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Invitro Antioxidant evaluation of *Psidium guajava* strem extracts

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Abstract

Dried powdered stem of *Psidium guajava* was successively extracted with acetone, methanol and water respectively and subjected to antioxidant activity. Methanolic and aqueous extracts was used for the evaluation of antioxidant activity. The activity was determined at ambient temperature by means of a 2,2-diphenyl1-1-picrylhydryzyl (DPPH) spectrophotometrically with detection scheme at 520 nm. The activity was evaluated by the decrease in absorbance as the result of DPPH colour change from purple to yellow. The higher the sample concentration used, the stronger was the free radical-scavenging effect. The results obtained showed that methanol extract has almost same antioxidant activity as of ascorbic acid while aqueous extract showed less antioxidant activity. This study revealed that guava stem extracts comprise effective potential source of natural antioxidants which might be helpful in preventing the progress of various oxidative stresses.

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Psidium guajava; Antioxidant; DPPH(1,1-diphenyl-2-picrylhydrazyl); Free radical scavenging effect.

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Introduction

Psidium guajava Linn, belonging to the family of Myrtaceae, has been used as health tea. Its stem contains copious amounts of phenolic phyto chemicals which inhibit peroxidation reaction in the living body, and therefore can be expected to prevent various chronic diseases such as diabetes cancer, heart-disease [1]. Furthermore, decreasing of free-radicals has antioxidizing effect in the body, meaning these guava stem polyphenols can prevent arterial sclerosis, thrombosis, cataract and inhibit senescence of the body and skin. Many people habitually take medicinal decoction of guava leaf for long treatment

of diarrhoea, and therefore, the safety of guava stem have empirically been confirmed[2].

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis [3] as well as in degenerative processes associated with aging [4] [5] Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage [6].

The antioxidative activity is conventionally used to indicate the ability of antioxidant to scavenge some radicals. Phenolic compounds are typical active oxygen scavengers in foods and have been evaluated by several methods. One among tests proposed for assessment of antioxidative activity (AOA) is DPPH" free-radical colorimetry [7], whose colour changes from purple to yellow in the presence of antioxidants. The kinetics of decolorization reactions directly relate to the types of antioxidants and to their different concentrations. The more rapidly the absorbance decreases, the more potent is the antioxidant activity of the antioxidants in terms of hydrogen donating ability [8]. The rapid reduction of DPPH" radical by antioxidants allows the evaluation of antioxidant power of different antioxidants.

Material and method

Collection and Identification of Plant materials

Psidium guajava stems (5kg) were collected from herbal garden of Lovely Professional University. The plant was authenticated at Regional Research Institute, Bangluru and a voucher specimen No.(NADRI/BNG/SMP/Drug Authentication/2010-11/644) has been retained at the Department of Pharmacognosy and Phytochemistry, Lovely Professional University, Phagwara, Punjab.

Preparation of Extracts

The stems were washed with water and air-dried at room temperature (30–40°C). Dried stems were ground and pass through a 1-mm sieve. The dried powdered stems (1 kg) was successively macerated with acetone, methanol and distilled water respectively. The extracts were filtered through Whatman filter paper and concentrated to about 10% of the original volume by a rotary evaporator at 40˚C.

Evaluation of Antioxidant activity by Free Radical- Scavenging Method

DPPH radical scavenging is considered a good in vitro model and is widely used to conveniently assess antioxidant efficacy. In its radical form, DPPH free radical has an absorbance at 520 nm which disappears when DPPH is reduced by an antioxidant compound or a radical species to become a stable diamagnetic molecule. As a result, the color changes from purple to yellow . This color change is taken as an indication of the hydrogen donating ability of the tested compounds.

The DPPH radical scavenging activity of the samples was estimated according to the methods of Brand-Williams et al., 1995 and Gamez et al., 1998 [9] with some modification. Samples in MeOH were added to a solution of DPPH radical in MeOH and the reaction mixture was left to stand for 30 min at room temperature in the dark. The scavenging activity of samples was estimated by measuring the absorption of the mixture at 520 nm, which reflects the amount of DPPH radical remaining in the solution. The scavenging activity was expressed as the IC_{50}, the concentration of samples required for scavenging 50% of DPPH radical in the solution. The

percentage of free radical scavenging effect was calculated as follows:-

Scavenging effect (%)

$$\text{Absorbance}_{\text{Control}} - \frac{\text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Control – DPPH in methanol

Sample - extract and DPPH

Results and Discussion

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity. Therefore, research for the determination of the natural antioxidants source is important.

The percentage of total antioxidant activity of methanolic extract of *Psidium guajava* presented in Table 1. The methanolic extract of *Psidium guajava* exhibited a maximum total antioxidant activity of 79.37 % at 100 µg/ml whereas for ascorbic acid (standard) was found to be 80.56 % at 100 µg/ml. The IC_{50} values of the methanolic extract of *Psidium guajava* and ascorbic acid were found to be 33.95µg/ml and 30.28µg/ml respectively.

Table 1: Total antioxidant activity of methanolic extract of *Psidium guajava* stem using DPPH scavenging method

S. No.	Concentration (µg/ml)	% of activity (± SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbic acid)
1	10	39.51±1.064	44.97±0.909
2	20	47.70±0.424	44.97±0.909
3	40	51.16±1.025	51.23±0.929
4	60	57.75±2.136	55.98±1.045
5	80	68.19±1.934	68.81±1.797
6	100	79.37±0.423	80.56±0.331
		IC_{50} =33.95 µg/ml	IC_{50} = 30.28 µg/ml

*All values are expressed as mean ± SEM for three determinations.

The percentage of total antioxidant activity of aqueous extract of *Psidium guajava* presented in Table 2. The aqueous extract of *Psidium guajava* exhibited a maximum total antioxidant activity of 73.56 % at 100 µg/ml whereas for ascorbic acid (standard) was found to be 80.56 % at 100 µg/ml. The IC50 values of the aqueous extract of *Psidium guajava* and ascorbic acid were found to be 40.16µg/ml and 30.28µg/ml respectively.

Table 2: Total antioxidant activity of Aqueous extract of *Psidium guajava* stem by DPPH scavenging method

S. No.	Concentration (µg/ml)	% of activity (± SEM)*	
		Sample (Aqueous extract)	Standard (Ascorbic acid)
1	10	36.53±0.887	44.97±0.909
2	20	44.97±0.577	44.97±0.909
3	40	49.87±1.239	51.23±0.929
4	60	55.42±1.622	55.98±1.045
5	80	66.18±1.230	68.81±1.797
6	100	73.56±0.734	80.56±0.331
		IC_{50} =40.16 µg/ml	IC_{50} = 30.28 µg/ml

*All values are expressed as mean ± SEM for three determinations

Based on the result showed the methanolic extract of *Psidium guajava* stem was found to more effective than aqueous extract. But when compare all the extracts with standard the methanolic extract showed almost same antioxidant activity as of ascorbic acid (Standard) whereas aqueous extract showed less antioxidant activity as compared to ascorbic acid. Both extracts from guava stem showed good free radical-scavenging activity depending on the concentration used. The higher the concentration used the higher the free radical-scavenging effect.

Conclusion

The results of the present investigation indicated that the methanolic extract of *Psidium guajava* can

be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. However, the methanolic extract of *Psidium guajava* was found high content of flavonoids. So it can be concluded that these components might be involved in the antioxidant activity of *Psidium guajava*. Therefore, it is suggested that further work should be performed on the isolation and identification of the antioxidant components in *Pidium guajava*.

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