



Research Article

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PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDIES OF *PSIDIUM GUAJAVA* L.

Namrata Dwivedi¹, Geeta Patel¹, Neelesh Dwivedi² and I.P. Tripathi^{3*}

¹Research Scholar, Faculty of Science & Environment, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna, (M.P.), India

²Research Officer, JRD Tata Foundation of Research in Ayurveda & Yoga Sciences, Chitrakoot, India

³Professor & Dean, Faculty of Science & Environment, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna, (M.P.), India

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***Corresponding author**

E-mail: tripathi.ip@gmail.com

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ABSTRACT

Psidium guajava L. is a small tree belongs to Myrtaceae family, traditionally used in treatment of several diseases like inflammation, diabetes, hypertension, wounds, pain and fever. The present study was carried out to investigate the physico-chemical properties and phytochemical analysis of leaves, stem bark and root of *Psidium guajava* L. Phytochemical analysis shows the presence of flavonoids, tannins, saponins, triterpenes, saponins; cardiac glycosides sterols, alkaloids and carbohydrates. The studies of these parameters ascertain the claimed medicinal or therapeutic effects of plants secondary metabolites and pigments that can have therapeutic actions in humans and will be boosting the research in the development of herbal medicine for combat diabetes.

Key words: *Psidium guajava*, diabetes, phytochemical analysis, HPTLC

INTRODUCTION

Medicinal plants have been used in primary health care over many centuries before the advent of modern medicine. *Psidium guajava* L belongs to Myrtaceae family is popularly known as 'poor man's apple of the tropics, has a long history of traditional use for a wide range of ailments. It is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs. It is a native of tropical America. It is a common vegetation cover by roads and in waste places in Hawaii. Guava is a tropical and semitropical plant. It is well known in the islands for its edible fruit. It is common in the backyards¹.

The fruit as well as its juice is freely consumed for its great taste and nutritional benefits. Much of the traditional uses have been validated by scientific research. The extracts of root, bark and leaves of guava are used as folk medicine to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions². Fruits are known to be a source of antioxidant³. Leaves contain phenolic compounds, isoflavonoids, gallic acid, catechin, epicatechin, rutin, naringenin, kaempferol having hepatoprotective, antioxidant, anti-inflammatory, antispasmodic, anticancer, antimicrobial, anti-hyperglycemic, analgesic actions⁴. *Psidium guajava* leaves showed that tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, polyphenol and glycoside present in both extract⁵.

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 processes⁶. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product.

The plant was used for long time in Indian tradition, but it wasn't studied to determine its chemical composition. The plants have complicated composition and the phytochemical studies can be performed using chromatographic and spectral methods that can separate and characterize the different compounds from complex matrix. Within a decade, there were several dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structure determination of Phytochemical⁷. The present study deals with physicochemical and phytochemical investigations of leaf stem bark and root of *Psidium guajava* L.

MATERIALS AND METHODS

Plant material

The plant specimens (leaves, root and stem bark of *Psidium guajava* L.) for the present study were collected from university campus at MGCGV, Chitrakoot, Satna, MP, India and authenticated by Dr. R.L.S. Sikarwar, Taxonomist, Deendayal Research institute. A voucher specimen no.101 is kept at Herbarium of Research Lab, M.G.C.G.V. Chitrakoot for further reference. The All chemicals and reagents used to include the solvents were of analytical grade.

Preparation of extracts

All the plant specimens were air dried at room temperature for one week to get consistent weight. The dried samples were ground to powder. 10 gm of each sample were extracted with 100 ml of methanol and water using cold maceration method⁸. The filtrate was concentrated under reduced pressure using rotary vacuum evaporator (Buchi type).

Physicochemical Analysis

The extracts of the plants were standardized according to WHO guidelines and other Pharmacopoeial procedures. Physicochemical standardization which includes extractive values in different solvents, total ash value, acid insoluble ash value, loss on drying, pH values (1% and 10% solutions) were checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia⁹⁻¹³.

Preliminary Phytochemical Screening

The physicochemical investigation of the ethanol and water extracts of *Psidium guajava* was carried out with standard procedures^{14, 15} for determining the presence and/or absence of phytochemicals.

Quantitative Analysis

(A)Determination of total polyphenol

The concentration of phenolics in methanol fraction of *P. guajava* was determined with the Folin–Ciocalteu's reagent (FCR) using spectrophotometric method^{16, 17}. Each sample (1 mL) was mixed with 0.5mL FCR (diluted 1:10, v/v) followed by 2mL of sodium carbonate (20.00%, v/v) solution. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 ug/ml) were prepared. The absorbance was measured for test and standard solutions against the reagent blank at 765nm after incubation at room temperature for 90 min. Results were expressed as mg of GAE/gm of extract.

(B)Determination of total proteins

Extraction of Protein from sample

Extraction is carried out with buffers used for the enzyme assay. Accurately weighed 500 mg of the sample and ground well with a pestle and mortar in 5-10ml of the buffer. Centrifuged (SORVALL RC 5B plus) 20 minutes at 10,000 per rpm and collected supernatant used for protein estimation.

Standard Protein solution

Weighed accurately 50mg of bovine serum albumin (fraction V) and dissolved in distilled water and the volume was made up to 50 ml in a standard flask. Diluted 10ml of the stock solution to 50ml with distill water in a standard flask. 1 ml of this solution contains 200 μ g protein.

Estimation of protein

Pipetted out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes. The volume was made to 1ml in all the test tubes. A tube with 1ml of water serves as the blank. Added 5 ml of Alkaline copper solution to each tube including the blank. Mixed well and allowed standing for 10 minutes. Then added 0.5 ml of freshly prepared Folin-Ciocalteau reagent mixed well and incubated at room temperature. Kept in the dark for 30 min. A blue color is developed. Took the readings at 660 nm by UV spectrophotometer and calculated the amount protein in the sample. Drawn a standard graph and expressed the amount of protein in mg/gm of sample.¹⁸

(c) Determination of total saponin

Determination of total saponin was done using anisaldehyde reagent. Sample solution was prepared in water. For total saponins estimation 500 μ l of sample, 500 μ l of 0.5% anisaldehyde reagent were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60°. After 10 min tubes were cooled and absorbance was taken at 435 nm by UV spectrophotometer. The amount of saponins¹⁹ was calculated as saponin equivalent from the calibration curve of standard sponging (100-1000 μ g/ml).

Determination of alkaloids

Calibration curve

The calibration curve was obtained with bismuth nitrate pentahydrate stock solution. Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of stock solution into separate 10ml standard flask and diluting to volume with distilled water. A 1ml amount of this solution was taken and 5ml thiourea solution was added to it the absorbance value of the yellow solution was measured at 435 nm against colorless reagent blanks²⁰.

Assay for alkaloids

A 5ml amount of the extract/solution was taken and the pH was maintained at 2-2.5 with dilute HCL. A 2ml amount of Dragendorff's Reagent was added to it, and the precipitate formed was centrifuged. The centrifuged was checked for complete precipitation by adding DR. after centrifugation the centrifuge was decanted completely and meticulously. the precipitate was further washed with alcohol. The titrate was discarded and the residue was then treated with 2ml disodium sulfide solution. the brownish black precipitate formed was then centrifuged. completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2mlconcentrate nitric acid with warming if necessary this solution was diluted to 10 ml in a standard flask with distilled water.1ml was then pipetted out and 5ml thiourea solution was added to it, the absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the solution was calculated using calibration curve. The amount of bismuth corresponds to the number of alkaloids present.

(E) High performance thin layer chromatography

The methanolic extracts of the samples T1 (leaf) T2 (stem bark) and T3 (root) of *Psidium guajava* L. were subjected to HPTLC fingerprint profile to check the presence of different phytoconstituents. HPTLC Fingerprinting was carried out on pre-coated silica gel HPTLC aluminium plates 60F254 (10 cm×10 cm, 0.2mm thickness, 5-6 μ m particle size, E-Merck, Germany) by using CAMAG HPTLC System (Switzerland). 4 μ L and 6 μ L of each of the extract was spotted as bands of 8 mm width by using a LINOMAT 5 Sample Applicator fitted with a 100 μ L Hamilton Syringe. The plates were developed using *Toluene: ethyl acetate* (7:3) as a mobile phase in CAMAG twin-trough chamber lined with filter paper and pre-saturated with 10 ml mobile phase. The resulted plates were dried in air and photo documentation was done at ultraviolet light at 254nm, 366nm and day light using CAMAG REPROSTAR 3 equipped with WINCATS Software. Numbers of bands, color of separated compound and Rf values were recorded²¹⁻²².

Table 1: Organoleptic Characters

| SN | Parameters | Observation | | |
|----|------------|----------------|----------------|----------------|
| | | Leaves | Stem bark | Root |
| 1 | Color | Green | Dark brown | Reddish brown |
| 2 | Odour | Characteristic | Characteristic | Characteristic |
| 3. | Taste | Bitter | Bitter | Bitter |
| 5 | Texture | Fine | Hard | Hard |

Table 2: Physicochemical parameters of *Psidium guajava*

| S.N. | Parameters | Value (n=3) | | |
|------|---|-------------|-------|-------|
| | | Leaves | Stem | Root |
| 1 | Loss on drying at 105°C (%w/w) | 6.09 | 7.44 | 5.30 |
| 2 | Total ash value (%w/w) | 6.39 | 5.32 | 4.32 |
| 3 | Acid-insoluble ash value (%w/w) | 5.36 | 3.09 | 3.66 |
| 4 | Water soluble extractive value (%w/w) | 20.2 | 22.23 | 16.54 |
| 5 | Alcohol soluble extractive value (%w/w) | 11.35 | 13.66 | 9.78 |
| 6 | pH (Filter of 10% w/v aqueous solution) | 4.77 | 4.9 | 5.6 |

* The results are expressed as mean (n=3) ± Standard deviation (SD)

Table 3: Phytochemical Analysis

| Phytochemicals | Tests | Observation | | | | | |
|--------------------------|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | Leaves | | Stem bark | | Root | |
| | | Ethanol extract | Aqueous Extract | Ethanol extract | Aqueous extract | Ethanol extract | Aqueous extract |
| Carbohydrates | Anthrone test, Fehling test | + | + | + | + | + | + |
| | | + | + | + | + | + | + |
| Resin | Acetone | + | + | + | + | + | + |
| Saponins | Foam tests | - | + | - | + | - | + |
| Alkaloids | Dragendorff's test, Wagners test | + | - | + | - | + | - |
| | | - | - | - | - | - | - |
| Steroids | Salkowski tests | + | + | + | + | + | + |
| Flavonoids | Shinoda test | + | + | + | + | + | + |
| Tannins | 5% FeCl ₃ | - | + | + | | | |
| Amino acids and Proteins | Biurate test | - | - | - | - | - | - |

Table 4: Quantitative estimation of Phytochemical Analysis

| SN. | Phytochemical | Concentration in ug/ml | | | Unit |
|-----|---------------|------------------------|-----------|---------|------------------------------------|
| | | leaf | Stem bark | Root | |
| 1 | Polyphenol | 1.004 | 1.1851 | 1.1569 | mg of GAE/g of extract |
| 2 | Protein | 2.3569 | 3.5166 | 9.8578 | mg of BSA/gm of extract |
| 3 | Saponin | 4.5314 | 6.9930 | 20.6993 | mg of Saponin/g of extract |
| 4 | Alkaloid | 18.499 | 15.4667 | 11.6926 | mg of Bismuth nitrate/g of extract |

Table 5: R_f values color of the bands resolved in test solutions of *Psidium guajava* Linn.

| Mobile phase: Tolune: Ethyl Acetate (9:1) | | | |
|---|--|---|---|
| 254nm | T1 (leaf) | T2 (stem bark) | T3 (root) |
| | 0.19,0.55 | 0.19,0.31,0.55 | - |
| 366nm | 0.16 (blue), 0.31(blue), 0.40 (blue), 0.48 (blue), 0.57 (red), 0.62 (sky blue), 0.75(red), | 0.16, 0.40 (red), 0.48 (blue), 0.68 (blue), 0.82 (red) | 0.16 (red), 0.31 (red), 0.44 (light red), 0.68 (red), 0.82 (red)) |
| 366nm AD | 0.17 (blue) ,0.56(brown) | 0.06,0.17 (blue) , 0.32 (light blue),0.40 (light blue), 0.56 | 0.06 light red, 0.17 (faint blue), 0.40(light yellow), 0.56 |

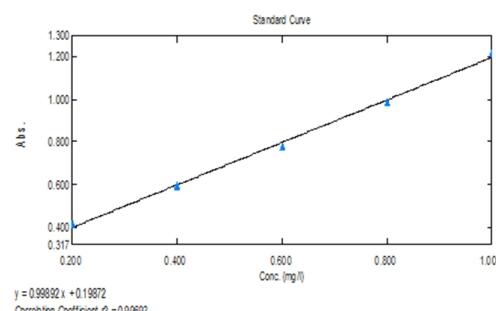
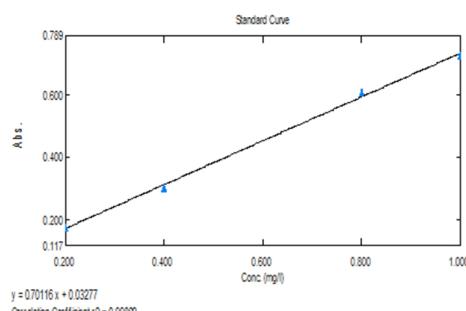


Figure 1: Calibration curve for protein

Figure 2: Calibration curve for saponin

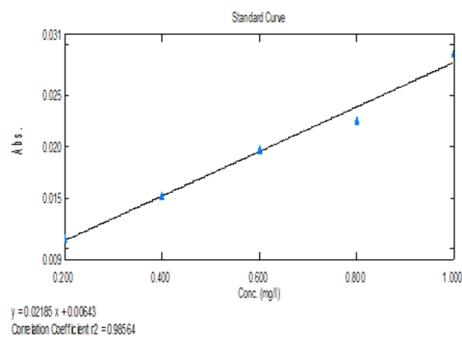


Figure 3: Calibration curve for alkaloids

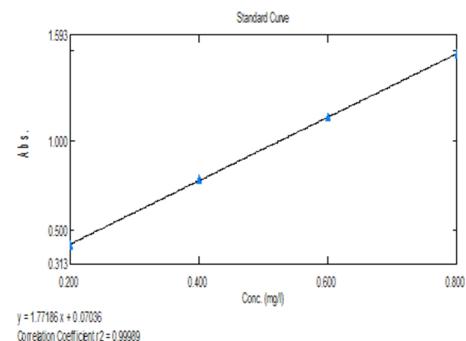


Figure 4: Calibration curve for total polyphenol

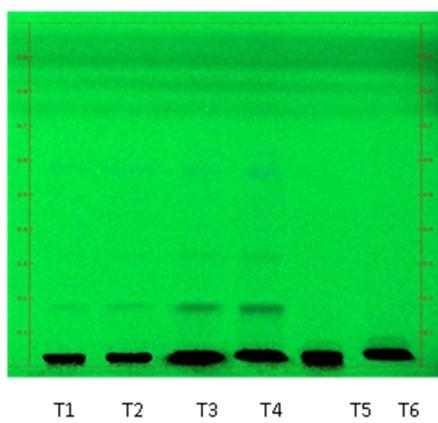


Fig-a. Observed at 254 nm

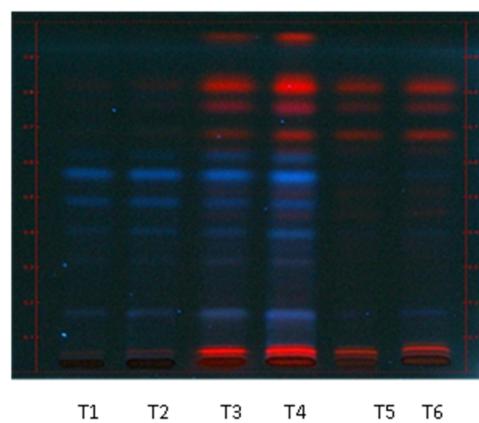


Fig-b. at 366 nm

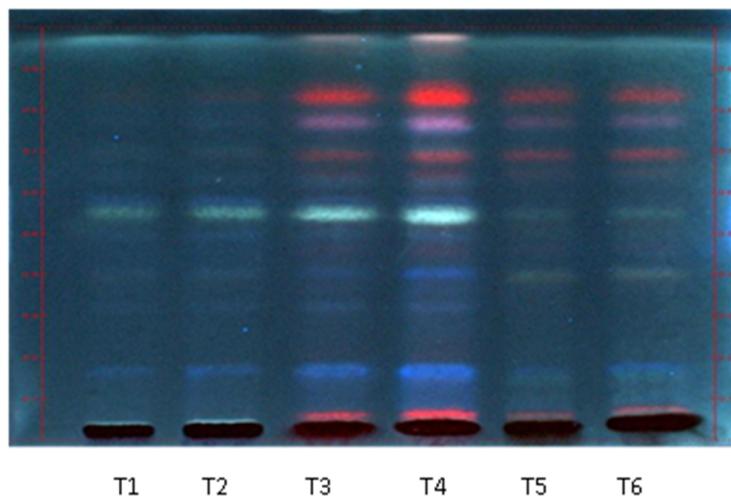


Fig-c. after derivatization at 366nm
Tracks=Where, T1, T2 = P. guajuva leaf, T3, T4 = P. guajuva stem bark and T5, T6 = P. guajuva root

Plate 1: HPTLC Finger print profile
Photo documentation of chromatograph of *Psidium guajava* L. (leaf)

RESULTS AND DISCUSSION

In present study, the leaves, stem bark and root of *Psidium guajava* L were evaluated for its physicochemical and phytochemical aspects. Organoleptic parameters revealed that the powder of leaves, stem bark and root of *Psidium guajava* are green, brown and reddish brown in color, with the characteristic odour, bitter taste and fine and hard texture (Table 1).

Physicochemical investigations for all parts of drug powder were performed for moisture content, ash content, acid insoluble ash, water soluble extractive; alcohol soluble extractive, pH and the results were tabulated in (Table 2). The results are expressed as mean ($n=3$) \pm Standard deviation (SD). The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of drug. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards²³. The extractive values names water soluble and alcohol soluble indicates the number of active constituents in given amount of plant material when extracted with respective solvent. The loss on drying value obtained is an indicative of amount of moisture content present in the drug. pH of 10% w/v solution revealed that the formulations are acidic. The less value of moisture content could prevent bacterial, fungal or yeast growth²⁴.

The results of preliminary phytochemical screening in the methanolic and water extracts of the drugs showed the presence of carbohydrates, flavonoids, alkaloids, resin, saponins and tannins (Table 3) which could make the drug useful for treating different ailment as having a potential of providing useful drugs for human use.

The quantitative analysis of phenolic contents, protein, saponin and alkaloid were carried out using spectrophotometer and results are given in (Table 4). The total phenolic contents in the examined plant extracts is expressed in terms of gallic acid equivalent (the standard curve equation: $Y=1.77186x+0.07036$, $r^2=0.99989$). The concentration of total phenols was measured 1.004, 1.1851 and 1.1569 ug/ml for leaf, stem bark and root respectively. The protein contents in the examined plant extracts is expressed in terms of BSA equivalent (the standard curve equation: $Y=0.05346x + 0.1614$, $r^2=0.99843$). The concentration of protein was measured 2.3569, 3.5166 and 9.8578 ug/ml for leaf, stem bark and root respectively. The saponin contents is expressed in terms of saponin equivalent (the standard curve equation: $Y=0.07150x + 0.01277$, $r^2=0.97804$). The concentration of saponin was found 4.5314, 6.9930 and 20.6993 ug/ml for leaf, stem bark and root respectively. Similarly, the alkaloid contents are expressed in terms of mg of Bismuth nitrate/g of extract equivalent (the standard curve equation: $Y=0.99892x + 0.19872$, $r^2=0.99693$). The concentration of saponin was found 18.499, 15.4667, 11.6926 ug/ml for leaf, stem bark and root respectively.

A simple qualitative HPTLC analysis was performed and Rf values were recorded. HPTLC Plate shows two bands at Rf 0.19 and 0.55 for test sample T1 & T2; four bands at Rf, 0.19, 0.31, and 0.55 under 254 nm; no bands observed for T5 & T6. Similarly, six spots at Rf 0.16 (blue), 0.31(blue), 0.40 (blue), 0.48 (blue), 0.57 (red), 0.62 (sky blue) and 0.75(red) for T1 &T2; five spots at Rf 0.16, 0.40 (red), 0.48 (blue), 0.68 (blue), 0.82 (red) for T3 &T4 and six spots at Rf 0.16 (red), 0.31 (red), 0.44 (light red), 0.68 (red), 0.82 (red) for T4 &T5 were observed under 366nm. After derivatization with 5% methanolic H_2SO_4 two spots at Rf 0.17 (blue) ,0.56(brown); five spots at Rf 0.06,

0.17 (blue) , 0.32 (light blue), 0.40 (light blue), 0.56 and four spots at Rf 0.06 light (red), 0.17 (faint blue), 0.40 (light yellow) and 0.56 were resolved under 366nm. The results of Rf values and colors of the resolved bands were shown in Table -5 & Plate-1(Fig- a, b & c). The current findings can be used as a reference standard for identification of genuine drug with same ingredients. Further marker based identification and pharmacological studies enhance its therapeutic potential to maintain Diabetes Mellitus.

CONCLUSION

The present study was carried out as per WHO/Ayurvedic Pharmacopoeial standards for various standardization parameters such as organoleptic, physicochemical parameters like total ash, acid insoluble ash, water & alcohol soluble extractive values, loss on drying, phytochemical analysis. Presence of various phyto-constituents can serve as basis for screening of different pharmacological activities, investigation and further research. Total phenol, protein, alkaloid and saponin contents were investigated in the drug. The pharmacological action of these plant drugs will be determined by the nature of these chemical compounds which are responsible for the desired therapeutic properties and definite physiological effects. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. The study of these parameters will be boosting the research in the development of herbal medicine for combat diabetes.

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