

IN VITRO ANTI-OXIDANT POTENTIAL OF PSIDIUM GUAJAVA LEAVES

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ABSTRACT

A study investigated the antioxidant capacity of various extracts from *Psidium guajava* leaves using in vitro models. The extract underwent various assays including DPPH assay, reducing power assay, nitric oxide assay, H₂O₂ assay, and SOD assay. The findings from all methods indicated that the ethanolic extract of *P. guajava* exhibits antioxidant potential in a concentration-dependent manner compared to the other two extracts. The antioxidant activity of the sample was compared with ascorbic acid as the standard reference. The study concluded that the ethanolic extract of *P. guajava* leaves is a rich source of antioxidants.

Keywords: *Psidium guajava*, invitro, antioxidant.

INTRODUCTION

In our bodily cells, oxidants are produced under both normal and pathological circumstances. These oxidants play a vital role in our body by eliminating microbes. However, at times, there can be an uncontrolled generation of oxygen-derived free radicals, known as reactive oxygen species (ROS). This ROS can lead to oxidative damage to macromolecules, contributing to the development of various illnesses, including cardiovascular diseases, cancer, aging, diabetes mellitus, rheumatoid arthritis, and cirrhosis (Polterat, 1997). The human body has innate protective mechanisms to mitigate the production of free radicals (Chandra et al., 1994).

These protective mechanisms can become impaired under different pathological conditions, necessitating the need for antioxidant supplements to minimize free radical formation. Antioxidants interact with the oxidation process through radical scavenging and chelation, preventing oxidative harm caused by free radicals. Although there are several synthetic agents available, such as butylated hydroxyanisole and butylated hydroxytoluene, these substances can lead to various side effects in humans and animals (Madhavi, D.L and Salunkhe, 1995). Compounds derived from plants, like flavonoids, tannins, proanthocyanidins, and phenols, exhibit strong antioxidant properties, prompting researchers to seek antioxidant solutions from natural sources that do not result in harmful effects.

Psidium guajava is a widely recognized medicinal plant from the Myrtaceae family, commonly known as guava, goiava, or guave (Killion, 2000). This plant can thrive in a variety of climatic conditions. The leaves of *P. guajava* are dark, simple, elliptic to ovate, and range from 5 to 15 centimeters in length. *P. guajava* leaves are utilized for their hypoglycemic (Manikandan et al., 2018), cardioprotective (Manikandan et al., 2023), antimicrobial, antifungal, and antispasmodic properties (Ross, 1999). The leaves contain compounds such as terpenoids, phenols, and tannins (Manikandan et al., 2013). The aim of the current study was to assess the antioxidant activity of the ethanolic extract of *P. guajava* Linn. using in vitro models.

MATERIALS AND METHODS

Plant Material and Extraction: The fresh leaves of *P. guajava* were collected locally and authentication was obtained from St. Joseph College, Trichy. The shade dried *P. guajava* leaves were powdered mechanically and stored in an air tight container. The plant was extracted by using ethanol. The extraction was carried out by hot percolation method using Soxhlet apparatus. About 100 gm of powder was extracted with 600 ml of ethanol. The extract was concentrated to dryness under controlled temperature 40- 50°C. The percentage yield was found to be 10.15%. The extract was preserved in refrigerator till further use. Then the in vitro antioxidant assays.

RESULT AND DISCUSSION

In the initial phase, leaves of *P. guajava* were gathered locally, and authentication was provided by St. Joseph's College, Trichy. The leaves of *P. guajava* were dried and then ground into a powder. The powdered leaves were used to extract compounds using hexane, petroleum ether, ethanol, chloroform, and water with a Soxhlet apparatus. These extracts were concentrated, dried, and stored in a desiccator for later use. Various extracts were prepared from the leaves of *P. guajava*, utilizing solvents like hexane, petroleum ether, ethanol, chloroform, and water, selected based on their polar characteristics. Subsequently, the extracts underwent preliminary phytochemical analysis. The findings indicated the existence of alkaloids, phenolic compounds, flavonoids, terpenoids, and tannins in the extracts of *P. guajava* leaves. The presence of these phytoconstituents may account for the diverse pharmacological activities observed.

In the following phase, in vitro antioxidant activity was evaluated by testing the inhibitory effects of both the *P. guajava* leaves and squalene against DPPH, reducing power, nitric oxide (NO), hydrogen peroxide (H₂O₂), and superoxide anion radical scavenging assays. In the DPPH assay, the IC₅₀ values for the ethanolic, aqueous, and chloroform extracts of *P. guajava* leaves were found to be 90.7 µg/ml, 97.5

$\mu\text{g/ml}$, and $99.4 \mu\text{g/ml}$, respectively, while ascorbic acid had an IC_{50} of $76.4 \mu\text{g/ml}$. During the reducing power assay, the IC_{50} values for the ethanolic, aqueous, and chloroform extracts of *P. guajavaleaves* were $100 \mu\text{g/ml}$, $102 \mu\text{g/ml}$, and $147.5 \mu\text{g/ml}$, respectively. The standard alpha-tocopherol exhibited an IC_{50} value of $50 \mu\text{g/ml}$. The IC_{50} concentrations for the NO assay were $108.5 \mu\text{g/ml}$, $146.5 \mu\text{g/ml}$, and $150.5 \mu\text{g/ml}$ for the ethanolic, aqueous, and chloroform extracts of *P. guajavaleaves*, respectively, while the IC_{50} for ascorbic acid stood at $90.5 \mu\text{g/ml}$.

The scavenging potency against superoxide radicals was observed in the ethanolic, aqueous, and chloroform extracts of *P. guajavaleaves*, with IC_{50} values of $135.5 \mu\text{g/ml}$, $150.5 \mu\text{g/ml}$, and $157.5 \mu\text{g/ml}$, respectively. BHT was utilized as a standard, showing an IC_{50} value of $100 \mu\text{g/ml}$. In the H_2O_2 assay, the inhibition concentrations for the ethanolic, aqueous, and chloroform extracts of *P. guajavaleaves* were recorded at $139.5 \mu\text{g/ml}$, $159 \mu\text{g/ml}$, and $173.5 \mu\text{g/ml}$. The standard alpha-tocopherol exhibited an IC_{50} value of $109.5 \mu\text{g/ml}$.

The results from this in vitro antioxidant study clearly demonstrate the antioxidant potential of the leaves of *P. guajava* across all extracts. This capacity may be attributed to the presence of various phytoconstituents in the extracts. Of all the extracts, the ethanolic extract of *P. guajavaleaves* exhibited the highest activity when compared to the other two extracts. Therefore, subsequent studies were conducted exclusively on the ethanolic extracts of *P. guajava* leaves.

Table 1: Phytochemical analysis of *P. guajava* leaves of various extracts

S.No.	Characteristics	<i>P. guajava</i> leaves Extracts				
		Ethan ol	Aqueo us	Chlorof orm	Hexane	Petroleu m ether
1.	Carbohydrates	+	+	+	+	+
2.	Protein	-	-	-	-	-
3.	Steroid	+	-	-	+	-
4.	Glycoside	+	+	-	-	-
5.	Tannin	+	+	-	-	+
6.	Flavonoids	+	+	-	+	+
7.	Alkaloids	+	+	-	+	-
8.	Saponin	+	-	-	-	-
9.	Starch	-	+	-	-	-
10.	Terpenoids	+	+	+	-	+
11.	Anthocyanin	+	-	-	-	-

12.	Coumarin	+	+	-	-	-
13.	Emodins	+	-	-	-	-
14.	Phytosterol	+	+	-	-	-
15.	Phlobatannins	-	-	-	-	-
16.	Cardial Glycosides	+	+	-	-	-
17.	Chalcones	+	+	-	+	-

a) + sign indicates positive test (presence of compound);

b) – sign indicates negative test (absence of compound)

Table 2: DPPH radical scavenging activity of *P. guajavaleaves* extract

S. NO	Concentration of plant extract and standard ($\mu\text{g/ml}$)	<i>P. guajavaleaves</i>			% of inhibition of Ascorbic acid
		% of inhibition of ethanol	% of inhibition of aqueous extract	% of inhibition of chloroform extract	

1.	25	20.63	19.12	17.23	26.18
2.	50	42.01	38.10	36.72	41.74
3.	100	54.15	52.59	51.27	65.19
4.	150	66.05	66.92	62.11	70.34
5.	200	75.04	72.04	69.25	82.15
6.	250	85.20	85.63	84.10	91.10
IC₅₀		90.7	97.5	99.4	76.4

Table 3: Reducing power assay of *P. guajavaleaves* extract

S. NO	Concentration of plant extract and standard (µg/ml)	<i>P. guajavaleaves</i>			% of inhibition of α-tocopherol
		% of inhibition of ethanol	% of inhibition of aqueous extract	% of inhibition of chloroform extract	

1.	25	32.16	28.25	24.56	35.17
2.	50	42.15	40.44	34.21	50.32
3.	100	50.54	49.22	43.51	64.27
4.	150	61.11	57.91	51.87	73.13
5.	200	67.14	65.32	60.18	82.03
6.	250	75.24	70.10	67.28	90.61
IC₅₀		100	102	147.5	50

Table 4: Nitric oxide assay of radical scavenging activity of *P. guajavaleaves* extract

	Concentration	<i>P. guajavaleaves</i>	% of
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S. NO	of plant extract and standard (µg/ml)	% of inhibition of ethanol	% of inhibition of aqueous extract	% of inhibition of chloroform extract	inhibition of Ascorbic acid
1.	25	19.72	18.21	16.27	22.56
2.	50	27.26	24.26	28.06	38.27
3.	100	48.10	40.76	40.16	52.47
4.	150	58.19	51.05	50.13	65.42
5.	200	69.20	63.27	60.28	78.03
6.	250	79.37	70.15	69.61	89.87
IC ₅₀		108.5	146.5	150.5	90.5

Table 5: Superoxide radical scavenging activity of *P. guajava* leaves extract

S. NO.	Concentration of plant extract and standard ($\mu\text{g/ml}$)	<i>P. guajava</i>			% of inhibition of BHT
		% of inhibition of ethanol	% of inhibition of aqueous extract	% of inhibition of chloroform extract	
1.	25	16.22	14.97	13.51	19.23
2.	50	24.40	20.65	19.46	28.14
3.	100	42.78	37.95	36.43	50.14
4.	150	58.14	50.21	48.34	61.87
5.	200	67.05	63.93	60.27	74.02
6.	250	78.09	73.59	70.65	90.87
IC ₅₀		135.5	150.5	157.5	100

Table 6: Hydrogen peroxide radical scavenging activity of *P. guajava* leaves extract

S. NO.	Concentration of plant extract and standard ($\mu\text{g/ml}$)	<i>P. guajava</i> leaves			% of inhibition of α -tocopherol
		% of inhibition of ethanol	% of inhibition of aqueous extract	% of inhibition of chloroform extract	
1.	25	14.62	13.10	12.11	18.82
2.	50	21.40	20.24	19.97	25.36
3.	100	36.76	34.68	32.21	43.05
4.	150	55.35	48.13	44.12	60.56
5.	200	70.03	63.11	60.15	75.19
6.	250	81.69	73.02	69.64	89.12

IC ₅₀	139.5	159	173.5	109.5
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DISCUSSION

The DPPH scavenging assay is a crucial method for assessing the antioxidant capacity of plant extracts in in vitro models. DPPH is a free radical that interacts swiftly with antioxidant substances. Antioxidative compounds have the ability to donate a hydrogen atom to DPPH, resulting in a color change. The color intensity is measured calorimetrically. A higher color intensity correlates directly with increased inhibition of DPPH. This study indicates that a higher concentration of the extract reduces DPPH activity, with the highest inhibition observed at 1500 µg/ml. The reducing power is associated with the capacity of the plant extract to transfer electrons. This assay measures the ability of Fe³⁺ to be converted to Fe²⁺ (Meir et al., 1995). The findings demonstrate that the extract possesses antioxidant properties in a concentration-dependent manner. According to the results, *P. guajavais* capable of converting Fe³⁺ to Fe²⁺, thus reducing oxidative damage to tissues. Nitric oxide is a free radical generated from sodium nitroprusside that reacts with oxygen to produce nitrite.

Antioxidant activity was evaluated based on the inhibition of nitrite formation, facilitated by the plant extracts that react with oxygen, nitric oxide, and various nitrogen compounds (Maccocci et al., 1994). This study confirms that higher concentrations of the extract exhibit maximum inhibitory effects on nitric oxide. Hydrogen peroxide is a significant reactive oxygen species, as it can be harmful if converted into hydroxyl radicals within cells. Antioxidant compounds that donate electrons to H₂O₂ can neutralize it into water molecules (Mathew and Abraham, 2006). This study confirms that hydroxyl radical inhibition from H₂O₂ occurs in a concentration-dependent manner. Superoxide dismutase plays a vital role in the antioxidant defense system. SOD transforms the superoxide anion into hydrogen peroxide, thereby diminishing its toxic effects. The percentage of superoxide inhibition by SOD may lessen cellular damage. This study shows that increasing concentrations of the extract lead to maximum SOD inhibitory activity.

CONCLUSION

The current investigation demonstrated the in vitro antioxidant properties of the crude ethanol extract derived from *P. guajavaleaves*. This plant exhibited considerable antioxidant

activity in an in vitro setting; therefore, additional research is required to identify the compounds responsible for this effect.

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