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Preliminary Phytochemical Analysis of the Extracts of Psidium Leaves

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Abstract: Amritphala (*Psidium guajava* L.), is a small tree in Myrtaceae family, traditionally used in treatment of several diseases (inflammation, diabetes, hypertension, wounds, pain and fever). The present study was carried out to investigate the phytochemical profile of leaves of *Psidium guajava* L. The leaves powder was successively extracted with petroleum ether, chloroform, ethanol, water, hydroalcohol. Phytochemical analysis shows the presence of flavonoids, tannins triterpenoids, saponins, sterols, alkaloids and carbohydrates. The result of the study could be useful for description and foundation of monograph of the plant.

Key words: Flavonoids · *Psidium* · Phytochemical

INTRODUCTION

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the traditional methods are procured and studied and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment.

Psidium guajava is a small tree upto 20 feet high, with spreading branches. Guava is easy to recognize because of its smooth, thin, copper-colored bark that flakes off, showing the greenish layer beneath and also because of the attractive, "bony" aspect of its trunk which may in time attain a diameter of 10 inch [1]. *Psidium guajava*, which is considered a native to Mexico, extends throughout the South America, European, Africa and Asia. It is mainly found in tropical and subtropical regions. In India it is distributed in Uttar Pradesh, Bihar, Maharashtra, Assam, West Bengal, Haryana and Andhra Pradesh [2].

The leaves are evergreen, opposite, short-petioled, oval or oblong-elliptic, somewhat irregular in outline; 7-15 cm long to 3-5 cm wide, leathery, with conspicuous parallel veins and more or less downy on the underside [3]. Flowers are white, borne singly or in small clusters in the leaf axils. Flowers are 2-3 cm wide, with 4 or 5 white petals which are quickly shed and a prominent tuft of perhaps 250 white stamens tipped with pale-yellow anthers [4]. The fruit, exuding a strong, sweet, musky odor when ripe, may be round, ovoid, or pear-shaped, 2.5-10 cm long, with 4 or 5 protruding floral remnants (sepals) at the apex with thin, light-yellow skin, frequently blushed with pink. Next to the skin is a layer of somewhat granular flesh, 1/8 to 1/2 inch thick, white, yellowish, light- or dark-pink, or near-red, juicy, acid, subacid, or sweet and flavorful. The central pulp, slightly darker in tone, is juicy and normally filled with very hard, yellowish seeds, 1/8 inch long, though some rare types have soft, chewable seeds. Actual seed counts have ranged from 112 to 535 but some guavas are seedless or nearly so. When immature and until a very short time before ripening, the fruit is green, hard, gummy within and very astringent. Bark is quite smooth, pale pinkish brown usually tinged with chlorophyll [5].

More recent ethnopharmacological studies show that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, for diabetes, hypertension, caries, wounds, pain relief and reducing fever. Some of the countries with a long history of traditional medicinal use of guava include Mexico and other Central American countries including the Caribbean, Africa and Asia [6].

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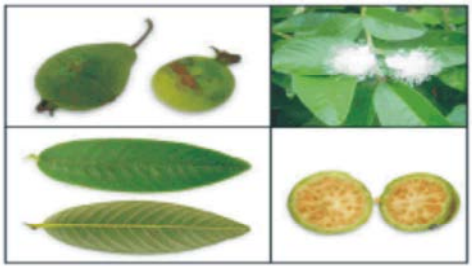

Fig. 1: Fruits, Flowers, Leaves and Seeds of Psidium guajava Linn

Taxonomic Classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	*Rosidae*
Order	*Myrtales*
Family	*Myrtaceae*
Genus	*Psidium* L.
Species	*Guajava L.*

MATERIALS AND METHODS

Collection of Plant Materials and Leaves: The leaves were collected from fields of Kangra, Himachal Pradesh, India. The collected leaves were shade dried for 35 days and finally pulverized in to coarse powder. It was stored in a well closed container free from environmental climatic changes till usage.

Preparation of Extracts: Successive extraction of plant material (Fig.1)

Preparation of Hydroalcoholic Extract: Hydroalcoholic (8:2) extract was prepared using leaves of *Psidium guajava*. Dried leaves (250 gm) were ground. The ground sample was soaked in hydroalcoholic solvent (8:2 *v/v*) and left for 24 h. The mixture was filtered and the filtrate concentrated by evaporation at 40°C. Then extract was dried and weighed.

Preliminary Phytochemical Screening: The preliminary phytochemical tests were performed for testing different chemical groups present in extracts [7, 8]. Extract (100 mg) was treated with few drops of Dragendroff's reagent [Potassium bismuth iodide solution]. Formation of orange

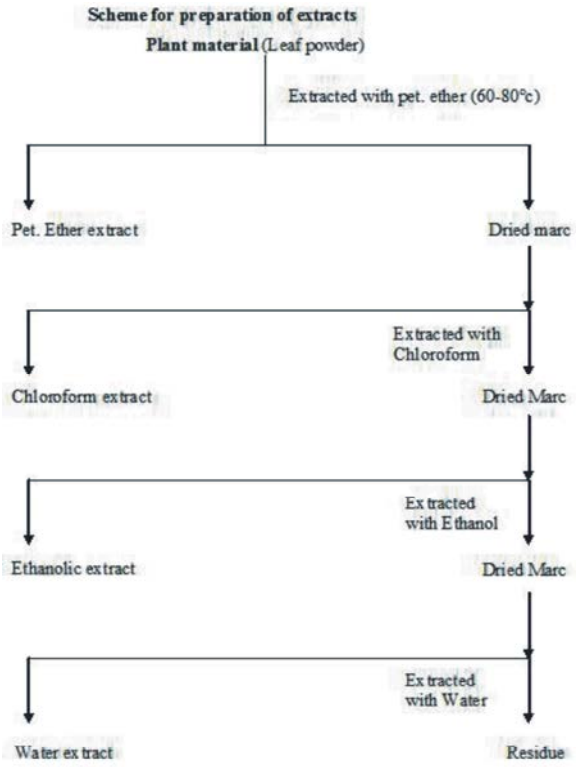

Fig. 1: A schematic representation of successive extraction

brown precipitate indicated the presence of alkaloids. To 100 mg of extract small quantity of Wagner's reagent [Solution of iodine in potassium iodide] was added. Presence of reddish brown precipitate if alkaloids are present. To 100 mg of extract small quantity of Hager's reagent [saturated solution of Picric acid] was added. Formation of yellow precipitate indicated the presence of alkaloids. Powdered drug extract on shaking vigorously with water results into persistent foam. 200 mg extract was boiled with 3 mL of dil. H_2S0_4 in a test tube for 5 min and filtered while hot. Cool and added the equal volume of C_6H_6 and $CHCl_3$, shake well and separated the organic solvent and added the NH_3. The ammonical layer turned pink or red. Alcoholic extract was treated with 1 mL pyridine and 1 mL of sodium nitroprusside. Pink to red colour appears. Extract (2 mL) was treated with 0.4 mL of glacial acetic acid containing a trace amount of $FeCl_3$ and 0.5 mL of concentrated H_2S0_4 was also added by the side of the test tube. Persistent blue color appeared in the acetic acid layer if cardiac glycosides were present. To 5 mL of extract few drops of 5% $FeCl_3$was added. Presence of deep blue black colour indicated the presence of tannins. When 5 mL of extract was treated with few drops of 5% lead acetate solution, white precipitates appeared.

Table 1: Successive and Hydroalcoholic Extraction of Powder of P*sidium guajava* Linn. Leaf

Type of extract	Amount of extract (gm)	Yield (% *w/w*)	Appearance
Petroleum ether	2.850	1.54	Oily yellowish black
Chloroform	10.175	4.47	Greenish Brown
Ethanolic	18.013	7.61	Brownish black solid mass
Aqueous	17.650	7.46	Brown
Hydroalcoholic	20.425	8.57	Brown

Table 2: Phytochemical Screening of Various Extracts of *Psidium guajava* Linn.

Chemical tests	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract	Hydroalcoholic extractAlkaloids
Mayer's reagent	-	-	-	-	-
Dragendorff's reagent	-	-	+++	-	-
Wagner's reagent	-	-	-	-	-
Hager's reagent	-	-	++	-	++
Saponins					
Froth test	-	-	-	-	++
Sterols					
Salkowaski test	-	-	++	-	++
Leibermann's reagent	-	-	++	++	++
Leibermann Burchard's	-	-	++	++	++
Carbohydrates					
Molisch's test	-	-	++	++	++
Fehling's test	-	-	++	++	++
Camnelisation	-	-	++	++	++
Anthraquinone glycosides					
Borntrager's test	-	-	-	-	-
Cardiac glycosides					
Legals test	-	-	-	-	-
Keller-killiani test	-	-	-	-	-
Tannins					
Lead acetate solution	-	++	+++	+++	+++
Ferric chloride solution	-	-	++	++	++
Proteins					
Xanthoproteic test	-	-	-	-	-
Biuret test	-	-	-	-	-
Flavonoids					
Ammonia test	-	-	++	++	++
Alkaline reagent test	-	++	++	++	++
Magnesium ribbon test	-	-	++	++	++
Triterpenoids					
Leibermann Burchard's test	-	-	++	++	++
Salkowski's test	-	-	++	++	++
Fats					
Stain test	-	-	-	-	-
Saponification	-	-	-	-	-

(+) Positive Test, (-) Negative test

To 5 mL of extract 5 mL of 95% ethanol was added along with dilute HCl from sides of test tube. Few fragments (0.5 g) of magnesium turnings were also added. Presence of slight pink colour indicated the presence of flavonoids. To 5 mL of extract few drops of NaOH solution was added. Formation of an intense yellow color, which turns to colorless on addition of few drops of dil. H_2SO_4 indicated the presence of flavonoids. A little extract was taken with 2 mL of water and 0.5 mL of concentrated HNO_3 was added to it. Yellow colour is obtained if proteins are present. To 5 mL of extract 4% NaOH was added along with few drops of 5% $CuSO_4$ solution. Violet or pink colour appeared indicated the presence of proteins. Extract (5 mL) was treated with 5 mL $CHCl_3$ with few drops of conc. H_2SO_4, shaked well and allowed to stand for some time. Formation of yellow colored lower layer indicated the presence of triterpenoids. Extract (5 mL) was treated with few drops of acetic anhydride, boiled and cooled, conc.

H_2SO_4 was added from the sides of the test tube showed a brown ring at the junction of two layers and the upper layer turns green which showed the presence of Steroids and formation of deep red color indicated the presence of triterpenoids. In a test tube containing 5 mL of extract, few drops of freshly prepared 10% alcoholic solution of α- naphthol was added and shaken/stirred for few min. Then 5 mL of conc. H_2SO_4 was added from sides of the test tube. Violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates. Small quantity of extract was pressed the between two filter papers, the stain on I^{st} filter paper indicated the presence of fixed oils. The extract was evaporated to get 10 mL of extract. To the extract 25 mL of 10 % NaOH was added, then it was boiled in water bath for 30min. The extract was cooled and excess of sodium sulphate was added. Soap was formed at the top and filtered. To the filterate H_2SO_4 was added which was evaporated. The extract was dissolved in ethanol and few drops of $CuSO_4$ and NaOH was added. Clear blue solution indicated the presence of fats.

CONCLUSION

Phytochemical screening of petroleum ether, chloroform, ethanol, aqueous and hydroalcoholic extracts revealed the presence flavonoids, tannins triterpenoids, saponins, sterols, alkaloids and carbohydrates by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituents in ethanolic and hydroalcoholic extracts.

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