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Evaluation of Invitro Anti-oxidant Activity with Tannin Fraction from *Psidium Guajava Linn*. Leaves and Bark

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ABSTRACT

The present studies are to review the Psidium guajava Linn leaves and bark is used for anti-oxidant activity. And compounds in tannin like poly-phenolic are served as a free radical scavenging activity in many bacterial associated diseases. Guva is most popular plant in India and it is freely available in all over India. As it is having high in medicinal values we choose these for our research. Methods: Extraction of tannins from Psidium guajava Linn leaves and barks. Find out the anti-oxidant activity.

Keywords: *Anti-oxidant activity; Guva; Tannins*

INTRODUCTION

Natural products plant resources are having great Medicinal values from ancient days these are used to cure disease. Now days the research has expanded and most of the people are looking for natural treatment. That's why we choose the natural products for our research.

Present generation people don't know about the Medicinal values of the natural products. Our main theme is to identify the medicinal properties in naturally available plant sources by conducting different test.

The main aim of the study is to explore the medicine values of the natural plants, and how it will helps to cure the chronic diseases. Earlier work on Psidium guajava Linn. With reference to literature from Vels university library and other well established libraries were collected and listed below.

Plant Profile

Botanical source-*Psidium guajava Linn.*, Family-Myrtaceae., Common names-Guava, common guava, yellow guava, apple guava., Parts used-Fruits, leaf, flower, root and bark.

MATERIALS AND METHODS

Collection and Authentication

The plant specimen (Leaves and bark) for the proposed study was collected during the month of July 2010 from the garden of Vels university, Pallavaram, Chennai. It was identified and authenticated by Dr. P. Jayaraman, Director of Plant Anatomy research center (PARC), Tambaram, Chennai. A voucher specimen No. PARC /2010/594 has been deposited for further reference.

Extraction

Tannins Fraction:

The leaves and bark of Psidium guajava Linn. were shade dried and coarsely powdered. About 300 gm of powdered drug was extracted with Acetone + Water (7:3) by cold maceration method after 72 hrs of maceration it was filtered. To this filtrate, Petroleum ether to be added in a separating funnel by removing the Chlorophyll. After removal of chlorophyll, petroleum ether layer was decanted. Again to these filtrate add a saturated solution of sodium chloride and vitamin-C (Ascorbic Acid), again Filtered these solution. The filtered solution to be added Ethyl Acetate solvent in a separating funnel. After gradual shaking of both these solvents in a separating funnel, decanted the ethyl acetate solvent. The separating funnel contains Aqueous layer to be taken, After complete extraction the extraction was concentrated by distilling off the solvent and then evaporated to dryness under reduced pressure using vaccum flash evaporator.

Phytochemical Screening

The leaf and bark tannin fractions were subjected to qualitative phytochemical test for identification of constituents.

Chromatography

The Results of TLC Presented in Table 2.

Pharmacological Study

Invitro Anti-Oxidant Study

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Free radicals can lead to a variety of physiological and biochemical lesions and induce degenerative diseases such as coronary artery disease, aging and cancer.

Although almost all organisms possess anti-oxidant defense and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely. Antioxidants are such substances that can delay or restrict oxidative cellular oxidizable substrates. Interest in finding naturally occurring anti-oxidants in foods or medicines to replace synthetic anti-oxidants has increased considerably, given that synthetic anti-oxidants are being restricted due to their side effects [1-5].

DPPH radical scavenging activity

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. About 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to in 3 ml of the different concentrations(50,100,200,400,800,1000 µg/ml) of Tannin leaf fraction(TLF),Tannin bark fraction(TBF),standard(vitamin c) and control (without the test compound, but with an equivalent amount of methanol) in different test tubes. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC50 value (50% of inhibitory concentration in µg/ml) and the TLF&TBF was compared with that of vitamin C, which was used as the standard.Decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage inhibition of DPPH radical was calculated using the formula,

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The result was given as percentage of inhibition in Table 3 and Figure 2.

Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations of TLF&TBF (50,100,200,400,800,1000 µg/ml),Vitamin C as reference standard (50,100,200,400,800,1000 µg/ml) and dissolved in phosphate buffer saline (0.025 M, pH: 7.4) and the tubes were incubated at 25oC for 5 hr. Control experiments without the test compounds but equivalent amounts of buffer were conducted in an identical manner. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine-dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm. All the determinations were performed in 6 replicates. Percentage inhibition of nitric oxide radical was calculated by using the formula [6-8].

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The result was exhibited as percentage of inhibition in Table 4 and Figure 3.

RESULTS AND DISCUSSION

The present work covers study on anti-oxidant activity of the leaves and bark of *Psidium guajava Linn*.

Extraction

Table 1: Isolation of tannin fraction

Extract/Fraction	Percentage Yield (% w/w)	Color	Consistency
Tannin leaf fraction	8.8	Light brown	Greasy
Tannin bark fraction	9.6	Brownish	Greasy

Phyto-Chemical Screening

The phytochemical test was carried out for the identification tannin fraction shows positive result in tannins chemical test.

Chromatography (TLC) of Extract

Table 2: Thin layer chromatography of leaf and bark fraction of Psidium guajava Linn

S. No.	Test extract	Solvent system	Detecting agent	Number of spots	Rf value
1	Standard (Gallic acid)	Toluene: acetone :Glacial acetic acid (3:1:2)	5% Fecl3	2	0.91
2	TLF	Toluene: acetone :Glacial acetic acid (3:1:2)	5% Fecl3	1	0.91
3	TBF	Toluene: acetone :Glacial acetic acid (3:1:2)	5% Fecl3	1	0.89

Rf _Retardation factor, TLF-tannin leaf fraction, TBF-tannin bark fraction.

TLC of TLF and TBF of *Psidium Guajava Linn*.

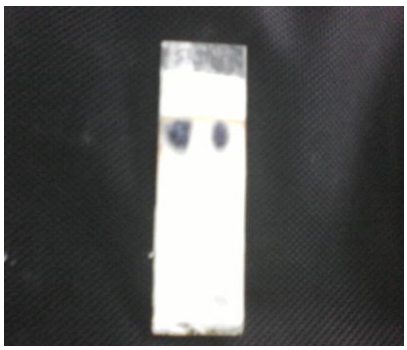

Figure 1: TLC of TLF and TBF of *Psidium Guajava Linn*.

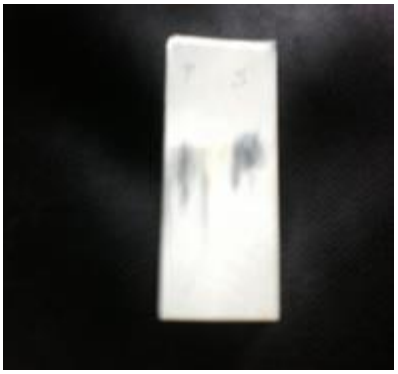

Figure 2: TLC of TLF and TBF of *Psidium Guajava Linn*.

T-Tannin leaf fraction (TLF)
T-Tannin bark fraction (TBF)
S-Standard

Anti-oxidant Activity

Table 3: Free radical scavenging activity of *Psidium guajava* bark and leaf by DPPH method

S.NO	CONCENTRATION (µg/ml)	% INHIBITION		
		STANDARD (Vitamin-C)	LTF	BTF
1	50	56.438±0.7557	17.642±0.3377**	14.356±0.7305**
2	100	65.55±0.679	20.202±0.3341**	17.446±0.7502**
3	200	70.256±0.8019	25.134±0.8562**	20.532±0.7631**
4	400	73.378±0.7377	41.134±1.516**	23.86±0.5514**
5	800	76.40±0.7823	57.216±1.545**	56.40±0.6994**
6	1000	82.36±0.7078	60.778±1.041**	61.39±0.7791**
7	IC_{50}	540 (µg/ml)	510 (µg/ml)	530 (µg/ml)

The values are expressed as Mean ± SEM, n=6 in each group. If * P<0.05, **P<0.01 and ***P<0.001 vs control.

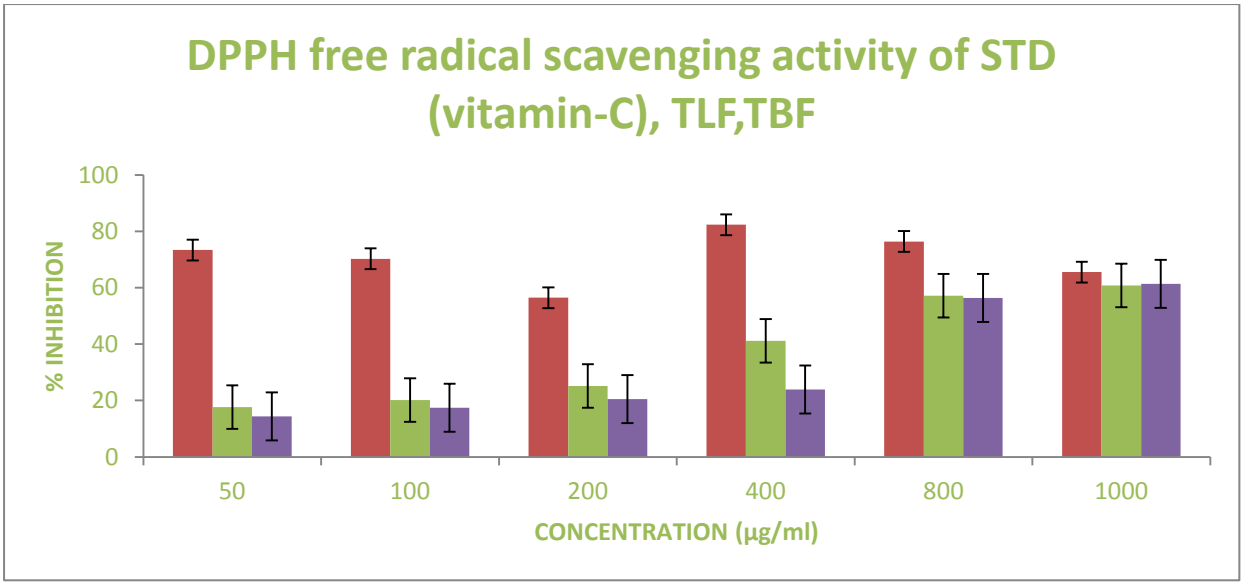

Figure 2: Dpph free radical scavenging activity of Standard (Vitamin C)

- **-vitamin-c -** standard
- **-TLF -** Tannin leaf fraction
- **-TBF -** Tannin bark fraction

Table 4: Free radical scavenging activity of *Psidium guajava* bark and leaf by Nitric oxide method

S.NO	Concentration (µg/ml)	% Inhibition		
		Standard (Vitamin-C)	TLF	TBF
1	50	8.30±0.45	49.438±0.4514**	60.108±0.0385**
2	100	16.66±0.90	51.85±0.3136**	60.332±0.0253**
3	200	27.77±1.08	56.688±0.3715**	60.646±0.1795**
4	400	52.77±0.60	59.16±0.2598**	61.232±0.0606**
5	800	61.11±0.51	60.204±0.2747**	61.71±0.0593**
6	1000	65.88±0.84	60.378±0,3989**	65.17±0.0244**
7	IC_{50}	380 (µg/ml)	590 (µg/ml)	620 (µg/ml)

The values are expressed as Mean ± SEM, n=6 in each group. If * P<0.05, **P<0.01 and ***P<0.001 vs control.

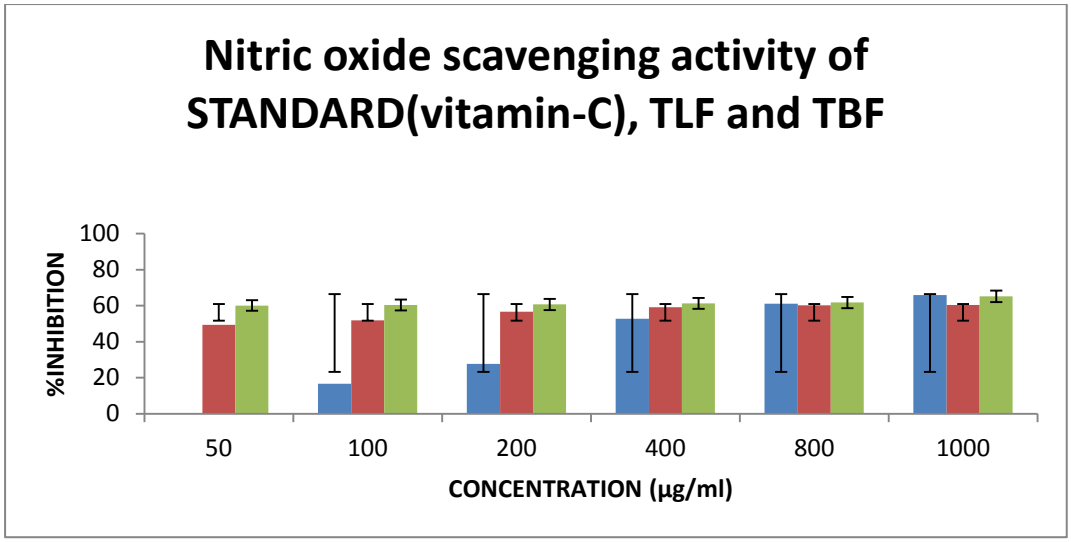

Figure 3: Nitric oxide scavenging activity of standard (vitamin c), TLF and TBF

- **-TLF -** Tannin leaf fraction
- **-TBF -** Tannin bark fraction
- **-Vitamin-C –** Standard

DISCUSSION

The Ethnobotanical studies and folklore claiming reviewed that the leaves and bark of the plant Psidium guajava L., are used for anti-oxidant ac tivity. So the present work was focussed to isolate tannin rich fraction and it was evaluated for anti-oxidant activity.

Phytochemical study

Phytochemical screening was carried out to identify the phytoconstituents present in the ethanolic extracts and its Fraction. Phytochemical screening of tannin isolation shows the presence of tannin.

TLC was done for the tannin fraction on support of the chemical test since it showed blue colour spot with 5% ferric chloride, confirmed the presence of tannin. It was identified as galllic acid by comparing its Rf value with that of standard gallic acid.

Invitroanti-oxidant study

Nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. Nitric oxide is a very unstable species under aerobic conditions. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates. It was estimated by using Griess reagent and in presence of test compound which was the scavenger. In this study the nitrite produced by the incubation of solutions of sodium nitro-prusside in standard phosphate saline buffer at 250c was reduced by the etanolic extract of psidium guajava (EEPG) and tannin fraction of Psidium guajava (TFPG) compared to ethanolic extract and tannin fraction produced a good significant free radical scavenging property which may be due to the presence of tannin (gallic acid).

DPPH assay is considered a valid method to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not have to generate as in other radical assays. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solutions loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of plant extract to act as free radical scavengers. DPPH assay of ethanolic extract and tannin fraction showed a dose dependent increase in the percentage of inhibition of free radicals. The tannin fraction was found to contain more total tannin content. It also showed a good anti-oxidant potential.

CONCLUSION

From this study, it is concluded that Psidium guajava Linn. Leaf and bark tannin fraction have significant anti-oxidant activity all in Invitro models, and then based on free radical scavenging property.

The anti-oxidant activity is probably due to the presence of tannin (Gallic acid). Further studies need to be isolate individual tannin and explore its biological potency by various pre-clinical and clinical trials of the isolated compounds.

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