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Evaluation of antimicrobial activity of the peel extract of Psidium Guajava Fruit on selected bacterial strains

Prakruthi K N*, Ahalyadevi K H, Krishna K R, Sindhu K B, Albert Paul

Department of pharmacology, Bharathi college of pharmacy, Bharathinagara, Maddur Taluk, Mandya District, Karnataka, India -571422

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*Corresponding author:

Email : prakruthiprakruthi16@gmail.com
Phone : +91 - 8904945230

ABSTRACT

Plants and plants extracts have important role in modern medicine as their chemical and medical constituents are found in natural form. Plants and plant based products are bases of many modern pharmaceuticals that are currently in use for various diseases. *Psidium guajava* is an Indian medicinal plant belongs to the family Myrtaceae. The present study has been designed to screen the pharmacological studies such as antimicrobial activity of fruit peels of *psidium guajava* extracts on bacterial strains *(E. coli* and *S. aureus)*. The ethanol extract of *P. guajava* fruit peel has demonstrated promising antimicrobial properties. Increasing awareness, promotion and utilization of this fruit for public benefits are highly encouraged and identification of active phytoconstiuents in the extracts will serve as a natural cytotoxic agent against various cancers.

INTRODUCTION

INTRODUCTION TO HERBAL PLANTS AND THEIR USES

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes. The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases [1]. They conducted thorough study about the same, experimented to arrive at accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure [2]. Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants [3].

Phytochemicals are a field of increasing attention, both in science and in commerce. As is now generally recognized, many plant compounds and pigments have effects on animals and human beings. There is a great effort now to study and understand at a fundamental level and significant health effects of these

Fig. 1 : *Psidium guajava fruits*

compounds. This field is maturing and the health effects of these compounds are now getting the careful study they warrant at both a chemical and a molecular biological level. Identifying bioactive compounds and establishing their health effects are active areas of scientific inquiry. There are exciting prospects that select bioactive compounds will reduce the risk of many diseases, including chronic diseases such as cardiovascular disease [4].

1.2 ANTIMICROBIAL ACTIVITY OF GUAVA

Several fruits and fruit extracts, as well as arrowroot tea extract and caffeine have been found to exhibit antimicrobial activity against *E. coli* and *S.aureus*. This suggests that plants 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 extract [5].

Several reports have demonstrated that guava fruits contain compounds associated with positive health benefits. Regular consumption of guava fruit significantly increases the level of high-density lipoprotein (good cholesterol) and significantly decreases serum total cholesterol, triglycerides, and blood pressures. Guava also contains several phytochemicals with many unique properties including high antioxidant activity [6].

The fruit is commonly eaten fresh or made into beverages, jams, and other foods. Various parts of the plant, including the leaf and the fruit, are used as medicine. People use guava leaf for stomach and intestinal conditions, pain, diabetes, and wound healing. The fruit is used for high blood pressure [7]. Guava is loaded with nutrients. Not only does it have more Vitamin C than oranges, guava is also rich in other antioxidants, and has been shown to have a number of great health benefits. Here are just a few of the benefits of eating this tropical fruit. One of the key nutrients found in guava is fiber[8].

This study is to highlight some biological activities of ethanol extract of *P. guajava* fruit peels. Hence search for an effective chemo preventive agent has led to the identification of various naturally occurring compounds like flavonoid and alkaloids (*P. guajava*, *L. Myrtaceae*) fruit peel which is known to possess number of pharmacologic properties such as antioxidant, antitumor, anti-allergic, anti-inflammatory, antimicrobial and neuroprotective activities. A thorough review of literature

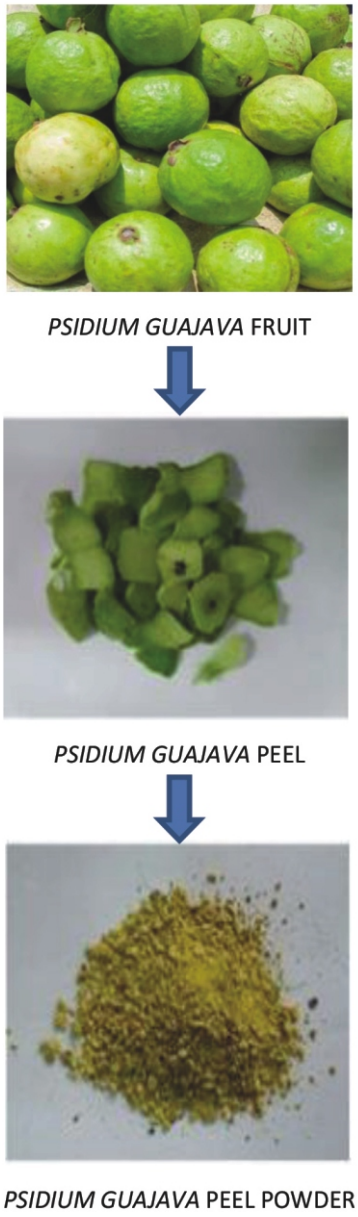

Fig. 2 : Processing of guava fruit peel.

revealed that no work has been taken on the antimicrobial activity. Hence, the present study was undertaken to fill in the lacunae and unravel the antimicrobial activity of extract of fruit peel of *P. guajava*. The objective of the present paper was to determine the antimicrobial activity of guava against *Escherichia coli* and *Staphylococcus aureus* in a liquid medium.

2. MATERIALS AND METHODOLOGY

2.1 MATERIALS AND METHODS

2.1.1 Collection Authentication of *psidium guajava*

Psidium guajava fruits were collected from natural habitat in and around Bharathinagara, Maddur Tq, Mandya Dist, Karnataka. The *Psidium guajava* fruits has authenticated by the Dr. Mahesh H M, Assistant Professor and Head, Department of Botany, Bharathi College, Bharathinagara, Maddur Tq, Mandya Dist, Karnataka.

2.1.2 Processing of medicinal Fruit Peel

The (P. guajava) diseased free fruit peel was used to prepare extracts for the study. The fruit were collected and washed thoroughly with running tap water and allowed it to remove the soil and unwanted dust particles. The peel was collected by using sharp knife. Then they were shade dried at room temperature for ten days and then the dried peel materials were powdered using motor and pestle. The powdered sample were stored in a clean glassware container and stored in low temperature until needed for analysis [9]. The fruit and fruit peel is exhibited in fig.2

2.1.3 Preparation of fruit peel Extract

The 50 gram of fruit peel powder was mixed with taken 500ml of solvent Methanol extracts. The fruit peel was kept in orbiter shaker for 48 hrs. Then the extracts were filtered and dried in hot air oven at 37°C. Then the extract was stored under refrigeration at 4°C for further analysis [9].

Extraction of fruit peel is done by simple maceration method and after this process extract filtered and it is used for further tests.

2.1.4 Preliminary phytochemical investigations

Analysis on the presence of both primary metabolites and secondary metabolites such as proteins, amino acids, carbohydrates, alkaloids, saponins, phytosterols, glycosides, phenols, tannins, flavonoids, steroids, terpenoids, and vitamin C were assessed according to the standard procedure, as described by Harborne, 1998.

2.1.5 Antibacterial activity of guava

Spread plate method

The pre-treated sample and the solidify media containing plate has been taken. 1 ml of sample is procured form the flask with the help of micropipette. Then lid of media containing plate has been opened and the sample is poured and the plate is closed with lid .The plate kept at rotating plate, then by using spreader the sample is spreaded evenly and plate is kept for drying.

A bacterial strain of interest is grown in pure culture. Using a

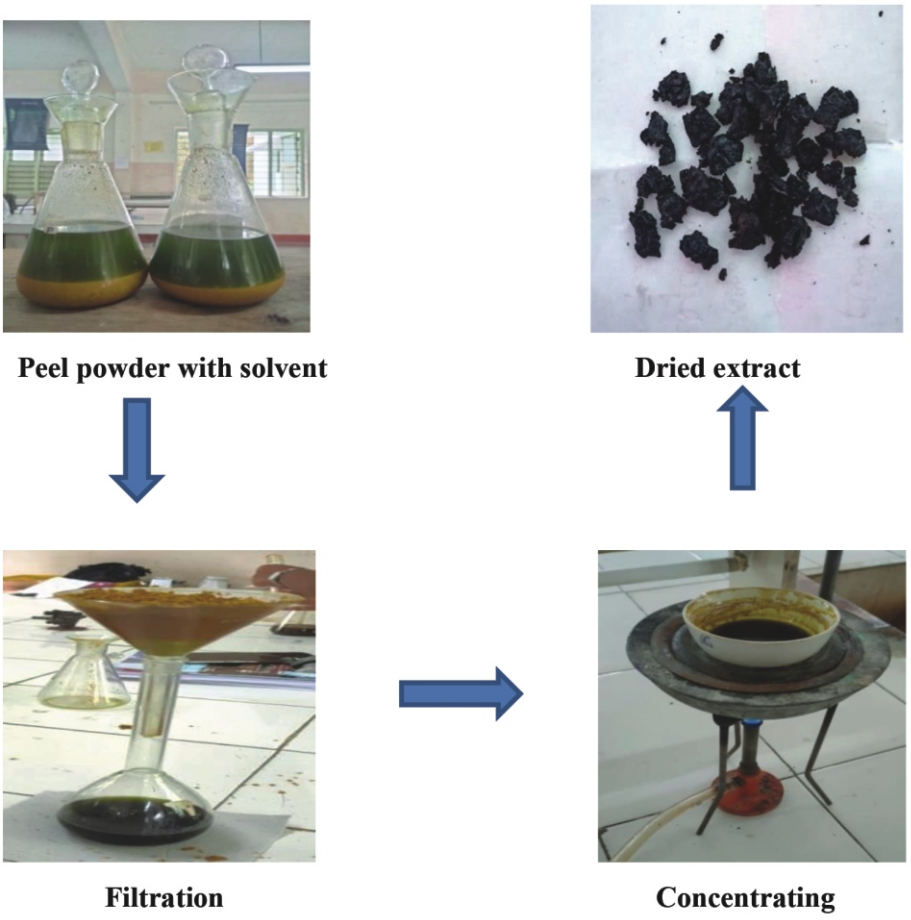

Fig. 3 : Ethanolic extract of *Psidium guajava* fruit peel.

sterile swab, a suspension of the pure culture is spread evenly over the face of a sterile agar plate. The antimicrobial agent is applied to the center of the agar plate (in a fashion such that the antimicrobial doesn't spread out from the center). A hole can be bored in the center of an agar for a liquid substance. The agar plate is incubated for 18-24 hours (or longer if necessary), at a temperature suitable for the test microorganism.

If antimicrobial agent came out from the object into the agar and then exerts a growth-inhibiting effect, then a clear zone (the zone of inhibition) appears around the test product. The size of the zone of inhibition is usually related to the level of antimicrobial activity present in the sample or product - a larger zone of inhibition usually means that the antimicrobial is more potent [10].

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Phytochemical analysis of *Psidium guajava*

In the phytochemical analysis, the phytochemicals present in the ethanolic extract of guava peel was studied.

Table 1 : Phytochemical analysis of *Psidium guajava* peels extract.

SL.NO	PHYTOCHEMICAL COMPOUNDS	ETHANOL EXTRACT
1	Flavonoids	+
2	Saponins	+
3	Phenols	+
4	Tannins	+
5	Alkaloids	+
6	Terpenoids	-
7	Carbohydrates	+
8	Anthraquinones	-
9	Glycosides	-
10	Steroids	+
11	Proteins	-

Note: (-) indicates Absence (+) indicates Presence of phytochemicals

3.1.2Antimicrobial activity

A Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth. Researchers who develop antimicrobial textiles, surfaces, and liquids use this test as a quick and easy way to measure and compare levels of inhibitory activity.

Zone of Inhibition Testing is a fast, qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of microorganisms. In the world of antimicrobial substances/surfaces, the degree to which these materials are inhibitory can be of vital importance to the health of the consumer. This test is an outstanding qualitative way for manufacturers of antimicrobial surfaces/substances to be able to compare the inhibition levels of their products.

In the figure 4 and figure 5 we can see that the extent of zone of inhibition and where the figure 4 represent the inhibitory effect of

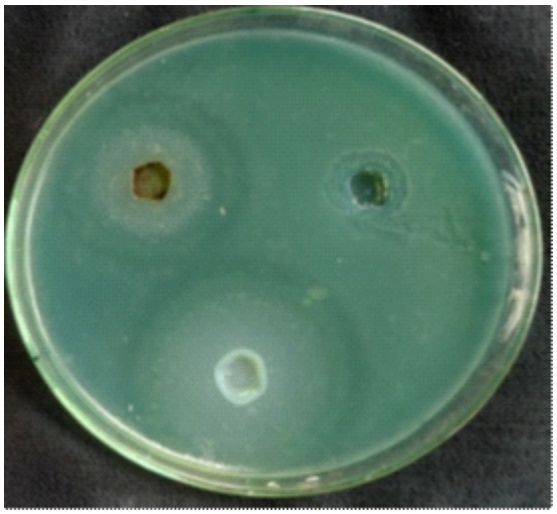

Fig. 4 : Inhibitory effect of *psidium guajava* fruit peel extract on *S. aureus.*

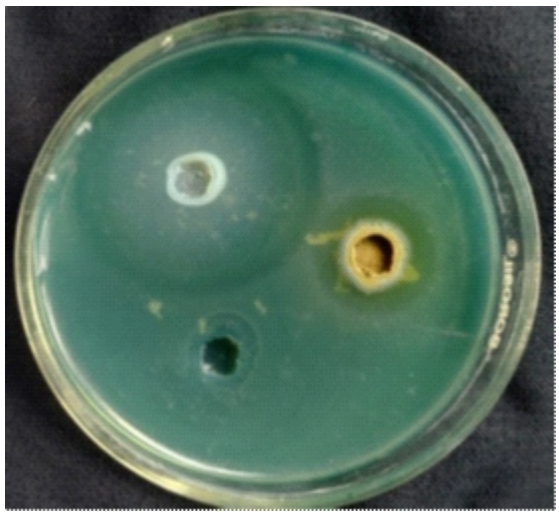

Fig. 5 : Inhibitory effect of *psidium guajava* fruit peel extract on *E. coli.*

psidium guajava fruit peel extract on *S. aureus* and figure 5 represents the inhibitory effect of *psidium guajava* fruit peel extract on *E. coli*. The zone of inhibition is more in figure 4 when compare to figure 5 that is comparatively E. coli has less zone of inhibition than the S. aureus.

Various activities are carried out in this experiment; it includes the phytochemical screening and antimicrobial activity of ethanolic extract of psidium guajava fruit peel. The phytochemical screening is done by performing a various tests. And the antimicrobial activity is done by spread plate method on bacterial strains like E. coli and S. aureus which are gram negative and gram positive bacteria's respectively. And here we measure the zone of inhibition and we done compare the effect of the extract on E. coli and S.aureus.

The results of the study indicated that the crude solvent extracts prepared from the fruit peel of *Psidium guajava*, ethanol, and showed inhibitory activity against bacteria. Only Gram-positive bacteria, *Staphylococcus aureus,* are susceptible to the extracts, while neither of the Gram-negative bacterium showed any inhibition, than ethanol extract with mean zone of inhibition 11.0 mm against *S. aureus*. The resistance of the Gram-negative bacteria could be attributed to its cell wall structure. Gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding the plant extract. It has been reported earlier that Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts.

3.2 DISCUSSION

Results obtained for qualitative screening of phytochemicals in peel of *psidium guajava* fruit is presented in Table 1. Of the eleven phytochemicals screened for, seven were found to be present in ethanolic extract. They are Flavonoids, Saponins, Alkaloids, Phenols, Tannins, Steroids, and Carbohydrates. And Terpenoids, Anthraquinones, Glycosides, and proteins are found to be absent in ethanolic extract.

The results of the study indicated that the crude ethanolic extracts prepared from the fruit peel of *Psidium guajava*, showed inhibitory activity against bacteria. Gram-positive bacteria, *Staphylococcus aureus,* are more susceptible to the extracts, while the Gram-negative, *Escherichia coli* bacteria are less susceptible when compare to Gram-positive bacteria, than ethanol extract with mean zone of inhibition 11.0 mm against *S. aureus* and 8.4 mm against *E. coli.*

This study supports previous findings in the literature that the antimicrobial activities have a direct relation to increasing the extracts concentration (%)[11]. As put forward that the difference in MIC of plant extracts is due to variation in their chemical constituents and volatile nature of their components [12]. In general, the ethanolic extracts had lower MIC values than most of the corresponding aqueous extracts. This lends support to previous findings in the literature that alcoholic extracts display higher antimicrobial activity than aqueous extracts [13]. Moreover, it has been reported that large number of different chemical compounds such as (phenolic compounds and its derivative compounds, the esters of weak acid, fatty acid, terpenes, and others) are presented in ethanolic extracts of spice, and thus these chemical components can affect multiple target sites against the bacterial cells [14,15]. Similar observations for MIC values, with minor variations, were observed in other studies [12, 16, 17].

Then cell wall disruption was observed in cells treated with plant extracts, suggesting a possible mechanism of antibacterial action. These findings indicate that the plant extracts tested in this study could be used as natural preservative agents in food to eliminate or control the growth of spoilage and pathogenic microorganisms[18].

The results in this study revealed that guava as natural ingredient could be used to improve the safety and quality of several consumable products. And the result obtained from antimicrobial activity of *psidium guajava* fruit peel ethanolic extract on selected microbial strains like E. coli (Gram negative bacteria) and S. aureus (Gram positive bacteria) is studied. And from this we came to know that ethanolic extract of *psidium guajava* will possess an antimicrobial activity.

4. SUMMARY AND CONCLUSION

In conclusion, the methanol extract of *P. guajava* fruit peel has demonstrated promising antimicrobial properties. Increasing awareness, promotion and utilization of this fruit for public benefits are highly encouraged and identification of active phytoconstiuents in the extracts will serve as a natural cytotoxic agent against various cancers.

This study confirms that *P.guagava* peels possess antimicrobial activity. From the entire experiment, it can be concluded that guava peel have antibacterial activity. The antibacterial activity was strong enough to inhibit *E. coli* (Gram negative bacteria) and *S. aureus* (Gram positive bacteria). This research indicates that guava peel have potential natural antibacterial compound. Further research is suggested to study the application of antibacterial activity of guava peel.

This inhibitory effect was strain and concentration dependent*;* Gram positive bacteria were more susceptible to the effect of *P.guagava* peel compared to Gram negative. This effect could be attributed to the structure of gram negative bacterial cell wall that provides a level of intrinsic resistance to certain hydrophilic substances and thus preventing the penetration of active materials in ethanolic extracts into the bacterial cell. This could provide an explanation for our results.

The present work demonstrates the antimicrobial potential of *Psidium guajava* fruit peel extract by using ethanol as solvent. The results indicate that ethanol extract of guava peel have antibacterial properties. The results also indicate that the plant extracts have less antibacterial effect on the Gram-negative bacteria. The observed inhibition bacteria suggest that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol 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. This study 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* fruit peel possesses the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *S. aureus* and *E. coli.*

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