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***Full Length Article***

# Antioxidant activity, Phenol and Flavonoid contents of *Acacia sinuata* (Lourr) Merr.

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**ABSTRACT**

The present investigation was carried out to evaluate the in-vitro antioxidant activity of methanol and aqueous extracts of leaves of *Acacia sinuata* using 1,1, diphenyl,2, picrylhydrazyl (DPPH) and Ferric reducing power assays. The methanol and aqueous extracts exhibited varying degrees of antioxidant activity ranged between 12.87 - 76.12. The methanolic extract showed significant activity than aqueous extract. The total phenol varied from 25432 – 1432.45 mg/100g of crude extracts in the extracts. Flavonoid contents were between 143.19 – 423.12 mg/100g of crude extracts. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *A.sinuata* extract.

**Key word:** dpph assay, Flavonoids, Phenols, Methanol, Ferric reducing power assay

## INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world (Geeta Singh and Padma Kumar, 2011). In developing country and particularly in India low income people such as former, people of small isolate villages and native communities use folk medicine for the treatment of common infections. Antioxidants are compound that can delay or exhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reaction (Doss *et al*., 2011). Hence, considerable attention has already been focused on the isolation, characterization and utilization of natural antioxidants as potential disease preventing agents. To screen antioxidants from natural products an *in-vitro* assay was performed using the free radical scavenging activity of the 1, 1, diphenyl-2-picrylhydrazyl radical (DPPH) and Ferric reducing power assay.

*Acacia sinuata* is a perennial, woody, large climbing shrub grows on other big trees. Leaves are bipinnate, leaflets small, sessile, flowers small heads, fruits thin pods with 6-10 seeds per pod. In this report, we described the phenol and flavonoid contents of aqueous extracts from *A.sinuata* leaves and their antioxidant activities determined using various assay systems.

## MATERIALS AND METHODS

Fresh plant parts (*Acacia sinuata*) were collected randomly from the villages of Tiruchirappalli District, Tamil Nadu from the natural stands. The botanical identity of these plants was confirmed by Dr.V.Sampath Kumar, Scientist – C, Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu. A voucher specimen has been deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamil Nadu, India.

**Preparation of extracts**

**Aqueous extraction**

100 grams of dried powder were extracted in distilled water for 24 h at Room Temperature. Every 2 h it was filtered through whatman No.1 filter paper and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume.

**Solvent extraction**

100 grams of dried plant powdered samples were extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at $4^{o}$C in airtight bottles for further studies.

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma chemical Co. (St., Louis, USA). Gallic acid, Ascorbic acid, Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany).

**DPPH Radical Scavenging Activity**

DPPH scavenging activity was carried out by the method of Blois, (1957). Different concentrations (1000, 500, 250, 125, 62.5 & 31.25 mg/ml) of *A.sinuata* crude extracts were taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2- Picrayl hydrazyl) was added to each of the test tubes and were shaken vigorously, to stand at $37^{0}$ C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

Percentage of Antioxidant Activity = Control OD – Sample OD / Control OD

**Reducing power**

Reducing activity was carried out by using the method of Oyaizu (1986). Different concentrations (1000, 500, 250, 125, 62.5 & 31.25 mg/ml) of *A.sinuata* crude extracts were taken in test tubes in triplicates. To the test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at $50^{0}$ C for 20 minutes. After incubation 2.5ml of 10% TCA was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chloride was added and was incubated at $35^{0}$ C for 20 minutes. The O.D (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample.

**Total phenolic content**

Total phenolic contents were determined by Folin Ciocalteu reagent (McDonald *et al.,* 2001). A dilute extract of each crude extracts (0.5 ml of 1:10g ml $^{-l}$) or garlic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g-l of dry mass), which is a common reference compound.

**Determination of Total flavonoids**

Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al*., 2002). Each crude fruit extracts (0.5ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solution at concentrations 12.5 to 100g ml $^{-1}$ in methanol.

**RESULTS AND DISCUSSION**

Natural antioxidants that are present in medicinal plants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress (Doss and Anand, 2013). In the present paper, we have evaluated the free radical scavenger and reducing power assays of methanol and aqueous extracts of *A. sinuata*.

**Table 1. Total phenol and Flavonoids contents in the crude extracts of *Acacia sinuata* (mg/100g of crude extracts)***

| Medicinal plants | Total phenols content | |
|---|---|---|
| | **Methanol** | **Aqueous** |
| *Acacia sinnata* | 1432.45 ± 2.54 | 254.32 ± 1.12 |
| | **Flavonoid content** | |
| | **Methanol** | **Aqueous** |
| *Acacia sinnata* | 423.12± 3.12 | 143.19 ± 0.21 |

*Each value in the table was obtained by calculating the average of three analyses ± standard deviation.

**Table 2. Antioxidant activity of *Acacia sinuata* (DPPH assay)**

| Concentrations (mg/ml) | Extracts (%) | | Standard (Ascorbic acid) |
|---|---|---|---|
| | **Methanol** | **Aqueous** | |
| 1000 | 76.12 ± 0.17 | 70.34 ± 3.09 | 69.43 ± 0.03 |
| 500 | 60.43 ± 0.02 | 59.76 ± 2.41 | |
| 250 | 49.32 ± 0.25 | 46.71 ± 0.08 | |
| 125 | 38.53 ± 1.20 | 31.38 ± 0.35 | |
| 62.5 | 21.76 ± 1.01 | 20.56 ± 1.01 | |
| 31.25 | 12.87 ± 0.03 | 10.12 ± 1.54 | |

Each Value is SEM ± 5 individual observations

**Table 3. Ferric Reducing capacity of *Acacia sinuata***

| Concentrations (mg/ml) | Extracts | | Standard (Ascorbic acid) |
|---|---|---|---|
| | **Methanol** | **Aqueous** | |
| 1000 | 0.823 ±2.12 | 0.716 ± 5.12 | 0.689 ± 0.05 |
| 500 | 0.612 ± 0.61 | 0.587 ± 1.04 | |
| 250 | 0.540 ± 1.01 | 0.462 ± 2.76 | |
| 125 | 0.356 ± 0.45 | 0.348 ± 1.03 | |
| 62.5 | 0.231 ± 1.02 | 0.202 ± 0.54 | |
| 31.25 | 0.117 ± 0.08 | 0.104b ± 0.03 | |

Each Value is SEM ± 5 individual observations

The total phenolic compound and flavonoids contents in the plant extracts are shown in Table 1. It appeared the methanol extract of *A.sinuata* had the highest content of flavonoid and phenol. The aqueous extract had the lowest content while compared to methanol extract. Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities.

The extracts, methanol as well as aqueous, exhibited an antioxidant activity in a dose-dependent manner. The methanolic extract of *A.sinuata* leaf at different doses exhibited significantly higher antioxidant activity as compared to aqueous extract. The extracts of all the tested extracts possessed free radical scavenging properties, but to varying degrees, ranging from 12.87 to 76.12% DPPH scavenging. Using the alcoholic extraction, generally methanol extract showed better DPPH scavenging activity. A maximum scavenging activity was offered by methanol extract (76.12 %), followed by Aqueous extract (70.34 %) (Table 2). The α, α,diphenyl-β-picrylhydrazyl (DPPH) a stable nitrogen centered free radical, has been used to evaluate the antioxidant activity of natural products by measuring the radical quenching capacity in a relatively short period of time.

Antioxidant activity as measured by Ferric reducing power assay showed a wide range of variation among the plants studied as well as among the extracts used (Table 3).

Methanol extract showed the highest Ferric reducing activity while aqueous extract showed the lowest Ferric reducing value (Table 3). Reducing power assay is often to evaluate the ability of natural antioxidant to donate electron or hydrogen (Dorman *et al*., 2003). Samples with high reducing power were reported to have a better ability to electrons. It has been widely accepted that the higher level of absorbance at 700 nm indicates greater reducing power of the test samples (Duh *et al*, 1977).

Natural antioxidant strengthens the endogenous antioxidant defenses from reactive oxygen species and restores an optimal balance by neutralizing the reactive species. They are gaining immense importance, by virtue of their critical role in disease prevention. In the present study, it is concluded that *Acacia sinuata* extract has free radical scavenging activity and improved antioxidant effect.

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