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Invitro antioxidant testing of the extracts of *Samanea saman* (Jacq.)Merr

P.Arulpriya¹, P.Lalitha² and S.Hemalatha³

Department of Chemistry Avinashilingam Deemed University for Women, Coimbatore

ABSTRACT

The petroleum ether, ethyl acetate, chloroform, aqueous and HCl extracts of Samanea saman (Jacq.)Merr were examined for their phytochemical constituents and for their invitro activities using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and reducing power assay. Higher antioxidant potential of the extracts was observed in both DPPH scavenging assay and reducing power assay.

Key words: Samanea saman(Jacq.)Merr, Antioxidant properties, DPPH assay, Reducing power assay

INTRODUCTION

Samanea saman (rain tree) is a tropically found common plant which mitigates multitude of diseases and ailments [1]. There are several folk remedies prepared from various parts of rain tree. The root decoction is used in hot baths for stomach cancer [2]. Rain tree is a folk remedy for colds, diarrhoea, headache, intestinal ailments, and stomach ache [3].

Recent evidence [4] suggest that free radicals, which are generated in any bioorganic redox processes, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids) and may also be involved in processes leading to the formation of mutations.

1 Research scholar

2 Asst. Professor (SS)

3 Research scholar

Furthermore, radical reactions play a significant role in the development of life limiting chronic diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts and others [5].Antioxidants may be classified according to their mode of action as being free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen closed system [6]. Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Several epidemiological, clinical and experimental data suggest that plant based antioxidants have beneficial effects on prevention on chronic diseases [7,8] As a result, there has been a keen interest in evaluating the role bioactive constituents from medicinal plants in reducing the risk of the aforesaid diseases. Recent studies have demonstrated the antioxidant activities and health benefits of the phenolic compounds occurring in plants [9,10].

In view of the aforesaid, in the present study the antioxidant activity of petroleum ether, ethyl acetate, chloroform, water and acid extracts of *Samanea saman* were assessed using DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical quenching and reducing capacity assay.

MATERIALS AND METHODS

Collection of plant

Combination of dried fallen leaves, flowers and stems of *Samanea saman* (Jacq.) Merr. were collected from Coimbatore.

Preparation of extracts

Solvent extracts (Petroleum ether, ethyl acetate, chloroform, Aqueous and HCl) were prepared by refluxing 940g of the fallen parts of *Samanea saman* with 1.5 litre of the appropriate solvent.

Evaluation of Antioxidant activity by *in vitro* techniques DPPH assay: (1, 1-diphenyl-2-picrylhydrazyl)

Principle

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

 $(DPPH) + (H-A) \longrightarrow DPPH-H + (A)$ (Purple) (Yellow)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [11].

Chemicals required:

0.1 Mm solution of DPPH in methanol was prepared and used in the study. Ascorbic acid (1%)

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Procedure

DPPH in methanol (0.1 Mm) was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to18 μ g/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

S.no	Ascorbic acid 1ml-3µg	Extract Concentration (1ml-3mg)
1	1ml	PE
2	2ml	EA
3	3ml	СН
4	4ml	Aq
5	6ml	HCl

Fable 2:	Volume of	[°] ascorbic ac	id and	extracts	taken for	DPPH	assav
abic 2.	volume of	ascorbic ac	iu anu	CALLACIS	taken 101		assay

DPPH Scavenged (%) =
$$\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} X 100$$

where $A_{control}$ is the absorbance of the control reaction and a test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the *Samanea saman* extract is expressed comparing with standard. The **Table 2** shows that the volume of ascorbic acid and extract taken for the present study.

Reducing power assay Principle

The reducing power of petroleum ether, ethyl acetate, chloroform, aqueous and HCl extract of *Samanea saman* (Jacq.)Merr. was determined by the method of Oyaizu, [12]. Substances, which have reduction potential, react with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanide (Fe²⁺), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Antioxidant

Potassium ferricyanide + Ferric chloride ------ Potassium ferrocyanide + ferrous chloride

Chemicals required

Potassium ferricyanide (1% w/v), phosphate buffer (0.2 M, pH 6.6), trichloro acetic acid (10%), ferric chloride (0.1%) and ascorbic acid (1%).

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Phosphate buffer preparation

Dibasic sodium phosphate (18.75 ml of 0.2M) is mixed with 31.25 ml monobasic sodium phosphate and diluted to 100 ml with water.

Procedure

Various concentrations of the plant extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50° C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. **Table 1** shows that the volume of ascorbic acid and extract taken for the presence study.

S.no	Ascorbic acid 1ml-300µg	Extract Concentration 1ml-3mg
1	2m1	PE
2	3ml	EA
3	4ml	СН
4	5ml	Aq
5	6ml	HCl

Table1: Volume of ascorbic acid and extracts taken for reducing power assay



where A_{test} is absorbance of test solution; A_{blank} is absorbance of blank. The antioxidant activity of the *Samanea saman* extract was compared with the standard ascorbic acid.

RESULTS AND DISCUSSION

DPPH assay

The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extracts. A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience [13].

Concentration of ascorbic acid 1ml=3µg	% of increase DPPH Scavenging of ascorbic acid	% of increase DPPH scavenging of extract
1ml	63.51	68.91
2ml	64.86	67.56
3ml	66.21	63.51
4ml	67.56	25.67
5aml	68.9	13.51

 Table 3: DPPH scavenging activity of ascorbic acid and Samanea saman extracts

The results of the assay are expressed in percentage (%) of inhibition of DPPH free (**Table 3**). The DPPH assay of reference compound ascorbic acid given in **figure 1**. The analysis of (**Table 3**) that the radical scavenging activity of the extracts of *Samanea saman* increases with increasing in concentration and follows the given orders PE > EA > CH > Aq > HCI.

Around 10-15mg of the extracts gave 68% DPPH scavenging activity. From the present results it may be postulated that the extracts of *Samanea saman* reduces the DPPH radical to corresponding hydrazine when its reacts with hydrogen donors in antioxidant principles.



Figure 1: DPPH scavenging activity of ascorbic acid

Reducing Power Assay

In this assay the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals (ie antioxidant) causes the conversion of the Fe $^{3+}$ / ferricyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue at 700nm, the Fe $^{2+}$ concentration

can be monitored; a higher absorbance at 700nm indicates a higher reducing power. The results of the reducing power assay are given in **Table 4**.

The reducing power assay of reference compound ascorbic acid given in **figure 2** the relative reducing power of the different solvent extract of *Samanea saman* from the results are PE > EA > CH > Aq > HCl.

Concentration of ascorbic acid 1ml=300µg	Absorbance Of ascorbic acid (700nm)	Absorbance (700nm) of extracts
2ml	0.22	0.23
3ml	0.32	0.25
4ml	0.45	0.26
5ml	0.55	0.38
6ml	0.65	0.40
7ml	0.75	

 Table 4: Reducing power capacity of ascorbic acid and Samanea saman extracts

Figure 2: Reducing power assay capacity of ascorbic acid



The higher absorbance of PE extracts may be due to its strong reducing power potential. The reducing power of the extracts may be due to the biologically active compounds in the extract which possess potent donating abilities. This assay further confirmed the antioxidant properties of the extracts.

CONCLUSION

In conclusion this work describes for the first time *invitro* antioxidant activity of the extracts of the fallen plant parts of *Samanea saman*. Most of the extracts showed excellent free radical scavenging activity. Literature reports are evident that the reducing power of bioactive compounds is associated with antioxidant activity. Thus a relation is evidenced between reducing power and the antioxidant effect.

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