International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 06 Issue 04 April 2026

Page No : 965-971

DOI: https://doi.org/10.47191/ijpbms/v6-i4-07, Impact Factor: 8.428

In Vitro Antioxidant Activity of *Psidium Guajava* L. Leaf Extract

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ABSTRACT

Psidium guajava L. (family Myrtaceae), has been traditionally utilized in herbal medicine for its diverse therapeutic properties. The present study aimed to evaluate the in-vitro antioxidant activity of *Psidium guajava* L. leaf extract using free radical scavenging assays. The dried leaf powder was extracted by maceration and Soxhlet extraction method using ethanol as solvent, followed by preliminary phytochemical study and the antioxidant potential was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay. The preliminary phytochemical study was performed to check the presence of active phytoconstituents in the extract. The extract demonstrated significant dose-dependent free radical scavenging activity in both DPPH and ABTS assays when compared with standard ascorbic acid. The concentration range used for leaf extract in DPPH is 50-500ug/ml and for ABTS is 50-250ug/ml against ascorbic acid (10-50ug/ml and 10-40ug/ml). The IC_{50} values obtained from the DPPH and ABTS were found to be 73.07µg/ml and 132.97µg/ml respectively against standard ascorbic acid (20.94µg/ml and 20.42 µg/ml). The leaf extract exhibited the highest percentage of inhibition at 500 µg/ml in the DPPH and 250 µg/ml in the ABTS. Further the preliminary phytochemical study also confirms the presence of many active phytoconstituents such as phenolic compounds and tannins, flavonoids, alkaloids, saponins, glycosides, steroids and triterpenoids, carbohydrates, proteins and amino acids. The findings confirm that *Psidium guajava* L. leaves possess potent in-vitro antioxidant properties and may serve as a natural source of antioxidants for pharmaceutical and nutraceutical applications. Further scientific validation through in vivo studies is required to evaluate the therapeutic effects, which could provide significant benefits to society.

ARTICLE DETAILS

Published On:
18 April 2026

Available on:
https://ijpbms.com/

KEYWORDS: Psidium guajava L.; Antioxidant; DPPH; ABTS; Extraction; Phytoconstituent

INTRODUCTION:

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Over 50% of all modern clinical drugs are of natural product origin and natural products play important roles in drug development in the pharmaceutical industry. The isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important [26].

Free radicals are unstable molecules formed during normal body processes and from external factors like pollution, smoking, and radiation. When their levels become too high, they can damage important molecules such as DNA, proteins, and lipids. This damage is called oxidative stress, and it plays a major role in aging and the development of diseases like diabetes, cancer, heart disease, and Alzheimer's. Antioxidants help to protect the body by neutralizing free radicals and reducing oxidative stress, thereby supporting better health. Antioxidants act primarily through two mechanisms-a) chain breaking activity (stopping ongoing oxidative chain reactions by neutralizing free radicals. b) Preventive activity-reducing the initiation of free radicals through metal ion chelation or enzyme activation [28].

Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent, or repair damage done to the cells of

the body by reactive oxygen species or reactive species generated from molecular oxygen. They act by one or more of the following mechanisms: reducing activity, free radical scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. The involvement of active oxygen and free radicals in the pathogenesis of certain human diseases, including cancer, aging and atherosclerosis is increasingly being recognized [17]. Active oxygen and free radicals, such as superoxide anion, hydrogen peroxide and hydroxyl radical, are constantly formed in the human body by normal metabolic action. Their action is opposed by a balanced system of antioxidant defences, including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death [9]. Therefore, much attention has been focussed on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation, or to protect against the damage of free radicals [27]. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers [12] and neurodegenerative diseases [5].

Psidium guajava L. also known as guava, is a fruit belongs to the family myrtaceae, that is native to tropical America but is now grown throughout the tropical areas. The chemical constituents of guava leaves are phenolic compounds, iso-flavonoids, gallic acid, catechin, quercetin, epicathechin, rutin, naringenin, kaempferol, caryophyllene oxide, p-selinene etc.

Plant Profile:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsid
Order: Myrtales
Family: Myrtaceae
Genus: *Psidium*
Species: *guajava* L.

Traditional uses: The leaves are used in traditional system of medicines for febrifuge, antispasmodic, wounds, ulcers and toothache, rheumatism, gargle for sore throats, laryngitis and swelling of the mouth and externally for skin ulcers, vaginal irritation, discharge, vaginal and uterine wash, especially in leucorrhoea. They are also used to treat diarrhoea, bacterial infections, blood cleansing, astringent, lung problems, dysentery, stomach pain, antibiotics, inflammations, diabetes management, hypertension, sores, boils, cuts and sprains.

MATERIALS AND METHODS

Extraction: The fresh leaves of *Psidium guajava* L. were collected from Baridua, Ri-Bhoi, Meghalaya. The freshly collected leaves were washed thoroughly with distilled water and kept for drying under shade for 15 days. After drying, the leaves were subjected to extraction using ethanol as the solvent by employing two methods: maceration and hot percolation.

Preliminary phytochemical study: The preliminary phytochemical analysis was conducted separately for each of the ethanolic extracts obtained through maceration and hot percolation methods, the following methods were used to confirm the presence of different phytochemicals:

1. Determination of Alkaloids

The procedure involved preparing solvent-free extracts from the ethanolic extracts using a water bath. 50g of the resulting extract was then combined with diluted HCl and filtered. The clear filtrate obtained underwent Dragendorff's test, Mayer's test and Wagner's test to detect alkaloids.

2. Determination of Flavonoids

Using the leaf extracts, alkaline reagent test, concentrated sulfuric acid test and lead acetate test were conducted to determine the presence of flavonoids.

3. Determination of Saponins

To detect saponins, the froth test was employed. Leaf extract was added to water and vigorously shaken to observe the formation of froth, which indicate the presence of saponins.

4. Determination of Glycosides

The detection of glycosides involved hydrolysing a 50g plant extract with concentrated HCI for 2 hours on a water bath, followed by filtration and utilizing the filtrate for Keller-Kilani and Bontrager's tests.

5. Determination of Carbohydrates

The procedure included dissolving 100mg of solvent-free extract in 5ml. of distilled water, followed by filtration. The resulting filtrate was then used for conducting Molisch's and Fehling's tests.

6. Determination of Proteins & Amino acids

The plant extract was hydrolysed with concentrated HCl for 2 hours, filtered, and then tested for proteins and amino acids by performing the Biuret and Ninhydrin tests.

7. Determination of Steroids and Triterpenoids

For assessing steroids and triterpenoids, equal parts of chloroform were mixed with the plant extracts, filtered and then used for the Salkowski's test and Sulphur test.

8. Determination of Tannins and Phenolic compounds

To analyse tannins and phenolic compounds, each extract's residue was mixed with water gently warmed and filtered. The resulting filtrate was then tested using Ferric Chloride and Lead Acetate tests.

Determination of *in vitro* Antioxidant Activity:

a)DPPH radical scavenging activity: The free radical scavenging activity of the leaf extract and ascorbic acid as standard was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl). 1mg/ml of the test sample of different concentrations (50,100,150,200,250 and 500µg/ml) in triplicates were prepared and 0.15 mM (6mg in 100 ml of ethanol) of freshly prepared DPPH solution was used. The mixture was incubated for 15 minutes at room temperature in dark condition. The absorbance was measured at 517 nm using UV-visible spectrophotometer.

The capability to scavenge the DPPH radical was calculated, using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample. The antioxidant activity of the samples was expressed as IC_{50}. The IC_{50} value

was defined as the concentration in µg of dry material per ml (µg/ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

b)ABTS radical scavenging activity: The antioxidant effect of the samples was determined by using ABTS (2, 2'-azino-bis3-ethylbenzthiazoline-6-sulphonic acid) radical cation decolourisation assay. ABTS radical (ABTS+) were prepared by reacting ABTS solution (7mM) [36mg/10ml H_2O] with 2.45mM ammonium Persulphate [5.6mg in 10ml of H_2O] was mixed. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. 250µl sample of different concentrations (50,100,150,200 and 250µg/ml) were prepared in triplicates, to it 1ml of prepared ABTS reagent was added and incubated in dark at room temperature for 30 minutes. Absorbance was measured at 734 nm using UV-visible spectrophotometer

The percentage of inhibition of ABTS radical was calculated by using the following equation:

% of inhibition= {(Ac- At)/Ac}x 100

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample. The inhibition activity was expressed as IC_{50}. Ascorbic acid is used as standard.

RESULTS AND DISCUSSION:

Extraction: The results for extraction were found to be 8% and 7.2% for maceration and hot percolation respectively.

Preliminary phytochemical analysis: The results for preliminary phytochemical study shown presence of various bioactive compounds such as alkaloids, Flavonoids, tannins and phenolic compounds, steroids and triterpenoids, glycosides, proteins and amino acid and carbohydrates. The results were given in the table no 1.

Table no 1: Results of preliminary phytochemical study

Phytoconstituents	**Tests**	**Results**	
		Maceration	**Percolation by Soxhlet**
Alkaloids	Dragendroff's Test	+	+
	Mayer's Test	+	+
Flavonoids	Alkaline reagent Test	+	+
	Lead Acetate Test	+	-
Saponins	Froth Test	+	+
Glycosides	Keller-Killiani Test	+	+
	Borntrager's Test	+	-
Carbohydrates	Molisch's Test	+	+
	Fehling's Test	+	-
Proteins and amino acids	Biuret Test	+	+
	Ninhydrin Test	-	-
Steroids and Triterpenoids	Salkowski's Test	+	+
	Sulphur Test	+	+
Tannins and phenolic compounds	Ferric chloride Test	+	+
	Lead acetate Test	+	+

Determination of *in vitro* antioxidant activity:

a) **DPPH radical scavenging activity:** DPPH is a free radical which is stable at room temperature and this method is often employed to determine the antioxidant activity of many plant extracts. The concentration in µg/ml of the sample to scavenge 50% of the DPPH radical is called IC_{50} and lower IC_{50} values indicate higher DPPH activity. The result indicates that *Psidium guajava* L. leaf extract exhibit DPPH activity, however, the standard ascorbic acid showed significantly higher anti-oxidant activity. The fig 1 shows standard ascorbic acid curve and fig 2 show that *Psidium guajava* L. leaf extract had high antioxidant activity as compared to the standard. The 500µg/ml of the guava leaf extract have shown 94.34% inhibition of the DPPH chromophore and hence has shown potential antioxidant property as compared to the untreated Blank sample. The IC_{50} of Ascorbic acid and guava leaf extract was found to be 20.94 µg/ml and 73.07 µg/ml respectively.

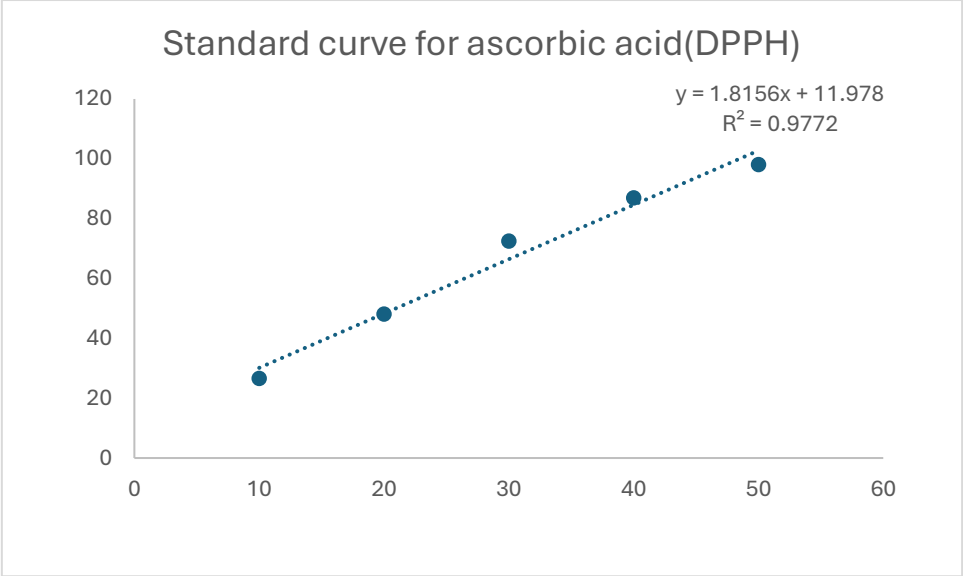

Fig 1: Standard curve for ascorbic acid (DPPH)

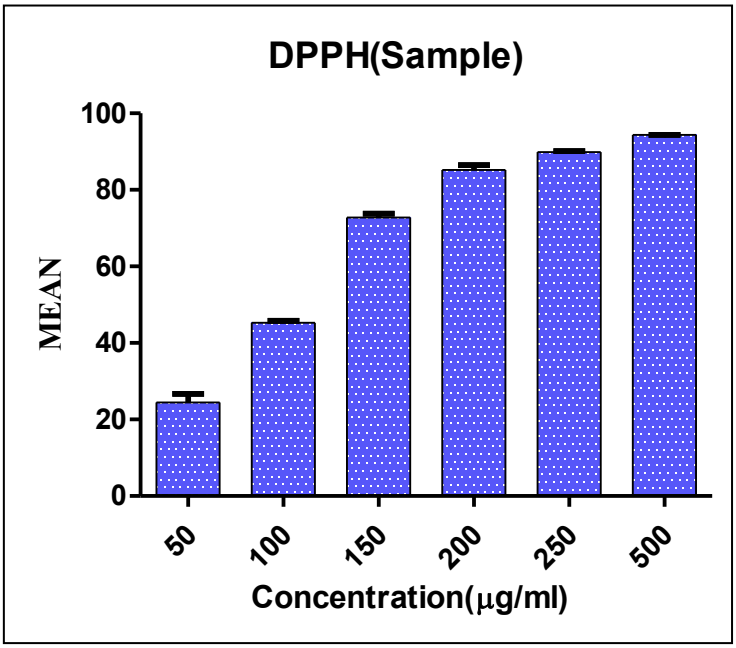

Fig 2: Graph showing Effect of leaf extract on various concentrations by inhibiting DPPH

b) ABTS radical scavenging activity:

The ABTS assay showed the antioxidant activity of plant extracts by scavenging the ABTS generated in aqueous phase and the values are expressed as IC_{50} when compared with ascorbic acid as standard. The fig 3 below represented standard ascorbic acid curve and fig 4 represented the *Psidium guajava* L. leaf extract showed high activity when compared to the standard.

The 250 µg/ml of the Guava leaf extract have shown 82.98% inhibition of the ABTS chromophore and hence has shown potential antioxidant property as compared to the untreated Blank sample. By comparing the values with ascorbic acid it is derived that 250µg/ml concentration of guava leaf extract is equivalent to 36.68µg of Ascorbic Acid. The IC_{50} of Ascorbic acid was found to be 20.42 µg/ml and the IC_{50} of Guava leaf extract was found to be 132.97 µg/ml.

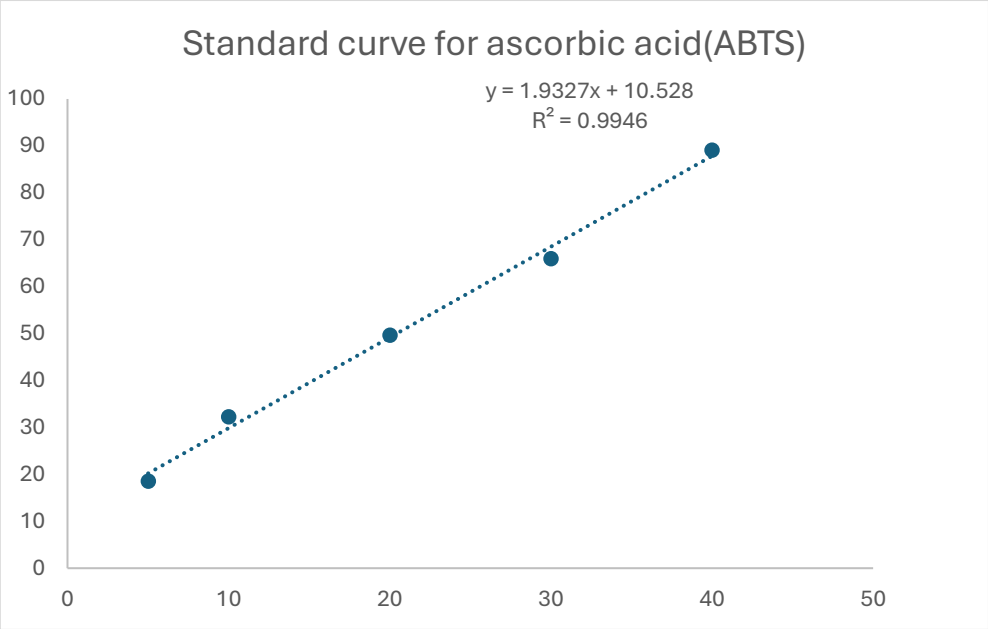

Fig 3: Standard curve for ascorbic acid (ABTS)

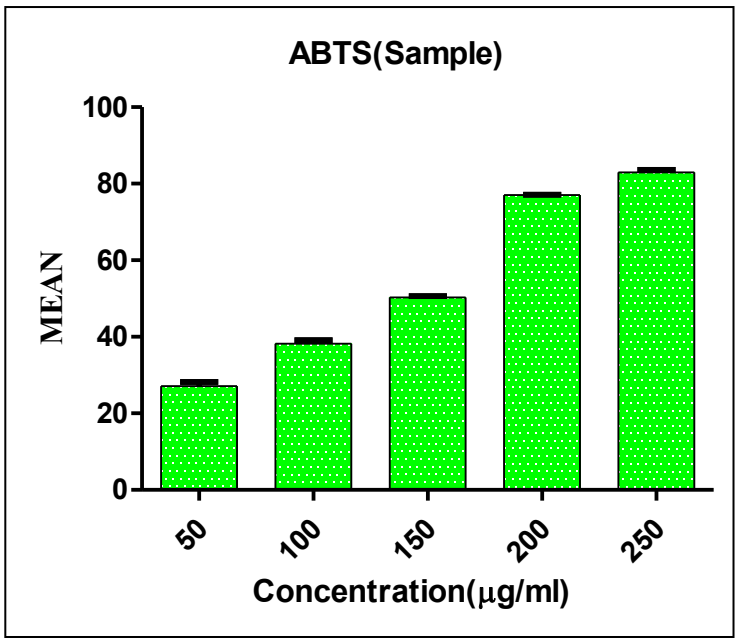

Fig 4: Graph showing Effect of leaf extract on various concentrations by inhibiting ABTS

CONCLUSION

The findings of the present investigation clearly establish that the leaf extract of *Psidium guajava* L. possesses potent *in vitro* antioxidant activity, as demonstrated by its effective scavenging of free radicals in both DPPH and ABTS assays. The preliminary phytochemical study further supports these results by revealing the presence of key bioactive constituents, including phenolic compounds, flavonoids, tannins, and other secondary metabolites.

Moreover, the results obtained in this study not only validate the traditional medicinal use of *Psidium guajava* leaves but also emphasize their potential as a cost-effective and natural alternative to synthetic antioxidants, which are often associated with adverse effects. The plant could therefore serve as a promising candidate for incorporation into pharmaceutical formulations, nutraceuticals, and functional foods aimed at combating oxidative stress–related disorders. Nevertheless, while the in vitro results are encouraging, they represent only an initial step. Further research is necessary to isolate, characterize, and quantify the specific active compounds responsible for the antioxidant activity. In addition, in vivo studies, toxicity assessments, and clinical evaluations are essential to confirm the safety, efficacy, and bioavailability of the extract. Such comprehensive investigations will help in fully elucidating the therapeutic potential of *Psidium guajava* L. and facilitate its application in modern medicine.

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