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PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITY OF *Psidium guajava* L.

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ABSTRACT

Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated. The medicinal value of the chosen plant *P. guajava* leaves has not been extensively worked out. Therefore, the present study was to investigate the phytochemical screening, histochemical, Fluorescence, UV-Visible analysis and antimicrobial activity of *P. guajava* leaves extract. The phytochemical screening *P. guajava* leaves showed that the presence of tannin, saponins, flavonoids, terpenoids and glycosides, steroids, alkaloids, phlopatannins, anthroquinones, triterpenoids, polyphenol, carbohydrate and protein in methanol and aqueous extracts. Quantitative analysis revealed that the plant has phenol(480mg/gm), flavonoids (370mg/gm), tannin (216mg/gm) and terpenoids (20mg/gm) were presented.The histochemical analysis further confirmed in the presence of flavonoids, tannin and saponin. The fluorescence behavior of leaves powder proved by this study. The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the *P. guajava* leaves extract. The results reveal that extract of *P. guajava* leaves were significantly effective against bacteria species *E. coli* and fungal species *C. albicans.*

Keywords: Phytochemical, Antimicrobial, *Psidium guajava* L.

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INTRODUCTION

According to the World Health Organization (WHO), most populations still rely on traditional medicines for their psychological and physical health requirements, since they cannot afford the products of Western pharmaceutical industries (Salie and Simon., 1996), together with their side effects and lack of healthcare facilities (Griggs and Govendarajan, 2001). These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu *et al*., 1999; Idu *et al*., 2007; Mann *et al*., 2008; Ammara *et al*., 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari *et al*., 1980).

Plant based drugs have been used world wide intraditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study to investigate the phytochemical analysis of *Psidium guajava* L. leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Psidium guajava* L. were collected in December 2017 from Thanjavur, Tamil Nadu, India. The *Psidium guajava* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Leaves was spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine powder using grinder mixture. The powder materials were used for further studies.

Preparation of plant extract:

2 gram of the powder of *Psidium guajava* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *P. guajava* leaves were shakeit well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrateused for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara, (1993) Trease and Evans, (1989) and Harborne, (1973 and 1984).

Quantitative analysis of phytochemicals

Determination of total phenols by spectrophotometric method

Total phenols estimated by the method of Edeoga *et al*., (2005)

Determination of flavonoid

Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994)

Determination of tannin

Tannin determination by method of Van-Burden and Robinson (1981)

Estimation of total terpenoid content

Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Qualitative analysis of vitamins Pearson, 1976; Patel, (2005).

Determination of fluorescence behavior of plant powder (Rao *et al.*, 2011)

Histochemical tests

A small quantity of dried and finely powdered leaves sample wasplaced on a grease free microscopic slide and treated with specific chemicals and reagentsandwaited for 1-2 minutes.Apositive test for histochemicals was indicated by the appearance of the appropriate colour change afterapplication of the reagent. Using a light microscope to observeand record any colour changes. The leaf powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids. Plant powder treated with ferric chloride to give Dark blue to black indicates the presence of tannin. Plant powder treated with H_2SO_4 (few drops) to give yellow colour indicates the presence of Saponin.

UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 340-960 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Determination Of Antimicrobial Activity

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly,

inoculums containing bacteria specie were spread on Nutrient agar plates and fungus strains were spread on potato dextrose agar. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

Qualitative analysis

The phytochemical characters of the *P. guajava* leaves investigated and summarized in Table-1.The phytochemical screening *P. guajava* leaves showed that the presence of tannin, saponins, flavonoids, terpenoids and glycosides, steroids, alkaloids, phlopatannins, anthroquinones, triterpenoids, polyphenol, carbohydrateand proteinin methanol and aqueous extracts.

Hassain *et al.,* (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedalium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi *et al.* (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline *et al.,* (2011) screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.

RESULTS AND DISCUSSION

In the present study was carried out on the *Psidium guajava* L. leaves revealed the presence of medicinally active constituents.

Reena Ganesan *et al*., (2013) aimed to carry out preliminary phytochemical of six different solvents extracts from leaf and leaf derived callus of *Sebastiania chamaelea*. The preliminary phytochemical analysis reflects the presence of phenolic compounds, carbohydrate, alkaloids, phytosterols, fats and oils, terpenoids. The result highlights among two extracts, leaf extract show negligible activity than callus extracts

Kumar *et al*., (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Table.1: Qualitative analysis of Phytochemicals in *Psidium guajava L.* leaves

S.No	Test analysis	Methanol extract	Aqueous extract
1	Tannin	+	+
2	Phlobatannin	+	+
3	Saponin	++	+
4	Flavanoids	+	+
5	Steroids	++	++
6	Terpenoids	++	+
7	Triterpnoids	++	++
8	Alkaloid	++	+
9	Carbohydrate	++	++
10	Protein	++	++
11	Anthroquinone	++	++
12	Polyphenol	++	++
13	Glycoside	++	++

(-) Indicates Absence; (+) Indicates Presence;(++) Moderately present

Quantitative analysis

Quantitative analysis revealed that the *P. guajava* leaves has flavonoids, saponin, and tannin. Significant amount of phenol(480mg/gm), flavonoids(370mg/gm), tannin(216mg/gm) and terpenoids (20mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine

chemicals are extracted and purified from plant materials (Das *et al.*, 2010).

Table.2: Quantitative phytochemical analysis of *Psidium guajava* leaves

S.No	Secondary Metabolites	Result (mg/gm)
1	Phenol	480±33.6
2	Flavonoids	370±25.9
3	Tannin	216±15.12
4	Terpenoids	20±1.40

Values are expressed as mean ± SD for triplicates

Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. Vitamins work with other substances in the body like enzymes and minerals. Together they perform such functions as strengthening bones, healing wounds, keeping the skin healthy, building cells, and helping to resist infections. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004).The vitamins of the *Psidium guajava* leaves investigated and summarized in Table-3.

Table.3: Qualitative analysis of vitamins in *Psidium guajava* leaves

S.no	Vitamins	Observation
1	Vitamin A	−
2	Vitamin C	+
3	Vitamin D	-
4	Vitamin E	+

(+) Presence (-) Absence

3.4 Histochemical analysis of leaves powder of *Psidium guajava L.* leaves

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.*, 2001). In the present study, *P. guajava* leaves were treated with specific chemicals and reagents. The *P. guajava* leaves powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids, treated with $FeCl_3$gave green colour indicates tannin and treated with concentrated H_2SO_4gave yellow colour indicates saponin (Table 4**)**. This results further confirmed the presence of phytochemicals.

Table.4: Histochemical analysis of leaves powder of *Psidium guajava L.* leaves

S.No	Charecterisation	Observation	Result
1	Tannin	Green	+
2	Flavonoids	Yellow	++
3	Saponin	Yellow	+
4	Steroids	Green	+
5	Terpenoids	Orange	+
6	Alkaloid	Reddish brown	++
7	Glycoside	Brown	++
8	Polyphenol	Blue green	+

Note: (+) Presence; (++) present with high intensity of the colour

Fluorescence behavior of *Psidium guajava* L. leaves powder

Fluorescence analysis of entire leaves of *P. guajava* has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *P. guajava* was carried out by the treatment of different chemical reagents such as $AlCl_{3g}$ H_2SO_4, HCl, NH_3, HNO_3, CH_3OH and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm) and the results were presented in Table 5.

In the fluorescence analysis, the plant parts or crude drugs may be examined as such or in their powdered form or in solution or as extracts. Although, in most of the cases the actual substances responsible for the fluorescence properties has not been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs (Denston, 1946).

Table.5: Fluorescence behavior of *Psidium guajava L.* leaves powder

S.No	Test	Visible Light	Short UV	Long UV
1	Plant powder	Light green	Dark green	Dark green
2	Plant powder treated with water	Light green	Dark green	Black
3	Plant powder treated with Hexane	Light green	Dark green	Black
4	Plant powder treated with Chloroform	Light green	Dark green	Black
5	Plant powder treated with Methanol	Light green	Dark green	Black
6	Plant powder treated with Acetone	Light green	Dark green	Black
7	Plant powder treated with 1N NaOH (water)	Light green	Dark green	Black
8	Plant powder treated with 1N HCl	Light green	Dark green	Black
9	Plant powder treated with sulphuric acid with an equal amount of water	Brown	Black	Black
10	Plant powder treated with Nitric acid dilute with an equal amount of water	Yellow and green	Orange and green	Black

3.6 Ultraviolet/visible (UV/VIS) spectroscopy

UV-Visible spectrophotometry technique is simple, rapid, moderately specific and applicable to small quantities of compounds. UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy. Phenolic compounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with ironthat have been detected by the ultraviolet/visible (UV-Vis) spectroscopy (Kemp, 1991).

The UV-VIS profile (Fig.1) of the *Psidium guajava L.* leaves extract was studied at awavelength range of 340 to 940 nm. Three major bands wererecorded at 340, 480 and 660 nm. The result confirms the occurrence of peaks at340-940 nm reveals that the absorption bands are due to the presence of flavonoids, phenol and its derivatives(Liu *et al.*, 2006). The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the extract of *Psidium guajava L.* leaves.

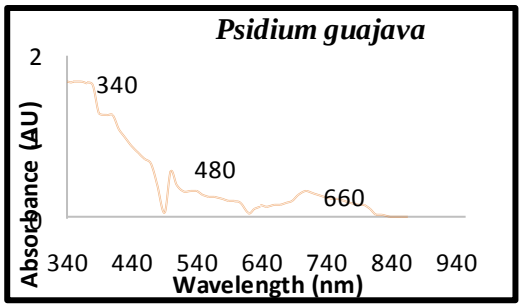


Fig.1: UV-Visible spectrum analysis of *Psidium guajava* leaves

3.7 Antimicrobial activity

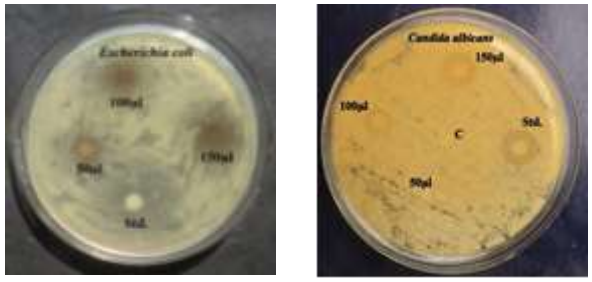
Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al.*, 2003).

This situation forced scientists to search for new antimicrobial substances with plant origin. Plant extract of *Psidium guajava L.* leaves was screened against *Escherichia coli* species of bacteria and *Psidium guajava L.* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Psidium guajava L.* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig.2. The inhibitory activities in culture media of the microbes reported in Table 6 were comparable with standard antimicrobiotic viz. chloramphenicol and fluconazole.

Table.6: Antimicrobial activities of *Psidium guajava L.* leaves

Microbial Organism	50µl	100 µl	150 µl	Standard
Escherichia coli (mm)	8.75±0.61	7.75±0.54	10.00±0.70	11.50±0.80
Candida albicans (mm)	4.00±0.28	4.25±0.29	5.50±0.38	11.00±0.77

Values were expressed as Mean ± SD.
Bacterial standard - Chloramphenicol
Fungal standard - Fluconazole



E. coli *C. Albicans*

Fig2: Antimicrobial activities of *Psidium guajava* leaves

CONCLUSION

Present investigation reported, *P. guajava* leaves extract is warehouse of chemo-diversity which will be useful in screening for medicines like steroids, alkaloids, phenolics, flavonoids and some other chemicals. The results are encouraging but scientific scrutiny is absolutely necessary before being put in practice. This study is the first scientific report that provides convincing phytochemicals and antimicrobial evidence for the relevance of *Psidium guajava* leaves thus providing scientific validity to its traditional consumption by the local populace of south India.

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