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Phytochemical analysis and antioxidant activity of ethanolic leaves extract 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. This study was conducted to assess the phytochemical constituents in *Psidium guajava* leaf extracts using standard methods. The ethanolic leaves extract of *Psidium guajava* was analysed by HPLC and GC to determine various phytochemicals. The free radicals scavenging activity of extract by using DPPH, NO and super oxide radicals generated *in vitro*. The ethanolic leaves extract of *Psidium guajava* was found to contain alkaloids (6.18 mg/gm), tannins (1.82mg/g), phenols (7.76 mg/gm) and flavonoids (5.15 mg/gm). The major flavonoids detected were kaempherol. The ethanolic extract of *Psidium guajava* was found to possess significant free radical scavenging activity against DPPH, NO and SOD anions the IC_{50} value of 39.0 µg/ml, 34.25 µg/ml and 30.05µg/ml respectively and comparable to that of their corresponding IC_{50} value. The medicinal property of *Psidium guajava* may be attributed to the presence of flavonoids and phenolic compounds with rich antioxidant potential.

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INTRODUCTION

Plants have been major source of medicine in all cultures from ancient times. In the traditional system, various indigenous plants are being used in the diagnosis, prevention and elimination of physical, mental or social imbalance. Phenolic compounds, ubiquitous in plants, are of considerable interest and have received more and more attention in recent years due to their bioactive functions. Polyphenols are amongst the most desirable phytochemicals because of their antioxidant activity. Natural therapy for various human ailments purified with plant products has gained much attention now days, due to various side effects associated with allopathic medicine these can be derived from any part of the plant like bark, leaves, stem, flowers, roots, seeds, etc., (Cragy and David, 2001). Medicinal plants are believed to be an important source of chemical substances with potential therapeutic effects (Farnsworth, 1989).

Free radicals play an important role in various pathological conditions such as tissue injury, inflammation, neurodegenerative diseases, cancer and aging. The Compound that can scavenge free radicals has great potential in ameliorating these diseases (Coban *et al.*, 2003).

Inflammation is a disorder characterized by invasion of leucocytes and production of proinflammatory cytokines (Mantri and Witiak, 1994).

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. Therefore the present study quantifies the secondary metabolites and the antioxidant potential of ethanolic extract of *P. guajava*. Considering all these facts, the present study was designed to investigate the presence of various phytochemicals in the different extracts of *P. guajava*, a plant which evokes various therapeutic effects.

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^oC 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).

Quantitative phytochemical analysis

HPLC-UV analysis (Total phenols)

Ethanolic extract of *Punica granatum* was subjected to solid phase extraction using column 5mm (4.6.mm), and peptides, small molecules were removed; fractionation of neutral and acidic phenolic acids was also carried out simultaneously. The resulting fraction was then subjected to reverse phase high performance liquid chromatography (RP-HPLC). The total phenolics in ethanolic extract of *P. granatum* was detected using, Stationary phase octadecylsil. Silica and mobile phase (A phosphoric acid: water (0.5: 99.5v/v) B acetonitrile). The UV detector was set at 220 nm with the flow rate adjusted to 1.0ms / min. The major peaks were identified and the retention times were compared with these of standards.

Fractionation of total alkaloids

Ethanolic extract of *P. granatum* was detected using monobasic phosphate as mobile phase (270ml. of Acetonitril). The liquid chromatography is equipped with 235 nm detector and 4.6nm x 150 mm column. The flow rate was adjusted to 1.8ml / minute. The major peaks were identified and the total alkaloids concentration was determined.

Fractionation of total flavonoids

HPLC chromatography (System Name: LACKROM L-7000 MERCK, Proc Method- HITECHI) total flavonoids. The total flavonoids in the extract was determined by using octadecysil silica gel as stationary phase and acetonitril, sodium dihydrogen phosphate with dilute orthophosphoric acid as mobile phase. UV detector was set at 350nm with flow rate of 0.5ml/min. The major peaks in ethanolic extract of *P. granatum* were determined in comparison to the retention time of standards run at identical conditions.

Free radical scavenging activity

Diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging assay is a commonly recommended method for assessment of antioxidant potential of plant extracts. The assay is based on the ability of DPPH, a free radical which get decolorized in the presence of antioxidants. To 200ml. of ethanolic solution of DPPH (1 µg/ml) various concentration of (20mg- 100 µg/ml) in water were added and incubated at 37^oC for 30 min in dark and the absorbance was measured at 517nm. Ascorbic acid was used as the reference standard. The percentage scavenging of DPPH free radical was calculated and compared with that of the standard ascorbic acid. The IC_{50} value also determined.

Superoxide anion scavenging activity

The method of Nishkimi *et al.* (1972) was applied for the measurement of MIT superoxide anion scavenging activity, Briefly 312µm Nitroblue tetarzolium in 120 µm phosphate buffer pH 7.4 were added to an aliquots of MIT (20-100µg/ml) the reaction was started by adding 100ml of phenazine metho sulphate (120mm preaperd in phosphate buffer pH 7.4) and the colour change was monitored at 560nm against water blank querceutin was used as the positive control.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of the aqueous extract was measured by taking various concentrations of MIT and standard. Ascorbic acid (20-100µg/ml) dissolved in phosphate buffer (0.025m, pH 7.4) and incubated with sodium nitroprusside (5mm) in standard phosphatebuffer at 25^oC for 5 hrs. After the incubation, 0.5ml of the reaction mixture was added with 0.5ml of Griess reagent (equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% napthlthyl ethylene diamine dihydrochloride in water). The absorbance of the chromophore formed was read at 540nm. The activity was compared with that of similar concentration of Ascorbic acid (Sreejavan Rao, 1997).

RESULTS AND DISCUSSION

Phytochemicals are naturally occurring biochemical compounds that plants developed, in order to protect themselves from oxidation, insect disease and other hazards in their environment. These phytochemicals give their characteristic colour, flavour, smell and texture. Epidemiological studies indicate that populations consuming high levels of plant derived foods have low incidence rates of various cancers.

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. Qualitative phytochemical analysis

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

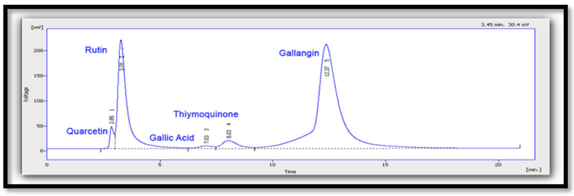
In this investigation, presence of quantitative phytochemicals in ethanolic extract of *Psidium guajava* expressed the value in mg/g phenols was (7.76 mg/g), tannins (1.82 mg/g), flavonoids (5.15 mg/g) and alkaloids (6.18). Finding the natural substance of medicinal plant that decrease the inflammation and reduce oxidative stress and there by counteracting the macromolecular damage. Flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells. Quantitative analysis of ethanolic extract of *Psidium guajava* was given in Table 2.

Table 2. Quantitative phytochemical analysis of *Psidium guajava*

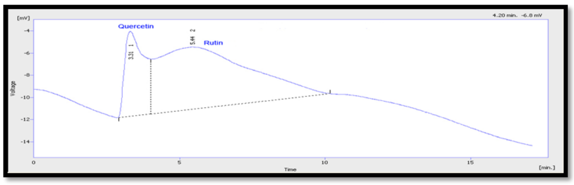
Phytochemical	Values in (mg/g)
Phenols (mg/g)	7.76
Tannins (mg/g)	1.82
Flavonoids (mg/g)	5.15
Alkaloids (mg/g)	6.18

HPLC analysis of ethanolic leaves extract of *Psidium guajava*

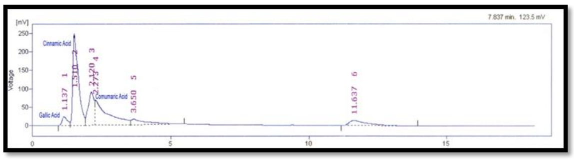
HPLC analysis reveals that the extract was found to be rich in Alkaloids (6.18 mg/g) terpenoids (1.82 mg/g) and phenols (7.76 mg/g). Ethanolic leaves extract of *Psidium guajava* also contain flavonoids such as Rutin (1.12 mg/g) and quercetin (1.19 mg/g).



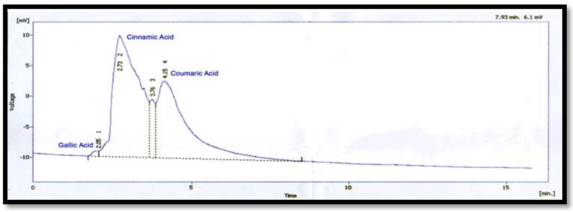
A. HPLC finger prints of standard flavonoids



B. HPLC finger print of flavonoids present in *Psidium guajava*



C. HPLC finger print of standard phenolics compound



D. HPLC finger prints of phenols present in *Psidium guajava*

Fig. 1 (A) to (D) many reports demonstrate that antioxidant principle present in medicinal plants are responsible for their therapeutic potential (Larson, 1988) flavonoic compound such as quercetin and

Rutin are formed to be responsible for anti-inflammatory and anticancer properties proliferates by their terminating action of free radicals. Alkaloids have many pharmacological activities including anti-cancer and anti-arthythmic effect (Cordell, 1983).

Alkaloids are known to reduce the inflammation level significantly. These results shows that ethanolic leaves extract of *Psidium guajava* containing which could be accounted for the antioxidant and anti-inflammatory effects. In the present investigation, HPLC chromatrographics pattern of the ethanolic extract of *Psidium guajava* showed 2 peaks of flavonoids and 3 peaks of phenolic compounds. In HPLC analysis of ethanolic extract of *P. guajava* was found to be rich in flavonoids such as quercetin, Rutin and phenols such as gallic acid, cinnamic acid and coumaric acid. The natural phytonutrients presents in fruits and vegetables scavenge the free radicals and protect the cells from oxidative damages. The phytonutrients present in ethanolic leaves extract of *P. guajava* migrates the responsible for the traditional claim by the test drug.

In vitro antioxidant assays

Antioxidants are known to exhibit their biochemical effects through numerous mechanisms, including the prevention of chain initiation, reductive capacity and radical scavenging mechanisms. Several methods have been used to measure the antioxidant activity of biological materials. It is essential to use more than one method to evaluate antioxidant capacity of plant materials simply because of the complex nature of phytochemicals present in them. Therefore, in the present study, DPPH free radical scavenging activity and nitric oxide scavenging activity assessment were done.

Table 3. Free radicals scavenging activity in *Psidium guajava*

Free radicals	Samples	Concentration of standard and extract in (μg/ml)				
		20	40	60	80	100
DPPH	Ascorbic acid	35.6	41.7	62.8	72.4	81.8
	P. guajava	25.5	32.4	45.6	56.5	65.2
Nitric oxide	Ascorbic acid	29.5	35.8	42.9	55.7	67.1
	P. guajava	26.8	31.7	36.8	47.9	57.5
Superoxide	Gallic acid	29.8	32.7	47.9	53.9	78.6
	P. guajava	18.8	24.8	35.3	42.2	58.4

The percent DPPH scavenging activity ethanolic extract of *Psidium guajava* were depicted (Table 3). Ascorbic acid was used as a positive control for comparison of plant materials. The IC_{50} value of ascorbic acid was found to be 52.25μg/ml. The IC_{50} value of DPPH scavenging activity was found to be ethanolic extract of *Psidium guajava* was 39.0 μg/ml. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The degree of discoloration indicated the scavenging potential of the antioxidants in the sample. The extract significantly inhibited the activities of DPPH radicals in a dose-dependent manner. The comparison of scavenging effects of ethanolic extract on DPPH radical showed consistently higher radical scavenging activity observed in seabuckthorm *Hippophae rhamnoidesl* seeds extracts of phenolic compounds (Heim *et al.*, 2002), Flavonoids (Apati *et al.*, 2003) and terpens (Saranya Panneerselvam *et al.*, 2010) might be taken in to account.

Activity evaluation of *Psidium guajava* extract showed the capacity of the plant towards nitric oxide scavenging in a dose dependent manner. Ascorbic acid, a natural antioxidant was used as a positive control for comparison. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with super oxides. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Incubation of solutions of sodium nitroprusside in PBS at 25°C for 2 hours resulted in linear time dependent nitrite production, which is reduced by the ECH. The IC_{50} of ascorbic acid was found to be 39.35μg/ml. The order of the scavenging activity was found to be ethanolic extract of *Psidium guajava* was IC_{50} 34.25 μg/ml. Nitric oxide scavenging activity of ethanolic leaves extract of *P. granatum* is on important chemical mediator generated by endothelial cells, macrophages, neuron and it is involved in the regulation of various physiological process like control of arthritis, cytotoxice effects alzhemer's disease (Sainani *et al.*, 1997). Thirunavukkarasu *et al.* (2011) previously studied higher free radicals compared to the results of 73.89±4.22 at 2mg/ml concentration of ethanol extracts of mangrove *A. officinalis* in Cuddalore region. Higher nitric oxide scavenging activity was reported by Athiperumalsami *et al.* (2010) in the methanol and water extracts of *H. ovalis* but the IC_{50} values were much lower than that of standard tocopherol.

Superoxide anion is a free radical created from the normal process of energy generation in the human body. Superoxide anion is toxic to cells and tissues and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen. The concentration of standard gallic acid to inhibit 50% of superoxide formation was found to be 40.3µg/ml. The scavenging activity of *Psidium guajava* extract was found to be IC_{50} 30.05µg/ml. The superoxide onions are toxic intermediates formed during inflammatory process and found to enhance the risk of inflammation related disorders such as arthritis and atherosclerosis. Super oxide anion is a free radio that plays an important role in the formation of reactive oxygen species such as hydrogen periode, hydroxyl / radicals, or singlet oxygen in living organism. Korycka *et al.* (1978) reported that the therapeutic activity of medicinal plants can be determined by superoxide activity. Superoxide anion is a free radical created from the normal process of energy generation in the human body. Superoxide anion is toxic to cells and tissues and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen (Nohl *et al.*, 2003).

CONCLUSION

Nowadays herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. The plant based compounds have the effective dosage response and minimal side effects when compared to the synthetic compounds Phytochemical screening of *Psidium guajava* leaves reveals it as a valuable medicinal plant with numerous medicinal properties. Since the ethanolic extract of *Psidium guajava* leaves contains more constituents it can be considered beneficial for further investigation. A typical research and developmental work needs to be carried out for its better therapeutic and commercial utilization. The free radical scavenging activity of *Psidium guajava* revealed that they can be used for the prevention or treatment of human diseases such as cancer, arthritis, diabetes mellitus which are associated with oxidative stress.

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