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Phytochemical screening of leaves of *Psidium guajava* L.

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Abstract

Psidium guajava is an important medicinal plant widely distributed in tropical and subtropical regions and traditionally used for the treatment of various diseases. The present study highlights the phytochemical composition and medicinal significance of guava leaves. Guava leaves are rich in bioactive compounds such as flavonoids, tannins, alkaloids, glycosides, saponins, terpenoids, steroids and phenolic compounds which contribute to their therapeutic properties including antioxidant, antimicrobial, antidiarrheal, anti-inflammatory and wound healing activities. Different solvent extracts of guava leaves, including n-hexane, carbon tetrachloride, chloroform and aqueous extracts were evaluated through standard phytochemical screening methods. The results revealed the presence of flavonoids and alkaloids mainly in aqueous extracts, glycosides and terpenoids in n-hexane extract, and tannins and saponins in carbon tetrachloride extract. These phytoconstituents are associated with significant pharmacological activities and support the traditional use of guava in the management of infectious and metabolic disorders. This present work emphasizes the importance of further scientific investigations to isolate and characterize active compounds for the development of safe and effective plant-based therapeutic agents.

Keywords: *Psidium guajava,* phytochemical screening, flavonoids, alkaloids etc.

Introduction

Guava is a small to medium-sized fruit-bearing tree native to the tropical regions of the Americas. The plant features evergreen, aromatic leaves arranged oppositely along the stem and attached by short petioles. Its stem is covered with thin, smooth, reddish-brown bark. Guava belongs to the subfamily Myrtoideae, whose members typically display dark green, simple, oppositely arranged leaves. These leaves are generally elliptical to oval, measuring approximately 5–15 cm in length. The plant produces white flowers with five petals and numerous stamens. The guava fruit, classified as a berry, contains many seeds. Among the various guava species, *Psidium guajava*, commonly known as lemon guava, is the most widely cultivated and recognized [1].

The biological classification of *Psidium guajava* is as follows:

Division **Magnoliophyta**
Class **Magnoliopsida**
Subclass **Rosidae**
Order **Myrtales**
Family **Myrtaceae**
Subfamily **Myrtoideae** [2].

Guava fruit is highly nutritious due to its rich content of vitamins, minerals, and antioxidants. Ripe guava provides approximately 100 mg of ascorbic acid (vitamin C) per 100 grams, which supports immune function. In addition to the fruit, guava leaves contain several important phytochemicals, including flavonoids, alkaloids, triterpenoids, tannins, and essential oils.

Flavonoids such as quercetin, along with tannins and alkaloids, are known for their antidiarrheal properties. The essential oils in guava leaves exhibit antibacterial effects. Moreover, experimental studies suggest that guava leaf extracts may help increase platelet counts and accelerate wound healing in animal models. Compounds such as ascorbic acid, flavonoids, and tannins have also been proposed to support platelet recovery in patients with dengue fever [3].

In traditional medicine, various parts of the guava plant have been widely employed to treat wounds, ulcers, digestive disorders, and diseases such as cholera [4]. Guava is extensively used for medicinal purposes across many regions. The plant typically produces axillary inflorescences with one to three flowers. Different parts of the guava tree are traditionally utilized to manage various diseases, especially in developing countries. In Nigeria and neighboring regions, guava leaves are commonly used in folk remedies for malaria, typhoid, and yellow fever. Guava is recognized as an important medicinal plant in several indigenous medical systems. Its fruits are often called "super fruits" because they are rich in dietary fiber, vitamins A and C, folic acid, and essential minerals such as potassium, copper, and manganese. Additionally, guava provides a broad range of nutrients while remaining low in calories. Notably, a single guava fruit contains approximately four times more vitamin C than an orange [5]. These constituents have been traditionally used to treat various ailments throughout history. Recent ethnopharmacological studies confirm that guava is used worldwide to treat inflammation, diabetes, hypertension, wounds, as well as for its analgesic and antipyretic effects [6]. Guava also serves as an anti-amoebic agent, typically prepared as an infusion or decoction. As a decoction or poultice, it is used to expel the placenta after childbirth and to treat skin infections, wounds, vaginal hemorrhage, fever, dehydration, and respiratory disturbances.

The leaf is the most commonly used part of the guava plant for medicinal purposes globally. In Nigeria and other African countries, guava leaves are used to treat malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, coughs, sore throats, inflamed gums, and other ailments [1]. The decoction or infusion of guava leaves serves as a febrifuge, antispasmodic, and treatment for rheumatism [7]. Guava is also used as an antibiotic and in managing diabetes and

hypertension in regions such as America, Central and West Africa, and Southeast Asia. In some areas, boiled leaf extract is applied to treat scabies-induced rashes.

Guava is a small tropical tree that can grow up to 35 feet tall and is widely cultivated for its fruit. It belongs to the Myrtaceae family, which includes about 133 genera and over 3,800 species. The leaves and bark of *Psidium guajava* have a long history of medicinal use, continuing to the present day [8]. Given the immense medicinal importance of *P. guajava*, as evidenced by the studies mentioned above and a recent review article [9], further research into the pharmacological activities of its extracts against common infectious diseases is strongly warranted. This is especially relevant because the plant is readily available in tropical regions and accessible to local populations. Guava contains a broad spectrum of phytochemicals, including polysaccharides, vitamins, essential oils [10], minerals, enzymes, proteins [11], sesquiterpenoid alcohols, triterpenoid acids [12], alkaloids, glycosides [13], steroids, flavonoids, tannins, and saponins [14].

Guava is a valuable source of bioactive compounds such as tannins, phenolics, triterpenes, flavonoids, saponins, carotenoids, lectins, vitamins, dietary fiber, and fatty acids. The fruit is particularly noted for its high vitamin C content, often exceeding that of many citrus fruits, providing about 80 mg per 100 grams. Additionally, guava contains considerable vitamin A, contributing to its nutritional and health benefits [1]. Guava fruits are also an excellent source of pectin, a form of dietary fiber beneficial for digestion. Guava leaves contain significant amounts of flavonoids, especially quercetin, which are believed to contribute to many of the plant's medicinal properties, including antibacterial activity [9]. Quercetin is considered responsible for the antidiarrheal effect by relaxing intestinal smooth muscles and reducing bowel movements. Other flavonoids and triterpenes in the leaves also exhibit antispasmodic properties. Research indicates that flavonoids are not present in non-polar solvents such as n-hexane, suggesting that extracts prepared with such solvents may not show significant antimicrobial activity [15].

Preparation of extraction

The collected guava leaves were thoroughly washed with distilled water to remove debris. The plant material was air dried at room temperature (28^0C) for two weeks until a constant weight was achieved. The dried samples were grinded into a fine powder through mixer. Powder was then extracted with 500 ml of solvent (1,2,3,4) using a Soxhlet apparatus for 8 hours (Harborne 1998). Where solvent 1 is n-hexane, 2 is carbon-tetra chloride, 3 is chloroform and 4 is water. The extract was filtered through Whatman no.1 filter paper under reduced pressure using rotatory evaporator at 40^0C. The concentrated extracts were stored in airtight containers at 4^0C for further analysis.

Phytochemical tests:

Materials for phytochemical analysis

Test tube, Conical Flask, Spatula, Weighing Balance, Shaker Machine.

Reagent Used: Sodium hydroxide, ferric chloride solution, Fehling solution, aqueous hydrochloric acid, Dragondroffs reagent, Conc. H_2SO_4, dilute H_2SO_4, chloroform, ethanol, alcoholic ferric chloride solution, ammonia solution, dilute HCl[16].

Test for flavonoids: A small amount of dilute ammonia solution (about 5 mL) was added to the aqueous filtrate of the plant extract, followed by the addition of a few drops of concentrated sulphuric acid. The development of a yellow coloration indicated the presence of flavonoids.

Test for Tannins: A small quantity of the plant extract was diluted, after which 4–5 drops of 10% ferric chloride solution were added. The appearance of a blue or green coloration confirmed the presence of tannins.

Determination of Saponins: Two milliliters of alcohol diluted with water were added to 2 ml of the plant extract and the mixture was shaken vigorously for about 15 minutes. The formation of persistent foam indicated the presence of saponins.

Determination of Glycosides: Five milliliters of the plant extract were mixed with 25 ml of dilute sulphuric acid and boiled for about 15 minutes. After cooling, the solution was neutralized using 10% sodium hydroxide. Subsequently, 5 ml of Fehling's solution was added. The appearance of a brick-red precipitate indicated the presence of glycosides.

Determination of Alkaloids: A small quantity of the plant extract was treated with a few drops of dilute hydrochloric acid and then filtered. The obtained filtrate was subsequently treated with Dragondroffs reagent. The formation of an orange-brown precipitate indicated the presence of alkaloids.[17]

Wagner's Test: Approximately 2 ml of 10% aqueous hydrochloric acid was mixed with 2 ml of guava extract and stirred thoroughly. The resulting solution was divided into two equal portions. One portion was treated with a few drops of Wagner's reagent, while the second portion was similarly treated with Mayer's reagent to test for the presence of alkaloids. [18]

Determination test for Steroids: (Salwoskitest): To 0.5 ml of the plant extract, 2 ml of chloroform was added, followed by the careful addition of 2 ml of concentrated sulphuric acid along the side of the test tube. The mixture was gently shaken for a few minutes. The appearance of a red coloration in the chloroform layer indicated the presence of sterols.[19]

Determination test of Protein:

Xanthoproteic test: To 3ml of sample, add 1ml of concentrated nitric acidand heated for 3min. Then cooled and added 0.5 ml ofNaOH. Reddish orange colour indicates the presence ofaromatic amino acids.

Biuret test: To 1ml of extract, equal volume of 5%NaOH solution and copper sulphate solution added. Appearance of blue colour indicates the presence of proteins.

Determination of Carbohydrates:

Molisch test: The test tube consisting of 2ml of the above filtrate and 2 drops of alcoholic α-naphthol solution was shaken vigorously. After that, 1ml of the concentrated H2SO4 was added along the side of the test tube and allowed to stand for a few minutes. The emergence of the purple violet ring at the intersection of the liquids suggested the presence of carbohydrates.

Benedict Test: Mixture of the 0.5ml each of Benedict's reagent and filtrate when heated on boiling water bath for 2 minutes. A characteristic colour precipitate was indicative of the presence of the reducing sugar**.**

Fehling Solution: When 1ml of the filtrate was boiled with 1ml each of the Fehling solution A and B. Red precipitate demonstrated the presence of the sugar [20].

Determination test for Terpenoids: Each extract (1 mg) was once combined with chloroform (2 mL) and concentrated sulphuric acid (1mL). The formation of reddish-brown colour at the interface suggests the presence of terpenoids [21].

Table: Phytochemistery of guava leaves

S.No.	Plant Constituent	n-Hexane extract	Carbontetrachloride extract	Chloroform extract	Water extract
1.	Flavonoids	-ve	-ve	-ve	+ve
2.	Tannins	-ve	+ve	-ve	-ve
3.	Saponins	-ve	+ve	-ve	-ve
4.	Glycosides	+ve	-ve	-ve	-ve
5.	Alkaloids	-ve	-ve	-ve	+ve
6.	Steroids	-ve	+ve	-ve	+ve
7.	Terpenoids	+ve	-ve	-ve	-ve
8.	Protein A. Xanthoproteic B. Biuret Test	 -ve -ve	 -ve -ve	 -ve -ve	 -ve -ve
9.	Carbohydrates A. Molisch Test B. Benedict test C. Fehling Test	 -ve -ve -ve	 -ve -ve -ve	 -ve -ve -ve	 -ve -ve -ve

Results and Discussion

Phytochemical screening of leaves of *Psidium Guajava L.* shows the presence of various phytochemical such as flavonoids, terpenoids, glycosides, saponins, alkaloids, steroids, in different solvent (n-Hexane, carbon tetrachloride, chloroform and water) Alkaloids are present in water only. Flavonoids are present in aqueous extract. Glycosides are present in n-hexane extract while terpenoids are present in extracts of n-hexane, saponin and tannin are present in CCl_4extract but absent in remaining extract.

Presence of tannin in leaves of *P. Guajava L.* contributing to antimicrobial and astringent properties, while the presence of flavonoids providing strong anti-oxidant activity. Presence of alkaloids and saponins attributes to its therapeutic potential; all these activities are due to presence of these phytochemicals. Further, investigation discovering a natural remedy for other diseases would be significant and advancement of modern science.

Conclusion

This study confirms that *Psidium guajava* contains a wide range of phytochemicals including flavonoids, phenolic compound, alkaloids, and tannins. These secondary metabolites are responsible for various biological activities such as anti-oxidant, anti-microbial, astringent and therapeutic effects.

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