



**PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL
ACTIVITY OF GUAVA SEED EXTRACT**

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ABSTRACT

Psidium guajava is a well-known medicinal plant that has been commonly used in traditional medicine because of its many healing benefits. Guava seeds are usually treated as waste, but they contain many important phytochemicals that show valuable biological and health-related activities. The present study was conducted to examine the phytochemical properties, chromatographic profile, and antibacterial activity of guava seed extract prepared using the maceration method. This work focused on studying the phytochemicals, chromatographic analysis, and antibacterial effect of guava seed extract prepared by the maceration method. The dried seed powder was extracted with ethanol and then tested to identify the presence of phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids. Thin-layer chromatography and paper chromatography were used to separate and study the bioactive compounds present in the extract. The TLC analysis showed an R_f value of 0.80, while paper chromatography gave an R_f value of 0.81, suggesting the presence of different phytochemical constituents. The antibacterial activity of the extract was tested against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. The extract showed good antibacterial effect against the tested bacteria. The findings show that guava seed extract is rich in bioactive compounds and may be useful as a natural source for developing pharmaceutical Products.

KEYWORDS: Guava seed extract, phytochemical screening, maceration, TLC, paper chromatography, antibacterial activity, medicinal plant.

INTRODUCTION

Medicinal plants have been relied on for centuries as a natural way to treat and manage different health problems. Plant-based natural compounds include a variety of secondary metabolites, known as phytochemicals, which are linked with several helpful biological effects such as fighting microbes, reducing oxidation, lowering inflammation, and helping in cancer-related activities. Among these plants, guava (*Psidium guajava*) is a member of the Myrtaceae family and is commonly found across tropical and subtropical regions around the world. Various parts of the guava plant—such as the leaves, bark, fruits, and seeds—are known to have medicinal importance and health benefits. Even though guava seeds are often thrown away during fruit processing, recent findings show that they are rich in important phytochemicals like alkaloids, flavonoids,

tannins, saponins, and phenolic compounds.

These compounds are responsible for different biological effects, making guava seeds a useful natural source for potential therapeutic applications. These compounds help produce a range of biological activities, which makes guava seeds a promising natural option for possible medicinal use. It gives useful insight into the chemical components that are linked to its medicinal properties. In addition, techniques like TLC and paper chromatography help in separating and identifying different phytochemicals present in the sample. This study was carried out to examine the phytochemical components, chromatographic pattern, and antibacterial activity of guava seed extract prepared using the maceration process.

Medicinal plants have been appreciated since ancient times for their natural healing ability and therapeutic benefits. Many plants contain natural bioactive substances called phytochemicals, which help support the body and protect it from various health issues. These natural compounds are commonly researched for their health benefits, including antimicrobial, antioxidant, anti-inflammatory, and other therapeutic effects. Among different medicinal plants, guava (*Psidium guajava*) is an important species because it is valued for both its nutritional content and its medicinal properties. It is a member of the Myrtaceae family and is widely grown in tropical and subtropical areas. Different parts of the guava plant, including its leaves, bark, fruits, and seeds, have long been used in traditional folk medicine for the treatment and management of a variety of diseases and infections, as they are believed to offer natural healing properties and health benefits. Guava seeds are usually thrown away as waste during fruit processing, but recently they have started gaining interest because they are found to contain a range of useful phytochemical compounds. Studies indicate that guava seeds contain a variety of beneficial compounds, including flavonoids, tannins, alkaloids, saponins, and different phenolic substances.

These compounds are thought to play a role in the plant's biological effects and may help protect against microbial growth as well as oxidative damage in the body. Phytochemical screening is a basic and important initial test used to identify whether bioactive compounds are

present in plant extracts. It gives important information about the chemical makeup of medicinal plants and also helps in understanding their possible healing or therapeutic value. Along with phytochemical analysis, chromatographic methods such as Thin Layer Chromatography (TLC) are widely used to separate and identify the different phytoconstituents present in plant extracts.^[1]

MATERIALS AND METHODS

Fresh, mature fruits of *Psidium guajava* were obtained from a nearby local market for collection and preparation of plant material. The seeds were carefully removed from the fruit pulp by hand, then rinsed well with distilled water and left to dry in the shade for about 7–10 days. The dried seeds were powdered using a mechanical grinder and stored in an airtight container for further analysis.^[1]

Extraction By maceration method

Approximately 50 g of the dried, powdered guava seeds was placed in a clean beaker, and 250 ml of ethanol was added to it. The mixture was left at room temperature for 72 hours, with occasional shaking to help the extraction process proceed effectively. After the maceration process, the mixture was filtered using Whatman filter paper. The filtrate was then concentrated in a water bath maintained at 40–50°C until a semi-solid crude extract was formed. The obtained extract was kept safely for further phytochemical analysis and antibacterial studies.^[1]



Figure No: 1 Extraction By Maceration Method.

Drug selected for antibacterial activity

Ciprofloxacin was used as the standard reference drug for antibacterial activity in this study. The antibacterial activity of the guava seed extract was tested using the well diffusion technique. Ciprofloxacin was taken as the standard drug to compare and evaluate the antibacterial activity of the guava seed extract. The activity was compared by measuring the zones of inhibition formed by both the plant extract and the standard drug.^[2]

Growth medium used

Mueller Hinton agar was selected as the growth medium to test the antibacterial effect of guava seed extract. The medium was prepared using standard lab methods and then sterilized in an autoclave before use. Once sterilization was completed, the melted agar was carefully poured into sterile Petri dishes and left undisturbed until it became solid. After the agar had solidified, the plates were used to inoculate the bacterial cultures and to carry out the agar well diffusion test.^[2]

Phytochemical Screening

The prepared extract was analyzed through qualitative phytochemical tests to identify different plant

constituents, including alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids, using standard laboratory methods.

Sr no	Phytochemical Tests	Test name	Procedure
1	Alkaloids	Mayer's Test Extract + Mayer's reagent Dragendorff's Test	Extract + Mayer's reagent. Extract + Dragendorff's reagent
2	Flavonoids	Shinoda Test	Extract + Mg ribbon + HCl
3	Tannins	Ferric Chloride Test	Extract + Ferric chloride
4	Saponins	Foam Test	Extract + Water (shake well)
5	Phenolics	Ferric Chloride Test	Extract + Ferric chloride
6	Terpenoids	Salkowski Test	Extract + Chloroform + Conc. H ₂ SO ₄

Result and discussion Phytochemical screening

Preliminary phytochemical screening of the guava seed extract was performed using standard qualitative tests to detect different bioactive compounds present in it. The screening results showed that the extract contained different important phytochemical groups, which were confirmed by noticeable colour changes and formation of precipitates during the tests. Alkaloids were identified in the guava seed extract using Mayer's test and Dragendorff's test. A cream-colored precipitate in Mayer's test and an orange precipitate in Dragendorff's test confirmed that alkaloids are present in the extract.

These results suggest that the extract contains bioactive compounds that have nitrogen in their structure. Flavonoids were identified using the Shinoda test, where a red color appeared after adding magnesium ribbon and concentrated hydrochloric acid. The red coloration confirms that flavonoid compounds are present in the extract. Tannins were detected using the ferric chloride test. A blue-colored complex formed when ferric chloride solution was added to the extract, confirming the presence of tannins. This result indicates that the

extract contains polyphenolic compounds that can bind and form complexes with iron salts.

Saponins were detected using the foam test. When the extract was shaken well with water, a stable and lasting foam formed, confirming the presence of saponins in the guava seed extract. Saponins were detected using the foam test. When the extract was shaken well with water, a stable and lasting foam formed, confirming the presence of saponins in the guava seed extract. Phenolic compounds were also identified through the ferric chloride test. The guava seed extract was found to contain a variety of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids. known for their antioxidant and antimicrobial effects. Terpenoids were detected using the Salkowski test. Overall, the phytochemical screening shows that the guava seed extract is rich in different bioactive compounds, including alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids. The phytochemicals present in the extract help explain the biological effects seen and give a scientific reason for its antibacterial activity.



Figure No: 2 Phytochemical Screening.

Table No: Qualitative Phytochemical Analysis Of Dried Psidium Guajava Seeds.

Sr. No.	Phytochemical	Test Name	Procedure (Simplified)	Observation	Result
1	Alkaloids	Mayer's & Dragendorff's test	Extract mixed with Mayer's reagent / Dragendorff's reagent	Cream precipitate / Orange precipitate	Present
2	Flavonoids	Shinoda test	Extract treated with magnesium ribbon and HCl	Reddish colour appears	Present

3	Tannins	Ferric chloride test	Extract mixed with ferric chloride	Blue colour develops	Present
4	Saponins	Foam test	Extract shaken well with water	Stable foam forms	Present
5	Phenolics	Ferric chloride test	Extract treated with ferric chloride	Blue colour appears	Present
6	Terpenoids	Salkowski test	Extract mixed with chloroform and concentrated sulfuric acid	Reddish-brown layer forms	Present

Paper chromatography

Paper chromatography was used to get an initial qualitative understanding of the phytochemical compounds found in the guava seed extract. Once the chromatography process was completed and the paper was fully dried, the chromatogram was carefully observed under normal visible light. A distinct yellow spot appeared on the chromatographic paper, showing the presence of a separated phytochemical compound.

The spot moved a noticeable distance from where it was initially placed, indicating good interaction between the

compound and both the stationary and mobile phases. The distance covered by the solvent front and the separated spot was measured carefully, and the R_f (retention factor) value was then calculated. The yellow spot showed an R_f value of 0.81. The presence of one clear, sharp spot suggests that the extract has a main phytochemical compound that was effectively separated under the chosen chromatographic conditions.



Figure No: 3 Paper Chromatography.

Thin layer chromatography

Thin layer chromatography was carried out to further study and confirm the phytochemical compounds present in the guava seed extract. After the plate was developed with a suitable solvent system, it was left to dry completely and then observed under normal visible light. A clear yellow spot appeared on the TLC plate, showing that a phytochemical compound from the extract was successfully separated.

The spot moved a noticeable distance from the original application point, showing proper interaction between the

compound and both the stationary and mobile phases. The distances covered by the compound and the solvent front were measured, and the R_f value was then calculated. The yellow spot showed an R_f value of 0.80. The appearance of one clear and distinct spot indicates that the extract likely contains a main bioactive compound that was effectively separated under the chosen chromatographic conditions.



Figure No: 4 Thin Layer Chromatography.

Antibacterial activity

The antibacterial activity of guava seed extract was tested using the agar well diffusion method against *Escherichia coli* and *Staphylococcus aureus*, and its effectiveness was assessed based on the observed inhibition zones. The extract showed noticeable antibacterial action against both organisms, which was confirmed by distinct clear

zones of inhibition forming around the wells. These findings suggest that the extract works against both Gram-negative and Gram-positive bacteria, showing a wide antibacterial effect and indicating the presence of active phytochemical compounds responsible for this activity.



Figure No: 5 Antibacterial Activity.

CONCLUSION

"The presence of bioactive compounds and antibacterial activity indicates that guava seed extract may have promising applications in the development of natural pharmaceutical and antimicrobial products." The study findings indicated that the guava seed extract possessed considerable antibacterial properties, effectively restricting the growth of *Escherichia coli* and *Staphylococcus aureus*." The qualitative phytochemical investigation confirmed that the guava seed extract contains various bioactive constituents such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids."#

"The aim of this study was to examine the phytochemical composition, chromatographic profile, and antibacterial potential of guava seed extract prepared through maceration." Overall, this study shows that guava seed extract is rich in various bioactive phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids, as confirmed by qualitative phytochemical screening. The chromatographic results also confirmed that the extract contains a variety of different chemical constituents. The antibacterial test showed good activity against both *Escherichia coli* and *Staphylococcus aureus*, suggesting that the extract can effectively slow down or prevent the growth of these harmful bacteria.

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