INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS OF *PSIDIUM GUAJAVA* LEAF EXTRACT COLLECTED FROM Dr. V. S. KRISHNA GOVERNMENT DEGREE COLLEGE CAMPUS

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ABSTRACT

Medicinal plants possess bioactive components called phytochemicals which are administered for treating various human ailments. Phytochemicals are of two categories i.e., primary and secondary constituents. Primary constituents include proteins, chlorophyll, sugar and amino acids. Secondary constituents contain alkaloids and terpenoids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities because of these phytochemicals. The aqueous extract of leaf samples of the plant Psidium Guajava is collected from Dr. V.S.Krishna College campus, Visakhapatnam in India. This extract is used for the phytochemical screening with an objective to check the presence or absence of the phytochemical constituents in the selected plant. The results of the phytochemical analysis of the plant leaf extract shows that terpenoids, tannins, reducing sugars, flavonoids and alkaloids are found to be present in aforementioned medicinal plant. The key phytochemical properties identified by this study will be very helpful to pharmaceutical industries for the fabrication of the novel drugs for treating various diseases.

KEYWORDS

Phytochemicals, Psidium Guajava , alkaloids, flavonoids, terpenoids and FTIR.

1.1 INTRODUCTION

The area of chemistry that deals with the chemical processes carried out by plant life is known as phytochemistry. Plants contain naturally occurring, biologically active compounds called phytochemicals. Alkaloids, terpenoids, phytosterols, flavonoids, glycosides, tannins, and phenolic compounds are the secondary constituents. Proteins, chlorophyll, and regular sugars are the primary constituents [1]. Plant cells are protected by phytochemicals from disease attack, drought, stress, and pollution [2]. Almost all plant parts, including the leaves, roots, bark, stem, fruits, flowers, seeds, and so on, are capable of producing phytochemicals [3, 4].

Phytochemicals are mostly responsible for providing plants with colour and organoleptic characteristics. According to recent studies, these phytochemicals are vital for defending against disease in humans. The process of extracting, identifying, and screening the bioactive chemicals present in plants is known as phytochemical screening [5, 6].

The guava, also known as *Psidium Guajava*, is a tiny tropical tree in the Myrtaceae family that is mostly grown for its edible fruits (Figure 1.1) It is typically found in India's agricultural areas. Iron, calcium, phosphorus, iron, and vitamin C are all present in guava fruit [7]. It exhibits strong antibacterial, anticancer [8], and anti-inflammatory [9] properties. The phytochemical screening of the leaf extract is the topic of the current paper.

Figure 1.1: *Psidium Guajava* plant

1.2 MATERIALS AND METHODS

1.2.1 Chemicals required

The chemicals required for the phytochemical screening of the leaf extract [9] are Mayers reagent (potassium mercuric iodide), Hager's reagent, Molisch's reagent, Benedict's reagent, Fehling's reagent, Schiffs reagent sodium nitroprusside, NaOH, ferric Chloride, benzene, H_2SO_4, chloroform, lead acetate, gelatin, HNO_3, acetic anhydride, ferric chloride, Ninhydrin reagent, copper acetate, sodium bicarbonate, hydrochloric acid, litmus papers, 2,4-DNP, Tollens reagent, iodine solution and deionized water.

1.2.2 Collection of *Psidium Guajava* leaves

From Dr.V.S.Krishna Government Degree College's botanical garden in Visakhapatnam, fresh leaves of the Psidium Guajava plant are obtained (Figure 1.2). 100 g of leaves are weighed, properly cleaned with running tap water to remove any dirt from the leaves' surface, followed by deionized water to remove any additional impurities, and then dried in the shade for five days, until the weight of the dried leaves stays consistent. These leaves are homogenised into a powder using a home blender after being thinly sliced. The resulting powder is kept for later use in an airtight container.

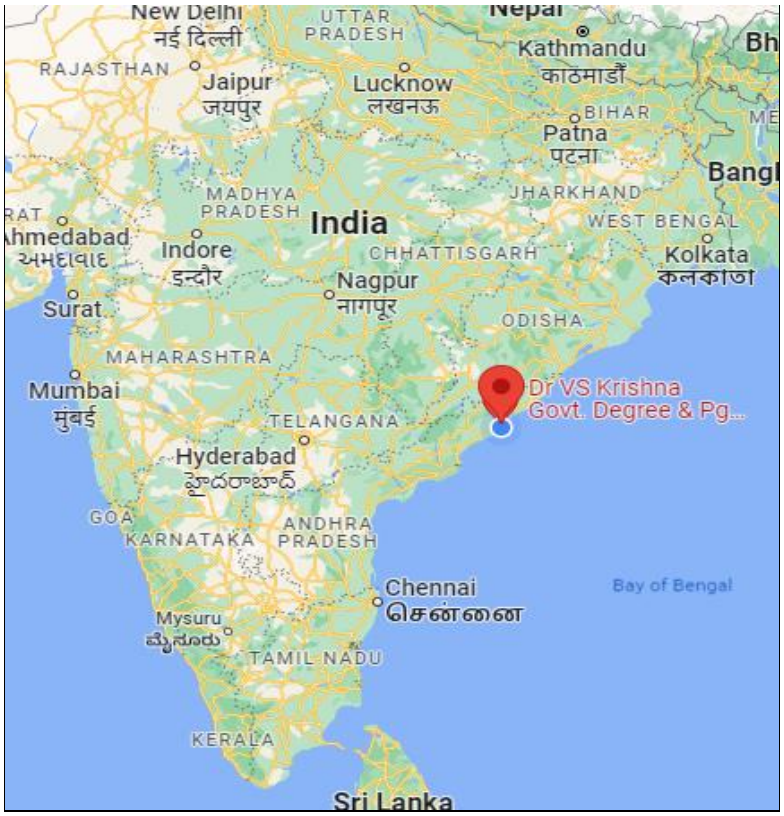

Figure 1.2: Map showing plant collection site in India

1.2.3 Preparation of leaf Extract

250 mL deionized water is taken in 500 mL beaker to this 10 g stored powder weighed and added. The contents in the beaker boiled for 20 minutes with occasional stirring with glass rod and then cooled to attain room temperature. The cooled leaf broth is filtered 2 times with Whatmann No.1 filter paper and reserved in refrigerator at 4°C. This is taken as leaf extract throughout the experiment (**Figure 1.3**).

Figure 1.3: Image of *Psidium Guajava* leaf powder

1.3 PHYTOCHEMICAL SCREENING TESTS

Aqueous extract of *Psidium Guajava* is screened to various phytochemical tests. Standard methods are used for phytochemical screening [10].

1.3.1 Test for Alkaloids

a) Mayers Test

To 4 mL of 2% HCl, 4 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of the filtrate is treated with three drops of Mayer's reagent. Development of yellow precipitate indicates the presence of alkaloids.

b) Hager's Test

To 4 mL of 2% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with 2 drops of Hager's reagent. Formation of yellow precipitate shows the presence of alkaloids.

c) Wagner's Test

To 4mL of 2% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with two drops of Wagner's reagent. Formation of brown colour precipitate indicates the presence of alkaloids in leaf extract.

1.3.2 Test for Carbohydrates

a) Benedict's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Benedict's reagent is added and heated gently for two minutes. Formation of red precipitate indicates the presence of carbohydrates (reducing sugars).

b) Molisch's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Molisch's reagent (alcoholic solution of α-naphthol solution) is added followed by the addition of concentrated sulphuric acid along the walls of the test tube. Formation of violet ring indicates the presence of carbohydrates.

c) Fehling's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 1mL of each Fehling solution A and B is added and boiled in a water bath for 2 min. Formation of brown precipitate indicates the presence of carbohydrates (reducing sugars).

1.3.3 Test for Glycosides

a) Modified Borntrager's Test

5 mL of extract is treated with 2 mL of $FeCl_3$ solution and immersed in boiling water for about five minutes. The mixture is cooled and extracted with equal volumes of benzene. The benzene layer is separated and treated with ammonia solution. Formation of rose-pink colour indicates the presence of anthranol glycosides.

b) Legal's Test

5 mL of extract is treated with 4mL of pyridine contained 2 mL of sodium nitroprusside solution. This is neutralized with 10% NaOH. Appearance of pink colour shows the existence of glycosides.

c) Keller-kilani test

The crude extract (2 mL) is reacted with glacial acetic acid (2 mL) containing 1-2 drops of 2% $FeCl_3$ solution. The mixture is then transferred into another test tube already containing 2 mL concentrated H_2SO_4. Appearance of brown ring at the interphase confirms the presence of cardiac glycosides.

1.3.4 Test for Saponins

a) Foam Test

To 5 mL of crude extract 10 mL of distilled water is added and this solution shaken vigorously in a 50 mL conical flask for 10 minutes. Persistent foaming on shaking confirms the presence of saponins.

1.3.5 Test for Steroids and Phytosterols

a) Salkowski's Test

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of conc. H_2SO_4, shaken and allowed standing. Appearance of golden yellow colour indicates the presence of steroids.

b) Libermann-Burchard's Test

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 is added. Formation of reddish brown colour indicates the presence of steroid ring.

1.3.6Test for Phenolic compounds

a) Ferric Chloride Test

The extract is dissolved in 5mL of distilled water and 2-4 drops of 5% $FeCl_3$solution is added. Formation of deep green colour specifies the presence of phenolic compounds.

b) Lead Acetate Test

2mL of 5% lead acetate solution is added to the extract solution. Formation of yellow precipitate indicates presence of phenolic compounds.

1.3.7Test for Tannins

a) Gelatin Test

To 1% gelatin solution containing 10% NaCl 5mL diluted algal extract is added. The formation of white precipitate indicates the presence of tannins.

1.3.8 Test for Flavonoids

a) Alkaline Reagent Test

5mL of extract is treated with few drops of sodium hydroxide solution. Formation of an intense yellow colour, which becomes colourless on addition of dilute acid connotes the presence of flavonoids.

1.3.9 Test for proteins

The extract is treated with few drops of Conc. HNO_3. Formation of yellow colour suggests the presence of proteins.

1.3.10 Test for Amino acids

a) Ninhydrin Test

5mL of extract is diluted by the addition of 15mL of distilled water. To the extract, 0.25% w/v Ninhydrin reagent is added and boiled for a few minutes. Formation of blue colour denotes the presence of amino acids.

1.3.11Test for Diterpenes

a) Crude extract (2 mL) is dissolved in chloroform (2 mL) and then evaporated to dryness. Concentrated H_2SO_4 (2 mL) is added and heated for 2 minutes. The appearance of grayish coloration indicates the presence of terpenoids.

b) Copper acetate Test

Extract is dissolved in water and treated with 2-4 drops of copper acetate solution. Formation of emerald green colour confirms the presence of diterpenes.

1.4 Detection of functional groups present in the leaf extract by FTIR analysis

The FTIR analysis is used to identify the functional groups of chemical constituents present in the leaf extract (**Figure 1.4**). The active components are separated based on its peak values in the region of IR radiation. The spectrum shows the signals at 3319 cm^{-1}, 2922 cm^{-1}, 1640 cm^{-1}, 1064 cm^{-1}, 636 cm^{-1} and 583cm^{-1} indicates the presence alcohols, phenols, Ketones, aromatic compounds, carboxylic acids, alkyl halides in the aqueous leaf extract of *Psidium Guajava* .

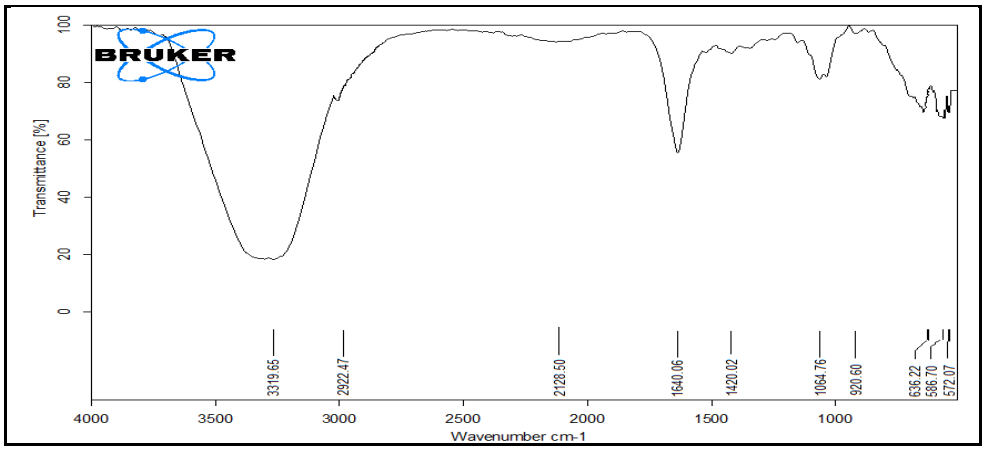

Fig. 1.4: FTIR spectrum of *Psidium Guajava* leaf extract

1.5 CONCLUSIONS

The phytochemical screening and FTIR spectroscopic analysis of *Psidium Guajava* leaf extract confirm the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, amino acids and terpenes. These are the plant secondary metabolites present in the leaf extract. The important phytochemical properties recognized by this study will be very useful in the development of new drugs for the treatment of various human diseases.

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