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EXTRACTION PHYTOCHEMICAL SCREENING AND TLC OF MEDICINAL PLANT EXTRACTS (PSIDIUM GUAJAVA)

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Abstract:
Psidium guajava L, belonging to the Myrtacea family, has been reported to have anti-diarrheal, hepatoprotective, hypoglycemic, lipid lowering, antibacterial and antioxidant activities. It contains important phytoconstituents such as tannins, triterpenes, flavonoid: quercetin, pentacyclic triterpenoid: guajanoic acid, saponins, carotenoids, lectins, leucocyanidin, ellagic acid, amritoside, beta-sitosterol, uvaol, oleanolic acid and ursolic acid. Thin layer chromatography has become widely used method for the separation of substances in a great variety of inorganic and organic studies. This investigation aimed to perform the extraction, phytochemical screening and TLC of Psidium guajava. The results showed that the yields were found to be (7.39% w/w of crude drug) of Methanolic extract Psidium guajava leaves. Obtained results were recorded in table 1. Results of Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Diterpenes, Saponins, Alkaloid and phenols (Table 2). Total Flavonoid content was 0.165mg/100mg quercetin equivalent in extract. The TLC was also performed to identify the presence of favanoids.It shows that the presence of such bioactive compounds has medicinal importance. Hence Psidium guajava was found to have medicinal properties.

Key words: *Herbal Medicine, Psidium guajava, Thin Layer Chromatography, Flavonoid*

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INTRODUCTION:

The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare. Although therapies involving these agents have shown promising potential with the efficacy of a good number of herbal products clearly established, many of them remain untested and their use are either poorly monitored or not even monitored at all. The consequence of this is an inadequate knowledge of their mode of action, potential adverse reactions, contraindications, and interactions with existing orthodox pharmaceuticals and functional foods to promote both safe and rational use of these agents. Since safety continues to be a major issue with the use of herbal remedies, it becomes imperative, therefore, that relevant regulatory authorities put in place appropriate measures to protect public health by ensuring that all herbal medicines are safe and of suitable quality [1].

Psidium guajava Linn. (family Myrtaceae), is commonly called guave, goyave or goyavier in French; guave, Guavenbaum, Guayave in German; banjiro in Japanese; goiaba, goiabeiro in Portugal; araçá-goiaba, araçá-guaçú, guaiaba in Brazil; guayaba, guayabo in Español and guava in English. *Psidium guajava*, which is considered a native to Mexico extends throughout the South America, European, Africa and Asia. It has been used widely and known in Peru since pre-Columbian times. It grows in all the tropical and subtropical areas of the world, adapts to different climatic conditions but prefers dry climates [2].

Thin layer chromatography (TLC) is an affinity-based method used to separate compounds in a mixture. TLC is a highly versatile separation method that is widely used for both qualitative and quantitative sample analysis. TLC can be used to analyze virtually any substance class, including pesticides, steroids, alkaloids, lipids, nucleotides, glycosides, carbohydrates, and fatty acids. In TLC, the stationary phase is a thin adsorbent material layer, usually silica gel or aluminum oxide, coated onto an inert plate surface, typically glass, plastic, or aluminum. The sample is spotted onto one end of the TLC plate and placed vertically into a closed chamber with an organic solvent (mobile phase) [3].

Experimental:

Materials:

Collection and storage of Plant:

The leaves of selected plant namely *Psidium guajava* was identified and collected from Moolchand-Phoolchand herbal store, Bhopal, Madhya Pradesh. The collected plant drug (leaves) was cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of Plant Drugs by Maceration [4]:

The Collected plant drug (leaves) was cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drug was converted into moderately coarse powder in hand grinder. Maceration was carried out in a closed conical flask for 72 h. (50 g) powdered plant drug sample and methanol as the extraction solvent was used. The solvent free methanol extracts obtained was evaluated.

Phytochemical Analysis:

In order to detect the various constituents present in the methanolic extract of *Psidium guajava*, was subjected to the phytochemical tests as per standard methods.

Estimation of total flavonoids content:

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatin. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 10 to 50 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extracts and Flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for determination of Flavonoid content as described[5].

Thin layer chromatography (TLC):

Thin layer chromatography has become widely used method for the separation of substances in a great variety of inorganic and organic studies. The separation of solutes is carried out a layer of adsorbent that is adhered to a flat surface of inert materials like a glass plate or a polyester film. Traditionally, analytical TLC has found application in the detection and monitoring of compounds through a separation process.

RESULTS AND DISCUSSION:

The yields were found to be (7.39% w/w of crude drug) of Methanolic extract *Psidium guajava* leaves.

Obtained results were recorded in table 1. Results of Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Diterpenes, Saponins, Alkaloid and phenols (Table 2). Total Flavonoid content was 0.165mg/100mg quercetin equivalent in extract. The TLC was also performed to identify the presence of favanoids.It shows that the presence of such bioactive compounds has medicinal importance.

Table 1: Extractive values obtained from *Psidium guajava*

S.N.	Solvent	% Yield
1.	Methanol	7.39%

Table 2: Preliminary phytochemical screening of *Psidium guajava*

S.N.	Phytoconstituents	Test Name	Methanolic Extract
1	Alkaloids	Wagner's Test	**+(ve)**
2	Carbohydrates	Fehling's Test	**+(ve)**
3	Flavonoids	Lead acetate	**+(ve)**
		Alkaline reagent test	**+(ve)**
4	Proteins & Amino acids	Precipitation test	**+(ve)**
5	Phenols	Ferric chloride test	**+(ve)**
6	Diterpenes	Copper acetate test	**+(ve)**
7	Saponins	Foam test	**+(ve)**

Table 3: Absorbance of standard and Methanolic extract of *Psidium guajava*

S. No	Concentration of Quercetin (µg/ml)	Mean absorbance
1	10	0.225
2	20	0.452
3	30	0.615
4	40	0.812
5	50	1.012

n=3, values are given in SEM

Table 4: Total Flavonoid content of Methanolic extract *Psidium guajava*

S. N.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mg/100mg
1	Methanolic extract (100µg/ml)	0.165

n=3, values are given in SEM

Results of TLC:
Long UV:

Table 5: R*f* value of various sports

Sport	R*f* value(cm)
1.	0.52
2.	0.58
3.	0.66
4.	0.84
Standard	0.52

Short UV:

Table 6 R*f* value of various sports

Sport	R*f* value(cm)
1.	0.520.236
2.	0.68
3.	0.88
Standard	0.52

CONCLUSION:

In the present study, we have found that the plant *Psidium guajava* showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Diterpenes, Saponins, Alkaloid and phenols rich in flavonoid, phenols in Preliminary phytochemical test. The results of TFC showed the presence of flavonoids in rich amount. The TLC was also performed to identify the presence of favanoids.It shows that the presence of such bioactive compounds has medicinal importance. As a promising source of bioactive compounds, it can be an excellent source of useful drugs.

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