

Phytochemical screening of leaf extracts of *Psidium guajava* and *Psidium guineense* (Myrtaceae)

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ABSTRACT: The methanolic extracts of leaves of *Psidium guajava* and *Psidium guineense* using qualitative and quantitative analytical protocols. The results of the qualitative analysis proved the presence of tannins, saponins, coumarins, terpenoids, phenolics, flavanoids, anthocyanin and quinines in both *Psidium guajava* and *Psidium guineense*. Similarly the results of the quantitative analysis of total phenolics, total flavonoids and total tannins in leaf extracts showed a higher levels of all these three ingredients in *Psidium guineense* than that of *Psidium guajava*. The bioactive compounds which are identified from the extracts of these plants are also supports the use of these plants in traditional medicine.

Key Words: *P. guajava*, *P. guineense*, Methanolic extract, Phytochemical screening.

INTRODUCTION

Phytochemicals are biologically active, naturally occurring, secondary metabolites found in plants. They provide different colour, aroma, flavor etc to different plants and plant parts. In addition to this, they are vital molecules involved in protecting plants from diseases and stress conditions. These phytochemicals work with nutrients and fibers to form an integral part of defense system of the plants [1]. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of an organism. Unlike primary metabolites, absence of secondary metabolites does not results in immediate death, but rather in long term impairment of the organism's survivability or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group and play an important role in plant's defense [2].

The medicinal properties of the plant are majorly due to the secondary metabolites which cause definite physiological actions on human body and these bioactive substances include tannins, alkaloids, terpenoids, steroids and flavanoids [3]. Now- a-days the use of these phytochemicals have become more popular. Phytochemicals play a vital role against number of diseases. Unlike pharmaceutical chemicals, these phytochemicals have only lesser side effects. Since the phytochemicals cure diseases without causing much harm to human beings, these can also be considered as "man-friendly medicines" [4]. The identification and evaluation of the drugs, based on phytochemicals and pharmacological approaches which lead to the drug discovery is referred as natural product screening [5].

There are different stages in natural product screening. First one is the qualitative phytochemical screening which helps to understand the variety of chemical compounds produced by plants and second one involves the quantification of those metabolites which helps to extract and purify the bioactive compounds in an economic manner [6]. *Psidium guajava* and *Psidium guineense* are medicinal plants belonging to the family Myrtaceae. *Psidium guajava* is a well known traditional medicinal plant used in various indigenous systems of medicine. The pharmacological actions and the medicinal uses of guava leaves in folk medicine include the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhoea, gastroenteritis, spasmolytic activity, dysentery, flatulence and gastric pain [7]. The present work is focusing on the identification and quantification of the phytochemical constituents in the leaves of *Psidium guajava* and *Psidium guineense*.

MATERIALS AND METHODS

The present study is an attempt to identify and quantify the phytochemicals present in two species of *Psidium* such as *Psidium guajava* and *Psidium guineense*. The description of plant materials, reagents used and procedure adopted in this study are described below.

ABOUT THE SELECTED PLANTS**A. *Psidium guajava*****Fig.1 Image of *Psidium guajava* L.**

Scientific name : *Psidium guajava* L.

Family : Myrtaceae

Fl. & Fr. : Mar- May

Common name : Guava

Malayalam Name : *Pera maram*

Small tree. Stem smooth with peeling bark. Young stem 4-angled. Leaves 16-11 x 2.5-5 cm, elliptic-oblong, base rounded to obtuse-cuneate, apex acute-apiculate, hirsute on both sides when young, glabrous on ageing except the nerves, thin-coriaceous, lateral nerves prominent; petioles 0.6-1 cm long. Cymes axillary, 1-3-flowered; peduncles 0.5-1.2 cm long; pedicel short or 0. Calyx tube 4-9 mm long, ovoid, densely hirsute; lobes 4, united and closed in bud. Petals 4, white, 1-1.5 cm long, broadly ovate, caducous. Stamens many. Ovary globose, many-celled; ovules numerous; style subulate. Berry 2.5-3.5 cm diam., globose crowned by persistent calyx lobes. Seeds many, embedded in fleshy pulp. (Fig 1) [8].

B. *Psidium guineense***Fig.2 Image of *Psidium guineense* Sw.**

Scientific name : *Psidium guineense* Sw.

Family : Myrtaceae

Fl. & Fr. : Jul- Oct

Common name : Guava

Malayalam Name : *Mundiri pera*

Shrubs to small trees, branchlets terete, pubescent. Leaves subopposite or opposite, subcoriaceous, 8-10 cm, broadly elliptic-oblong, pellucid dotted, entire, pubescent beneath; lateral nerves 8-10 pairs, looping; petiole 1-1.5 cm long. Flowers slightly fragrant. Calyx tube adnate to the ovary, imperfectly 5-lobed, green, pubescent without. Petals 5, caducous, white, spatulate, 1.5 x 1 cm. Stamens c. 200, white; filaments 1-1.2 cm long; anthers oblong, 0.1- 0.5 cm long, introrse, dehiscing longitudinally. Ovary many-celled, ovules many in each locule; style 1.3 cm long, white, stigma capitate. Berry globose, 2-3 cm diameter, pubescent, yellow when ripe; seeds many, embedded in the creamy-yellow flesh. (Fig 2) [8].

Preparation of Methanolic extracts

Mature leaves were used for the preparation of methanolic extract. The leaves of *Psidium guajava* were collected from Calicut district, Kerala and were authenticated by using available Floras and Literature. The fresh leaves were collected and were cleaned properly using running tap water. The damaged and

diseased leaves were discarded. Water was removed from the leaves by blotting using tissue paper. Then the leaves were chopped into small pieces and spread in steel trays. It was kept in oven for 2-3 days. The dried pieces were made into powder using motor and pestle. The powder was stored in glass bottles. 25g of the leaf powder was added to 250ml methanol and kept on magnetic stirrer for 3 hours for proper mixing. Later the suspension was centrifuged at 3000 rpm for 5 minutes. The supernatant was transferred to glass beakers and was kept in hot air oven at 35°C to evaporate the methanol completely. The methanolic extract thus obtained was scrapped from the beaker and was transferred to amber coloured bottle. The bottles were labelled properly and were stored under refrigeration.

Qualitative phytochemical analysis [9].

1. Test for steroids

5ml of 1% plant extract was taken in the test tube. 5ml of concentrated sulphuric acid was added along the sides of the tube.

2. Test for Tannins

3 drops of 1% lead acetate was added to 2ml of 1% plant extract taken in a test tube. The reaction mixture was mixed well.

3. Test for saponins

About 5ml of 1% plant extract was shaken with 20ml of water.

4. Test for coumarins

2ml of plant extract (1%) was taken in the tube and added 3ml of 10% sodium hydroxide to the tube and was mixed well.

5. Test for carbohydrate

2ml of 1% plant extract was mixed with 2ml of Molisch's reagent. To this mixture 2ml of concentrate sulphuric acid was added along the wall of the tube.

6. Test for protein

Added 3ml of Biuret reagent to 2ml of 1% plant extract and mixed well.

7. Test for Terpenoids

5ml of plant extract (1%) was added 2ml of chloroform and mixed well. To this mixture 3ml of concentrate sulphuric acid was added along the wall of the tube.

8. Test for Glycosides

5ml of 1% plant extract was taken in a test tube and added 2ml of glacial acetic acid and 2ml of 2% ferric chloride. The contents were mixed well. To this mixture added 2ml of concentrate sulphuric acid along the side of the tube carefully

9. Test for cholesterol

To 2ml of 1% extract, added 2ml of chloroform and 10 drops of acetic anhydride. The contents were mixed vigorously. Then added two drops of concentrated sulphuric acid.

10. Test for Phenolics

Added 5 drops of 2% ferric chloride to 10ml of 1% plant extract. The contents were mixed well.

11. Test for flavonoids

3ml of 10% ammonium solution was added to 3ml of 1% plant extract and the tube was shaken to mix the contents.

12. Test for Anthocyanin

2ml of 2N hydrochloric acid and 2ml of ammonia were taken in a test tube and added 2ml of 1% plant extract to this mixture.

13. Test for amino acids

Added 2ml of ninhydrin to 2ml of 1% plant extract. The mixture was heated on a boiling water bath.

14. Test for phlobatinins

1ml of 1% solution of plant extract was mixed with 1ml of 1% hydrochloric acid. The mixture was boiled.

15. Test for Fatty acids

1% solution of the extract was prepared in ether. This solution was allowed to evaporate on a filter paper.

16. Test for Leucoanthocyanins

5ml of 1% of aqueous extract was added to 5ml of isoamyl alcohol.

17. Test for Quinones

To 2ml of 1% extract added 3ml of concentrated hydrochloric acid.

Quantitative phytochemical analysis**1. Estimation of total tannins**

5 ml of the plant extract was mixed with 2 ml of ferric chloride solution and 2 ml of potassium ferrocyanide solution. The reaction mixture was mixed well and the absorbance was measured at 120 nm against pure tannin as standard. Blank solution was prepared without plant extract [10]. All the experiments were done in triplicate and the results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

2. Estimation of total phenolics

The total phenolics present in the plant extracts were estimated by the method developed by Singleton and Rossi [11]. 100 µL of plant extract was mixed with 500 µL of Folin-Ciocalteu reagent. The tubes were incubated at 30°C for 4 minutes. After incubation 400 µL of 7% sodium carbonate solution was added, mixed well and was incubated under darkness for 30 minutes. The absorbance of the reaction mixture was noted at 756 nm. Reaction mixture without plant extract was used as the blank and gallic acid was used as the standard. All the experiments were done in triplicate and the results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

3. Estimation of total flavonoids

The total flavonoid content was estimated using the method described by Kumaran and Karunakaran [12], using quercetin as the reference compound. 1 ml of the plant extract was mixed with 1ml of aluminium trichloride and a drop of acetic acid. It was diluted to 25 ml with ethanol. The reaction mixture was incubated at room temperature for 40 minutes and the absorbance was measured at 415 nm. Blank was prepared without aluminium chloride solution. All the experiments were done in triplicate and the results were analyzed statistically and were expressed in terms of arithmetic mean and standard error.

RESULTS AND DISCUSSION

The present study was aimed at the qualitative and quantitative analysis of the phytochemicals present in the methanolic extracts of leaves of *Psidium guajava* and *Psidium guineense*. The results obtained during the present investigation are tabulated below.

Qualitative Analysis

Preliminary phytochemical analyses for the presence of seventeen different compounds such as steroids, tannins, saponins, coumarins, carbohydrates, proteins, terpinoids, glycosides, cholesterol, phenolics, flavonoids, anthocyanins, amino acids, phlobatinins, fatty acids, leucoanthocyanins and quinones (Table:- 1) were conducted during the present study.

Among these compounds tannins, saponins, coumarins, terpinoids, phenolics, flavanoids, anthocyanin and quinines were present in extracts of both *Psidium guajava* and *Psidium guineense*. The intensity of the colour developed during these tests, in both the species was not much different. The remaining bioactives such as steroids, carbohydrates, proteins, glycosides, cholesterol, amino acids, phlobatinins, fatty acids and leucoanthocyanins were absent in both *Psidium guajava* and *Psidium guineense*.

Table:1 Table showing the results of qualitative analysis of leaf extracts of *Psidium guajava* and *Psidium guineense*

Sl. No	Name of the Bioactive compound	<i>Psidium guajava</i>	<i>Psidium guineense</i>
1	Steroids	-	-
2	Tannins	++	++
3	Saponins	+	+
4	Coumarins	+	+
5	Carbohydrates	-	-
6	Proteins	-	-
7	Terpinoids	+	+
8	Glycosides	-	-
9	Cholesterol	-	-
10	Phenolics	++	++
11	Flavanoids	+++	+++
12	Anthocyanin	+	+
13	Amino acids	-	-
14	Phlobatinins	-	-
15	Fatty acids	-	-

16	Leucoanthocyanins	-	-
17	Quinones	+	+

Quantitative Analysis

During the current study, among the eight compounds which gave positive response in qualitative analysis, only three compounds such as phenolics, flavonoids and tannins alone were subjected to quantitative analysis. The selection of these three compounds were based on the relatively higher colour intensity developed during the qualitative analysis. The results obtained during the quantitative analysis of the leaf extract of *Psidium guajava* and *Psidium guineense* are tabulated in Table 2. The results of the quantitative analysis of total phenolics, total flavonoids and total tannins in leaf extract of *Psidium guajava* and *Psidium guineense* showed a higher level of all these three ingredients in *Psidium guineense*.

Table:2 Table showing the results of quantitative analysis of leaf extracts of *Psidium guajava* and *Psidium guineense*

Sl. No	Name of the bioactive compound	Concentration of bioactive compound ($\mu\text{g}/\text{mg}$ extract)	
		<i>Psidium guajava</i>	<i>Psidium guineense</i>
1	Total phenolics	21.86 \pm 0.34	49.36 \pm 0.52
2	Total flavonoids	38.30 \pm 0.12	76.50 \pm 0.55
3	Total tannins	10.31 \pm 0.45	32.90 \pm 0.11

Psidium is commonly known for its food and nutritional values throughout the world. The medicinal properties of guava fruit, leaf and other parts are also well known in traditional system of medicine. Since each part of guava tree possesses economic value, it is grown on commercial scale. The different parts of guava plant are used for the development of various industrial and pharmaceutical products [13]. Regarding the curative effects of plant products, phenolics play a major role. In addition to phenolics, some other phytochemicals such as tannins, flavonoids, saponins, also play important roles in exerting clinical effects [14].

The plants analyzed during the present study, especially *Psidium guineense* showed very high levels of tannins, phenolics and flavonoids. These phytochemicals can have a cumulative effect on the therapeutic value of *Psidium*. In the case of *Psidium guajava* there are so many reports on the phytochemical ingredients of different plant parts [15, 16, 17].

But in the case of *Psidium guineense* there are only very few reports on the phytochemical ingredients or the therapeutic values of this species. The present study clearly showed the presence of 2 to 3 time more bioactive compound in *Psidium guineense* than in *Psidium guajava*. This observation gives an indirect proof for an unexpectedly higher therapeutic value of *Psidium guineense*. At present the fruits of *Psidium guineense* are used against diabetics in folk medicine. *Psidium* fruits are relatively cheap which makes it readily accessible even for the poor. It is commonly called as the apple of the tropics. It may be even considered as super fruit due to the presence of high levels of soluble dietary fibers. The biochemical investigations of methanolic extracts of leaves of two species of *Psidium* has revealed the presence of phenolics, flavonoids, tannins and terpenoids. The results obtained from this investigation indicate the high protective potentials of this plant. The findings of this study suggests that these plants could be a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing or slowing down oxidative stress related degenerative diseases.

CONCLUSION

The current investigation is an attempt to screen the methanolic extract of leaves of *Psidium guajava* and *Psidium guineense* using qualitative and quantitative analytical protocols. The present study concluded that the extractives of the leaves of *Psidium guajava* and *Psidium guineense* possess significant amounts of bioactive compounds. Further investigations are required to isolate the active compounds for the utilization of the medicinal and protective potentials of these plants completely.

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