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Phytochemical, proximate, anti-inflammatory and antimicrobial activity of *Psidium guajava*

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ABSTRACT

Psidium guajava, or guava, is used in traditional medicine for a wide range of conditions, including diarrhea, diabetes, high blood pressure, respiratory issues like coughs, and various infections. The present study aimed to address phytochemical studies, *in vitro* antioxidant activities, screening of antimicrobial activity and *in vitro* anti-inflammatory activity of *Psidium guajava*. The phytochemical and proximate analysis was done by usual method. Agar well diffusion method and protein denaturation method was carried out for antimicrobial and anti-inflammatory screening. The results showed that the presence of various phytochemical. The proximate composition shows that the plant has high carbohydrate (38.25%), moderate concentration of protein (6.56%) and low concentration of fat (5.15 %). Alkaloids were found to be the most abundant one followed by tannins and flavonoids while phenols and saponins were least in concentration. In our knowledge aqueous extract of *Psidium guajava* showed a potential antioxidant, antimicrobial and anti-inflammatory activity because of its high secondary metabolites.

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INTRODUCTION

Plants are main source of ancient medicine and are useful in the treatment of many diseases with some side effects (Bako *et al.*, 2005). Most of the population used medicinal plants for their basic health care because of their low cost and ease in availability (Shazadi *et al.*, 2010). Traditional medical practices are not only useful in disease healing but also have significant contribution in the discovery of pharmaceutically active substances from plants (Pearce and Puroshothaman, 1992) which can contribute in the commercial production of drugs. The role of plant biodiversity in health care has been well documented from ancient time (Posey, 1999). Various active ingredients like alkaloid, glycoside, saponin, tannins and flavonoids have been isolated from various plant parts such as root, stem, leaves, bark, fruit and seeds; these phytochemicals determine a definite curing physiological response in the treatment of various humans and other animal ailments (Adhikari *et al.,* 2010). Due to easy availability, less side-effects and sometimes only source of health care, the demand for medicinal plants is increasing in both developing and developed countries.

Plants are a predominant natural source of numerous bioactive compounds (Newman *et al.*, 2003; Kumar *et al.*, 2021). Several diseases have been cured using a variety of plant preparations in folk medicine since ancient times (Sharma *et al.*, 2017) and, presently, cosmetic, pharmaceutical, and nutraceutical industries are paying more attention to plant preparations and pure phytochemicals. The projected growth of the plant preparation market is around USD 86.74 billion by 2022, with the largest market share belonging to the pharmaceutical sector, followed by the nutraceutical industry. Interestingly, the utilization of plant preparations for cosmetics, beverages, food, and medicine is mainly dependent on plant leaves. Among all plant organs, leaves are the largest accumulators of bioactive compounds, such as secondary metabolites. Several recent studies reported phytochemical profiles and biological activities of leaf extracts of various cultivated plants (Amat-ur-Rasool *et al.*, 2020; Mannino *et al.*, 2020; Mateos-Maces *et al.*, 2020). Hence, although plant leaves are considered as agricultural waste, they are a rich source of high-value nutra-pharmaceutical compounds.

Psidium guajava (guava) exhibits a wide range of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, anti-diabetic, anti-spasmodic, anti-ulcer, anti-allergic, anti-cancer, and cardioprotective effects. These activities are attributed to its rich content of bioactive compounds such as flavonoids, terpenoids, polyphenols and ascorbic acid. These properties support its traditional use for various ailments, including diarrhea, diabetes, and gastrointestinal issues.

Psidium guajava Linn. is commonly called guave, goyave in French; guave, guavenbaum, in German; banjiro in Japanese; goiaba, in Portu gal; arac¸ guaiaba in Brazil; and guava in English (Killion, 2000). *P. guajava* used as an important food as well as a medicinal plant in tropical and subtropical countries, therefore its nickname as the poor man's apple. The scientific evidences of the medicinal uses of *P. guajava* began in 1940's and reports, maintain a tradition of repeating the data each decade.

Many people habitually take *P. guajava* leaf decoction for its antispas modic and antimicrobial properties for the treatment of dysentery and diarrhea (Gutierrez, 2008).

Therefore, the efficacy and safety of *P. guajava* leaves have empirically been confirmed (Hamada and Kitanaka, 1999). *P. guajava* leaf contains plenty of phenolic compounds which inhibit the peroxidation reaction in the body, and so it can be expected to prevent various chronic diseases such as diabetes, cardiovascular disease and cancer. Furthermore, decreasing of free radicals in the body, means that the polyphenols in the leaf of *P. guajava* can prevent atherosclerosis, cataract and also inhibits biological aging of the body and skin (Okuda, 1982).

P. guajava leaves contains triterpenes, cineol and tannins. Additionally, three lavonoids (avicularin, guaijaverin, and quercetin) have been isolated from the leaves (Khadem and Mohammed, 1959). In mature leaves, the greatest concentrations of flavonoids are found in: Quercetin> Myricetin> Kaempferol> Luteolin (Vargas *et al.*, 2006). Most of the medicinal activities of *P. guajava* are credited to the flavo noids and these phytoconstituents are well-known for their multi-di rectional medicinal activities. This review focuses on the phytochemical and pharmacological benefits of *P. guajava* from the internet data base PubMed and the most relevant articles are considered for review. In this present study an attempt has been made to study the phytochemical, proximate and vitamin constituents present in the medicinal herb. A research was made to determine the antioxidant, anti-inflammatory and its antimicrobial potential against human pathogenic microbes in *P. guajava*.

MATERIALS AND METHODS

Collection of plant and extraction

The leaves of *Psidium guajava* were collected in the MPC garden, Thanjavur. The leaves were air dried under natural conditions for five days and dried leaves were powdered by using home blender and aqueous extract was prepared and stored in refrigerator under 4^{o}C for further analysis.

Qualitative phytochemical analysis

The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Sofowora, 1993).

Proximate analysis

Proximate analysis such as moisture content, Ash content (AOAC, 2000), crude fibre, crude lipid (AOAC, 1990), Protein (Lowry's method, 1951) and Carbohydrate (Dubois *et al.*, 1956) was determined.

Quantitative analysis

The extract was subjected to quantitative analysis for the determination of secondary phytochemical constituents Determination of total alkaloids (Obadoni and Ochuko, 2001), Total phenolic content (Kaur and Kapoor, 2002), total flavonoids content (Chang *et al.,* 2002), total tannins (Siddhuraju and Manian, 2007) and total saponins (Makkar *et al.,* 2007).

Screening of antimicrobial activity

In-vitro antimicrobial screenings were carried out under laboratory conditions, for this various microorganism were collected from our microbiology laboratory, with bacterial strain of *E. coli* and *B. subtilis* and fungal strain of *Aspergillus niger* and *Candidas albicans*. All the suggested microorganisms were cultured on recommended cultural medium and finally transfer and maintained on agar broth for O/N. Antimicrobial activity of plant *Psidium guajava* have been carried out by using disc diffusion method (Kavanagh, 1972). The inhibitory effect of each extracts was compared with the standard antibiotics penicillin and mycostatin against bacteria and fungi respectively.

Anti-inflammatory activity

Anti-inflammatory activity of the plant extract was determined by *in vitro* method such as protein denaturation (Mizushima *et al.,* 1968; Elias and Rao, 1988).

RESULTS AND DISCUSSION

The phytochemical analysis of *Psidium guajava* was shown in Table 1. The phytochemical analysis showed the presence of alkaloids, flavonoids, phenols, steroids, tannins, saponins sugar and aminoacids with the absences of terpenoids and cardiac glycosides. Phytochemicals in the plants are non-nutritive chemicals that have protective or disease preventive properties.

It is well documented that the presence of these chemicals is responsible for various medicinal properties and there are many reports available to support the role of phytochemical constituents and their activity against specific disease (Suresh and Nagarajan, 2009).

Table 1. Preliminary phytochemical analysis of extracts of the leaf of *psidium guajava*

Constituents	Observation
Alkaloids	+
Flavonoids	+
Phenols	+
Steroids	+
Tannins	+
Saponins	+
Terpenoids	-
Cardiac glycosides	-
Sugar	+
Aminoacids	+

The proximate compositions determined in the leaves are summarized in Table 2. It shows that the plant has a high moisture content (12.5%), crude fibre (15.76%) and carbohydrate (38.25%), moderate concentration of protein (6.56%) and ash (4.90%), and low concentration of fat (5.15 %).

Table 2 Proximate composition of *Psidium guajava* leaves

Parameters	Value (%)
Moisture content	12.2
Total ash	4.90
Crude fibre	15.76
Crude lipid	5.15
Protein	6.56
Carbohydrates	38.25

Water determines the energy value in terms of moisture (Rahimi and Rabani, 2010).The proximal parameters studied would be used to characterize and standardize the experimental plant. A Moisture content of 75% in the leaves primarily explains the higher degree of food spoilage and microbial contamination. The percentage of ash content defines the quality of a food material which gives an identity to a substance of its carbon free nature and also denotes the organic, inorganic matter and impurities present in the sample. The total ash content predicts the soluble and insoluble minerals in the sample (Llodibia *et al.*, 2016).

The crude fiber is the organic residual content remaining after digesting with enzymes, acid and base. It is an important constituent of balance diet that decreases blood cholesterol level, heart risks, colon cancer and diabetes (Ishida *et al.*, 2000). The RDA values of fibers for children are 19-25% and for lactating mother is 29 %. Belewu and Babalola (2009) stated that crude fibers can be used for useful purposes if treated with microorganisms. Crude fats and oils is the part of a complex organic material that is soluble in ether consists chiefly of fats and fatty acids. It is a measure of the fat or oil (lipid) of plant which is considered as medicinal or nutritious feed and extremely rich sources of energy. Oils impede microbial fermentation; ruminant diets should be limited to about 4% fat.

Protein contents in the plant vary according to climatic and habitat conditions. Proteins are considered to be the building block of cells and carbohydrate is a group of organic compounds that includes sugars, starches, cellulose, and gums. It serves as a major source of energy in the diet of human. Carbohydrates performs numerous important roles in human and animal bodies. Polysaccharides serve for the storage of energy (e.g. starch and glycogen) and as structural components (e.g. cellulose in plants and chitin in animals).

Table 3. Quantitative phytochemical composition of *Psidium guajava* leaves

Parameters	Value (mg/100g)
Alkaloids	6.58 ± 0.17
Flavonoids	3.22 ± 0.19
Phenols	1.76 ± 0.21
Tannin	6.32 ± 0.09
Saponin	1.56 ± 0.18

Table 3 represented the data of quantitative determination of secondary metabolites as showed that phytochemicals determined from the leaves of *Psidium guajava*, alkaloids were found to be the most abundant one followed by tannins and flavonoids while phenols and saponins were least in concentration. Phenols and tannins have anti-oxidant properties.

Various reports indicate that regular flavonoids may trim down the risk of several chronic diseases including neurodegenerative diseases, atherosclerosis, and cancer (Mahesh *et al.*, 2013). The flavonoids also reported to have antiviral, anti-

allergic, anti-platelet and anti-inflammatory properties.

Antimicrobial analysis

The antimicrobial analysis (Table 4) showed a remarkable activity against the bacterial and fungal pathogens with aqueous extract of *Psidium guajava*. The maximum activity compared to the control shows the potential of the plant and is an indicator for determining the significance of the activity against the pathogens. The overall antibacterial analysis reveals maximum against the *B. subtilis* and minimum activity was noted against the *E. coli*. Against fungal pathogens activity was maximum towards *Aspergillus niger* and minimum activity was seen against *C. albicans*. Overall observation reveals that the plant has inhibitory activity against all the pathogens studied. *Psidium guajava* is a potential source of broad-spectrum antimicrobial agents due to the presence of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes (Cushnie and Lamb, 2005).

Table 4. Antimicrobial activity of *Psidium guajava* leaves

Microbes	Control	Standard (Penicillin & mycostatin)	Extract
Escherichia coli	-	18.02 ± 0.69	15.02 ± 0.3
Bacillus subtilis	-	22.82 ± 0.65	21.18 ± 0.2
Aspergillus niger	-	12.32 ± 0.18	10.3 ± 0.12
Candidas albicans	-	11.18 ± 0.27	8.16 ± 0.57

Anti-inflammatory activity

The protein denaturation method was used for the *in vitro* anti-inflammatory activity of the aqueous extract of the *Psidium guajava* which was presented in Table 5. % inhibition of protein denaturation activity was exhibited on the basis of concentration dependent manner. The % inhibition of protein denaturation was 18.2% for 100µg/ml and 33.75% for 500µg/ml. Sodium diclofenac was used as a standard. During inflammation the lysosomal enzymes is released which produced a variety of disorders and these enzymes is said to be related to acute or chronic inflammation. The diclofenac drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The aqueous extracts of leaves *Psidium guajava* showed biphasi effects on protein denaturation method.

Table 5. Anti-inflammatory activity of *Psidium guajava* leaves

Concentration (µg/ml)	% inhibition of protein denaturation
100	18.22 ± 0.16
200	22.60 ± 0.03
300	24.50 ± 0.22
400	30.21 ± 0.15
500	33.75 ± 0.14
Standard (Diclofenac)	74.62 ± 0.16 4

On the basis of the results obtained in the present study, it is concluded that aqueous extract of *Psidium guajava* leaves has potent anti-inflammatory and anti-microbial activities. Thus the *Psidium guajava* extract may be attributed to the presence of phenolic compounds and flavonoids etc., Therefore, further investigation is needed to isolate and identify the active compounds present in the plant extract and its efficacy.

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