod Letters. 2002; 16(3):173-7.
13. Begum S, Hassan SI, Siddiqui BS. Two new triterpenoids from the fresh leaves of *Psidium guajava*. Planta Medica. 2002; 68(12):1149-52.
14. Abdel WSM, Hifawy MS, El GoharyandM HM Isak. Study of carbohydrates, lipids, protein, flavonoids, vitamin C and biological activity of *Psidium guayava* L. Growing in Egypt. Egypt J Biomed Sci. 2004; 16:35-52.
15. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. The Journal of Antimicrobial Chemotherapy. 2008; 61(6):1295-301.