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Physicochemical Parameters and Phytochemical Screening of Guava (Psidium Guajava) Leaves

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Abstract:
Psidium guajava Linn. Family- Myrtaceae (guava) is a short tree or shrub and folk medicinal plant. It is found in all over India. Each part of the guava tree is useful and has biological importance. Many phytochemicals are present in guava leaves such as flavanoids, alkaloids, tannins, saponins, glycosides, oil and fats, steroids, phenols, proteins, carbohydrates. It is used in various diseases like cough, diabetes, cardiac diseases, diarrhea, wound healing. Guava has various biological activities like antispasmodic, antimicrobial, hepato-protective, antioxidant, anti-diarrheal, anti-inflammatory, anti-allergy. Petroleum ether, chloroform, ethanol is for the extraction of the guava leaves. Soxhlet extraction method is used for the extraction. Liquid-liquid extraction is used for the extraction and purification of the extract and ethyl acetate used for extraction. Physicochemical parameters are studied such as ash value, extractive matter, crude fibre content, foreign matter, moisture content.

Keywords: *Psidium guajava* L., Physicochemical Parameters,Phytochemical Screening, extraction

1. Introduction:

The Guava is generally native to central America or Mexico and it is later spread across the world by man and nature. In India it was introduced by the Portuguese in the early 17th century. Its botanical name is *Psidium guajava* and it belonging to the large family of Myrtaceae that also includes the Eucalyptus tree and several tropical species like Cloves, Nutmeg, Allspice, Cinnamon and also Jamun. The Guava is known for the “apple of tropics” because it contains vitamin C, pectin and other nutrients in higher amount. Guava is also known as ‘Poor man’s apple’.

The Guava is very popular fruit and available almost all throughout the year, but it mainly bears fruit twice a year, ripening during the rainy and winter season. In rainy season the production of fruit is high and the quality of fruit is inferior which grows in winter. Guavas are evergreen fast growing trees relatively easy to grow and capable of high fruit yield with very little care. Its production cost is low because it does not require much fertilizer, irrigation and plant protection.

Cultivation: Owing to its hardy nature, Guavas can grow in a wide range of soil in tropical and subtropical regions. The best quality guavas are grown where low night temperature is higher in winter season. Again it tolerates high temperature and draught conditions during summer in north India. pH of soil is 4.5 to 8.2 to grow the plant and the roots can usually penetrate only up to 25cm under the ground. This is why the surface soil should provide enough nutrients to the plant. In orchards, pit is prepared of 60 cm diameter and depth and guava is planted at a distance of 5 to 6 meters. In each pit manure mixture

of 10kg of cowdung or farmyard manure is applied, 300g of super phosphate and 150g of potash mixed with soil before planting.

2. Material and methods:-

2.1 Plant material

Fresh green guava (Psidium guajava L.) leaves were collected from the farm at Yeola, Dist Nashik, Maharashtra, India. The leaves were washed with water and dried in shade for 14 days. The leaves were powdered using blender to coarse powder and stored in polythene bags for further studies.

2.2 Physicochemical parameters

2.2.1. Ash Value

The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash, and water soluble ash.

2.2.1.1 Estimation of Total Ash of Leaves of *P. guajava* L.

2 gm of powdered drug was accurately weighed and taken in tare silica crucible. The drug was spread uniformly in crucible and ignited until the total drug is burned. The temperature of furnace was gradually increased up to 500-600°C until it was white which indicated absence of carbon. It was kept in desicator for 30 min to cooling and weighed. The percentage w/w of ash with reference to the air-dried plant material was calculated.

2.2.1.2 Estimation of Acid-insoluble Ash of Leaves of *P. guajava* L.

The total ash was boiled with 25 ml of 2M HCl for 5 min. Then filtered with ash-less filter paper and collected the acid insoluble ash on the filter paper and washed with hot water. Transferred the filter paper in crucible and ignited again at 450°C to constant weight. Then kept in desicator for 15 min for cooling and weighed. The percentage w/w of acid insoluble ash with reference to the air dried plant material was calculated.

2.2.1.3 Estimation of Water-soluble Ash of Leaves of *P. guajava* L.

The total ash was boiled with 25 ml of distilled water for 5 min. Then filtered it with ash-less filter paper and collected the water insoluble ash on the filter paper and washed with hot water. Transferred the filter paper in crucible and ignited at 450°C to constant weight. Then kept in desicator for 15 min for cooling and weighed. Then subtract this residue from total ash. The percentage w/w of water soluble ash with reference to the air dried plant material was calculated.

2.2.2. Extractive Matter

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.

2.2.2.1 Estimation of Alcohol Extractive Matter of Leaves of *P. guajava* L.

1 gm of powdered drug was weighed accurately and taken in glass stopper conical flask and macerated with 25 ml of ethanol specified for the plant material concerned for 6 hours, shake frequently, then

allowed to stand for 18 hours. Filtered and 5 ml solvent transferred to the previously weighed flat-bottomed dish and evaporated to dryness on water bath. Then the dish was kept in desicator for 30 min and weighed immediately. The percentage w/w of alcohol extractive matter with reference to the air dried plant material was calculated.

2.2.2.2 Estimation of Water Extractive Matter of Leaves of *P. guajava* L.

1 gm of powdered drug was weighed accurately and taken in glass-stopper conical flask and macerated with 25 ml of water specified for the plant material concerned for 6 hours, shake frequently, then allowed to stand for 18 hours. Filtered and 5 ml solvent transferred to the previously weighed flat-bottomed dish and evaporated to dryness on water bath. Then the dish was kept in desicator for 30 min and weighed immediately. The percentage w/w of water extractive matter with reference to the air dried plant material was calculated.

2.2.3. Foreign Matter

50 gm of medicinal plant material was accurately weighed. It was spread in a thin layer. Plant material was sorted the foreign matter by visually with the help of sieve no. 100. Remained sample was sifted through sieve no. 250. The portions of sorted foreign matter was weighed. The percentage w/w of foreign matter with reference to the air dried plant material was calculated.

2.2.4. Moisture Content (Loss on Drying)

1 gm of powdered drug was weighed accurately and taken previously weighed glass-stopper shallow plate. The shallow plate without lid was kept in hot air oven at 105°C for 1 hour. The shallow plate was then kept in desicator for 30 min for cooling and weighed immediately. The percentage w/w of moisture content with reference to the air dried plant material was calculated.

2.2.5. Crude Fiber Content

0.255 ± 0.005 N sulphuric acid solution and 0.313 ± 0.005 N sodium hydroxide solution was prepared.
2 gm of plant material was weighed and extracted with pet ether. 2 gm of dried material was boiled with 200 ml of H_2SO_4 for 30 min with bumping chips. The material was filtered and washed with boiling water. Again the material was boiled with 200 ml of NaOH solution for 30 min and filtered again, washed with 25 ml of 1.25% H_2SO_4, three 50 ml portions of water and lastly 25 ml alcohol. The residue was removed and transferred to pre-weighed ashing dish (W1). The residue was dried for 2 hours at 130°C and cooled the dish in desicator and weighed (W2). It was ignited for 30 min at 600°C, cooled in desiccator and reweighed (W3). % crude fiber content was calculated by –

Formula:

$$\frac{\text{Loss in weight on ignition (W2-W1) – (W3-W1) X 100}}{\text{Weight of the sample}}$$

2.3 Guava leaves extraction process

Guava leaves were extracted with petroleum ether 60-80ºC, chloroform, alcohol and ethyl acetate by successive extraction method.

Coarsely powdered leaves of *P. guajava* L. were weighed

↓

Powder was defatted with pet Ether (60-80°C) in Soxhlet extractor and concentrated (PG-PE)

↓

The obtained mark was dried and further extracted with chloroform and again concentrated (PG-C)

↓

Again dried marc was extracted with alcohol and concentrated (PG-A)

↓

Alcoholic extract was made hydro-alcoholic by adding water

↓

Hydro-alcoholic extract was extracted with ethyl acetate by liquid-liquid extraction

↓

Ethyl acetate layer was collected and concentrated (PG-EA)

2.4 Estimation of Phytochemical Screening of *P. guajava* L. Leaves Extract

Preliminary phytochemical screening

2.4.1. Test for carbohydrates

Molisch's test: To the T.S., few drops of α-naphthol solution was added in alcohol, shake and added conc. H2SO4 from side of the test tube. Violet ring is formed at the junction of two liquids which shows presence of carbohydrates.

2.4.2. Test for Proteins

Biuret test: To 3 ml of T.S. 4% of NaOH was added and few drops of 1% CuSO4 solution. Pink or violet color appears which shows presence of proteins.

2.4.3. Test for Amino Acid

Ninhydrin test: 3 ml T.S. + 3 drops 5% Ninhydrin solution was heated in boiling water bath for 10 min. Purple or bluish color appears.

2.4.4. Test for Fats and Oils

Solubility test: Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water.

2.4.5. Test for Steroids

Liberamann –Burchard reaction: 2 ml extract + chloroform + 1-2 ml acetic anhydride and 2 drops conc. H2SO4 from the side of the test tube. First red, then blue and finally green color appears.

2.4.6. Test for Glycosides

Keller-killani test: 2 ml T.S. + glacial acetic acid + 1 drop 5% ferric chloride and conc. H_2SO_4. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green.

2.4.7. Test for Saponin

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent stable foam observed.

2.4.8. Test for Flavonoids

Shinoda test: 2 ml T.S. was taken in test tube and to it magnesium powder and few drops of conc. HCl were added which gives pink color, indicates presence of flavonoids.

2.4.9. Test for Tannins and Phenolic compounds

Ferric chloride test: 2-3 ml T.S. + 5% $FeCl_3$ solution gives deep blue- black color indicates presence of tannins and phenolic compounds.

Lead acetate test: 2-3 ml T.S. + lead acetate solution gives white ppt indicates presence of tannins and phenolic compounds.

2.4.10. Test for Alkaloids

Dragendorff's test: 2-3 ml T.S. + few drops of Dragendorff's reagent. Orange brown ppt formed indicates presence of alkaloids.

Mayer's test: 2-3 ml T.S. + few drops of Mayer's reagent gives ppt indicates presence of alkaloids.

2.4.11. Anthraquinone glycosides

Brontrager's test: 3 ml of extract + dil. H_2SO_4, boiled and fltered. To cold filtrate add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer layer turns red or pink indicates anthraquinone glycosides.

3. Result and discussion:

3.1 Physicochemical parameters

Physicochemical parameters were done as per the Indian Pharmacopoeia and World Health Organization. Physicochemical parameters was done on powdered guava leaves.

Parameter	Percentage
Total ash	8.25 % w/w
Water soluble ash	1.5 % w/w
Acid insoluble ash	1.3 % w/w
Water extractive matter	7% w/w
Alcohol extractive matter	15% w/w
Foreign Matter	0.052 %w/w

Crude fiber content	19.65 %w/w
Moisture content	7.6% w/w

Table No. 1 Physicochemical parameters of *P. guajava* leaves

3.2 Successive extraction of *P. guajava* leaf powder

Solvents	Extract	Color	Yield	Consistency
Pet. ether	PG-PE	Dark green	1.63%	Semisolid
Chloroform	PG-C	Dark bottle green	2.23%	Semisolid
Ethanol	PG-A	Red	18.13%	Semisolid
Ethyl acetate	PG-EA	Green	6.6%	Solid

Table No. 2 Successive extraction of *P. guajava* leaf powder

3.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening of guava leaves extract showed that carbohydrates, alkaloids, flavanoids, saponins, tannins, phenolic compound, terpenoids were present in all extracts such as pet. ether, chloroform, alcohol and ethyl acetate.

Sr. No.	Phytochemical test	Extract			
		Pet. Ether	Chloroform	Alcohol	Eth. Acetate
1.	Carbohydrates	+	+	+	+
2.	Protein	-	-	+	-
3.	Amino acid	-	-	-	-
4.	Fats & oil	+	+	+	-
5.	Steroids	-	-	+	-
6.	Glycosides	-	-	+	-
7.	Saponin	+	+	+	+
8.	Flavanoids	+	+	+	+
9.	Tannins and Phenolic compounds	+	+	+	+
10.	Alkaloids	+	+	+	+
11.	Anthraquinine glycosides	-	-	-	-
12.	Terpenoids	+	+	+	+

+ : Positive - : Negative

Table No. 3 Preliminary Phytochemical Screening of *P. guajava* leaves

Discussion:

Leaves of the P. guajava were collected from the farm. The leaves are deep green in color with white veins, eliptic in shape, aromatic odor, leathery texture, 12-13 in length, 5-6 cm in width, characteristic taste. Physicochemical parameters of guava leaves were studied and found to be Total Ash was 8.25% w/w, Acid-insoluble ash 1.3% w/w, Water soluble ash1.5%w/w, Water extractive matter7%w/w,

Alcohol extractive matter 15%w/w, Foreign matter 0.052%w/w, Moisture content (Loss on drying) 7.6% w/w, Crude fiber content 19.65%w/w. Extraction of guava leaves is by chloroform, pet.ether and ethanol by soxhlet extraction method. Liquid- liquid extraction is done with ethyl acetate. Phytochemical screening was studied. Guava leaves extract contains many phyto constituents such as carbohydrates, saponins, alkaloids, flavanoids, steroids, glycosides, tannins and phenolic compounds.

Conclusion:

Guava is a Indian folk medicine. Guava leaves extract contains carbohydrates, saponins, alkaloids, flavanoids, steroids, glycosides, tannins and phenolic compounds. Guava leaves shows antibacterial, anti-inflammatory, antifungal, hepato-protective, antioxidant activities. It can be used to treat many diseases such as diabetes, diarrhea, mouth ulcer.

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