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Phytochemical Analysis And Antimicrobial Activity Of *Psidiumguajava* L. Leaves

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ABSTRACT

This study was designed to identify the phytochemical constituents and *in vitro* antimicrobial potential of methanolic extract of *Psidiumguajava* Linn (Myrtaceae). The phytochemical screening *Psidiumguajava* leaves showed that the presence of saponins, flavonoids, polyphenol, triterpenoids steroids, alkaloids,carbohydrate, protein, anthroquinone, terpenoids and glycosides whereas alkaloids and phlopatannins was absent in methanol extract. The inhibitory effect of methanolic extract of *P. guajava* was tested against five bacterial and three fungal strains by using the paper disc diffusion method. Antimicrobial activity was directly proportional to the concentration *of Psidiumguajava* extract. The *Psidiumguajava* extract shows highest antimicrobial activity was observed against bacteria when compared with fungi. The high doses (150µl) of *Psidiumguajava* extract possess similar activity to standard drug as chloramphenicol for bacteria and Fluconazole for fungi. This study is the first scientific report that provides convincing phytochemicals and antimicrobial activity evidence for the relevance of *Psidiumguajava* leaves thus providing scientific validity to its traditional consumption by the local populace of south India. *Psidiumguajava* leaves extract had a good potential for therapeutic use against the bacterial and fungal pathogens.

Keywords:*Psidiumguajava*, Phytochemical screening, Antimicrobial activity

INTRODUCTION

Microbes are truly the most underappreciated living organisms on Planet earth. Billions of them can fit on a fingernail, and they make up more than half of the living biomass on the planet. The world we live in is one full of microbes. Microbes, whether they are good, bad, or benign, are certainly everywhere. This includes on our body, in our homes, far below the earth's

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surface and up to the atmosphere, in cold, cool, warm and hot and very hot places, and even in places without oxygen. Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. Microorganisms always live in water (directly in aquatic environments, in water inside animals or plants, or in water around soil particles). They can eat all sorts of things, including oil, rocks, dead and living plants and animals (Needham, 2000). There are 4 major types of Microbes: bacteria, fungi, protists and viruses (Lynch and Hobbie, 1988).

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Ashis Ghosh, 2003).

In the mainstream, medicine is increasingly receptive towards the use of antimicrobial and other drugswhich is derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. There is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably. There is a scientific discipline known as ethno botany or ethno pharmacology, whose goal is to utilize the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health (Lewis *et al.*, 1995).

There is, therefore, an urgent need to investigate the biological properties of additional medicinal plants in order to develop new drugs. This prompted us to evaluate plants as a source of potential chemotherapeutic agents for antimicrobial activity based on their ethnomedical use. In the present study to investigate the antimicrobial activity of *Psidiumguajava* leaf extract against bacteria and fungi.One of the most gregarious of fruit trees, the guava of the Bottlebrush family, is almost universally known by its common English name or its equivalent in other languages.The Pharmacological studies of *Psidiumguajava* such asInfectious and Parasitic Diseases(Milyani, 2012),Diseases of the Blood and Immune System(Kailehet *al.*,2007).The objectives of the present study are to screen the phytochemicals in *Psidiumguajava*leaves and to

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monitor the ethanolic extract of *Psidiumguajava against*Gram – positive Gram– negative bacteria and against fungi as *Candida albicans,Aspergillusflavus* and *Aspergillus niger.*

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Psidiumguajava* were collected in December 2017 from Paravakottai, Thiruvarur district, Tamil Nadu, India. The *Psidiumguajava* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder materials were used for further studies.

Preparation of plant extract

2 g of the powder of *Psidiumguajava* leaves were transferred into different conical flask (250/ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Psidiumguajavaleaves* were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) and (1984).

Microorganisms

The microbial strains employed in the biological assays were Gram – positive bacteria: *Staphylococcus aureus* (MTCC 3160), *Bacillus cereus* (MTCC 8312), *Bacillus subtilis* (MTCC 2423) and Gram – negative bacteria: *Escherichia coli*, (MTCC 732) and *Pseudomonas aeruginosa* (MTCC 1035) and fungi as *Candida albicans* (MTCC 183), *Aspergillus flavus* (MTCC 10180) and *Aspergillus niger* (MTCC 1783) were obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of sample solutions for the experiment

The samples were weighed (10mg/10ml) and dissolved in sterile distilled to prepare appropriate dilution to get required concentrations of about 50µl (50µg), 100µl (100µg) and 150µl (150µg).The samples were kept under refrigerated condition unless they were used for the experiment.

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Preparation of dried filter paper discs

Whatman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization After sterilization, the discs were loaded with 50µl, 100µl and 150µl solutions and again kept under refrigeration for 24 hrs. Standard solution as Chloramphenicol for bacteria (25mg/ml distilled water- 30µl) to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka*et al.*, 2007) using plant extracts. NA medium were prepared by pouring 30 ml in petri plates for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates. In the agar plates ,usingsterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Each sample was tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS AND DISCUSSION

In the present study was carried out the phytochemical analysis on the *Psidiumguajava* leaves revealed the presence of medicinally active constituents.The phytochemical characters of the *Psidiumguajava* leaves investigated and summarized in Table-1. The phytochemical screening *Psidiumguajava* leaves showed that the presence of saponins, flavonoids, polyphenol, triterpenoids , steroids, alkaloids, carbohydrate, protein, anthroquinoneterpenoids and glycosides whereas alkaloids and phlobatannins was absent in methanol extract.

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Phytochemicals are derived chemicals from plants which are beneficial to human health and disease prevention andthese chemicals that may have biological significance. Scientists estimate that there are thousands of known phytochemicals having the potential to affect diseases such as cancer, stroke and metabolic syndrome and those caused by microorganisms (Anderson *et al.*, 2004).

Table1:Qualitative Phytochemical analysis of *Psidiumguajava*

S.No	Phytochemicals	Methanol Extract
1	Tannin	+
2	Phlobatannins	-
3	Saponnin	+
4	Flavonoids	+
5	Steroids	++
6	Terpenoids	++
7	Triterpenoids	+
8	Alkaloids	-
9	Carbohydrate	+
10	Protein	+
11	Anthroquinone	++
12	Polyphenol	++
13	Glycoside	+

- Indicates absence; + Indicates presence; ++ Indicates high concentration

Antimicrobial activity

The plant derived antimicrobials has enormous therapeutic potential and has been used since time immemorialand have been proved to be effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics.

Ethanolic extract of *Psidiumguajava was* screened against Gram – positive bacteria: *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* and Gram – negative bacteria:

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Escherichia coli and *Pseudomonas aeruginosa* and fungi as *Candida albicans* , *Aspergillus flavus* and *Aspergillus niger* were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Psidiumguajava*leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 1& 2.The inhibitory activities in culture media of the *Psidiumguajava reported* in Table 2 were comparable with standard antimicrobiotic viz. chloramphenicol and Fluconazole .

The results showed that the antimicrobial activity was directly proportional to the concentration of *Psidiumguajava*extract. The *Psidiumguajava*extractshows highest antimicrobial activity was observed against bacteriawhen compared with fungi. The high doses (150µl) of *Psidiumguajava extract* possess similar activity to standard drug as chloramphenicol for bacteria and Fluconazole for fungi.

Table 2: Antibacterial activity of *Psidiumguajava* leaves extract

Organism	50µl	100µl	150µl	Standard (30µl)
	Bacteria			
Staphylococcus aureus (mm)	4.75	5.0	7.25	70
Bacillus cereus (mm)	9.70	2.86	5.31	612
Bacillus subtilis (mm)	9.50	1.92	2.85	3.60
Escherichia coli (mm)	9.5	11.25	9.75	10.5
Pseudomonas aeruginosa (mm)	1.30	2.17	3.02	2.37
	Fungi			
Candida albicans(mm)	2.30	4.20	8.40	9.42
Aspergillus flavus (mm)	2.60	6.50	7.50	10.50
Aspergillus niger(mm)	3.10	4.40	5.10	11.20

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Figure 1: Antibacterial activity of *Psidiumguajava* leaves extract

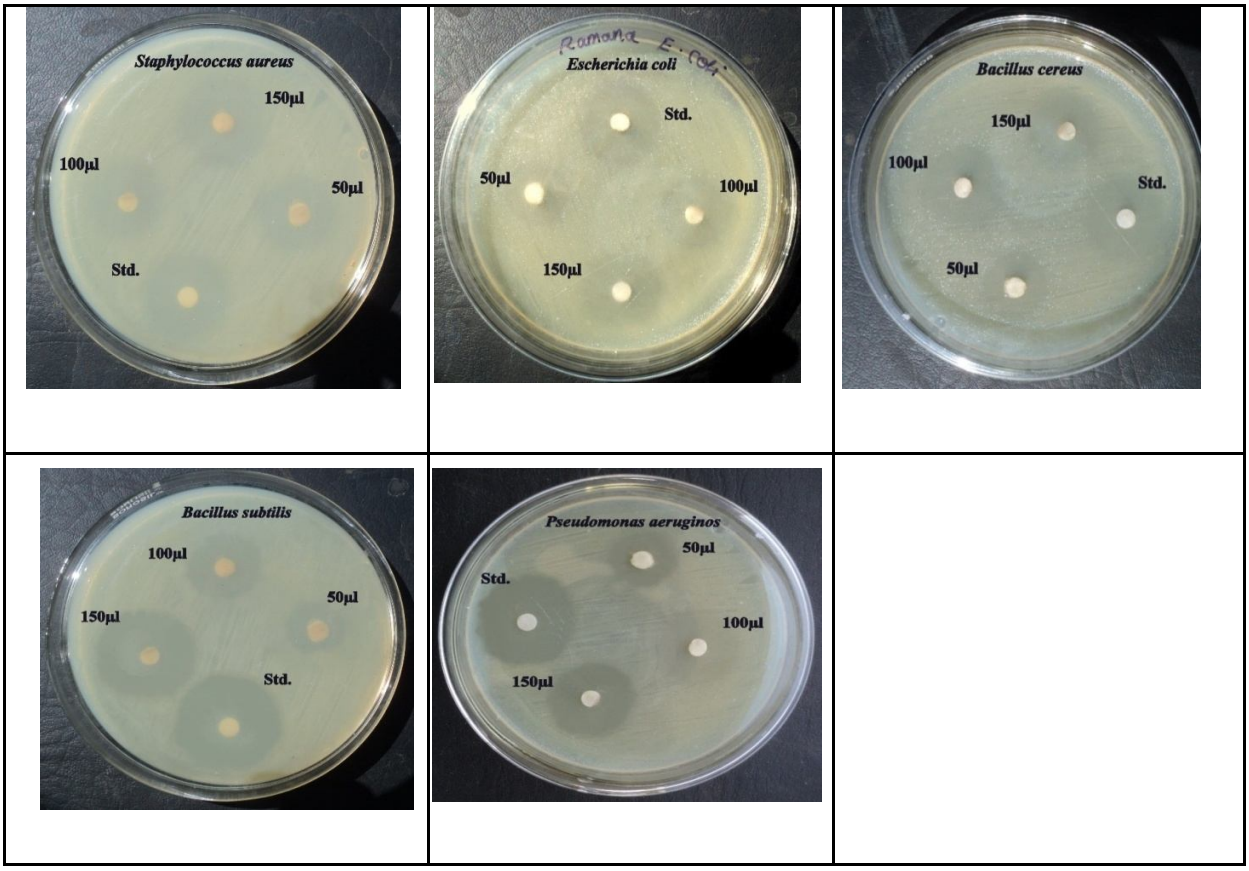
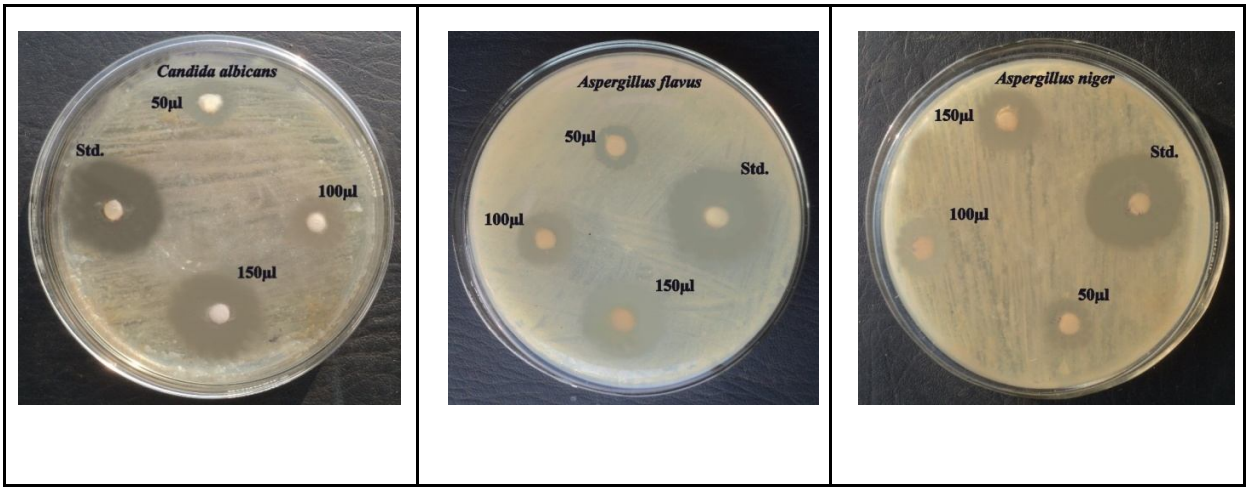


Figure 2: Antifungal activity of *Psidiumguajava*leaves extract



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It can be concluded from the present study that *Psidiumguajava*leavescontains rich source of phytochemicals. Antimicrobial activity was directly proportional to the concentration of *Psidiumguajava extract*. The *Psidiumguajava*extract shows highest antimicrobial activity was observed against bacteriawhen compared with fungi. The high doses (150µl) of *Psidiumguajava*extract possess similar activity to standard drug as chloromphenical for bacteria and fluconazole for fungi. This study is the first scientific report that provides convincing phytochemicals and antimicrobial activity evidence for the relevance of *Psidiumguajava*leavesthus providing scientific validity to its traditional consumption by the local populace of south India. *Psidiumguajava*leaves extract had a good potential for therapeutic use against the bacterial and fungal pathogens. Further studies are warranted for the isolation and characterizations of antimicrobial compounds are needed for understanding their mechanism of action as antimicrobials.

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