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ANTIOXIDANT ACTIVITY OF *Sophora interrupta* Bedd.

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ABSTRACT

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Because of potential health benefits of natural antioxidants they are expected to be an alternative to synthetic ones. The studies also showed that some medicinal plants possessed more potent antioxidant activity than common dietary plants. In this present study the methanolic extract of whole plant *Sophora Interrupta* were investigated by using DPPH scavenging test and reducing power method. The preliminary phytochemical screening was performed and the total phenolic content had been estimated. preliminary phytochemical Analysis of methanolic extract of *Sophora interrupta* plant revealed the presence of Poly phenols, alkaloids, glycosides, carbohydrates and proteins. The total phenolic content was found to be 172 mg quercetin per g weight of extract. The MESI exhibited a significant dose dependent inhibition of DPPH activity. The IC50 values of the MESI and reference standard ascorbic acid were found to be 30 µg/mL and 37 µg/mL respectively and the similar activity was shown in the other method also.

Keywords: *Sophora interrupta,* Antioxidant, DPPH radical scavenging, Total Phenolic content.

INTRODUCTION

There are valuable evidences that oxidative stress imposed by reactive oxygen species plays an important role in many diseases, such as cancer, diabetes mellitus, neurodegenerative diseases and ageing (Azizova *et al*., 2002). Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies *et al.*, 1997).

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The antioxidant defence system of the body is made up of some antioxidants, such as vitamin C, vitamin E, vitamin K and glutathione (Chae *et al*., 2004). On the other hand, it has been reported that natural antioxidants in fruits and vegetables were proved to treat effectively all the degenerative diseases (Duthie *et al*., 2000). Because of potential health benefits of natural antioxidants (Eberhardt *et al*., 2000), they are expected to be an alternative to synthetic ones. So, there is an increasing interest in investigation for new resources of natural antioxidants. Phytochemicals, phenolic compounds are dietary constituents widely existing in plants and have been considered to have high antioxidant capacity and free radical scavenging capacity (Kahkonen *et al*., 2001). In recent years, researches on antioxidant activities of medicinal plants have been considerable raised by increased interest in their potential high antioxidant capacity and positive health benefits (Katalinic et al., 2006). The studies also showed that some medicinal plants possessed more

potent antioxidant activity than common dietary plants (Cai et al., 2004).

Sophora interrupta Bedd belongs to the family, Fabaceae (Leguminaceae, Papilonaceae) is commonly called as *Edwardsia maderaspatana* Wight, Pili Girgoli. There are approximately 219 species in this genus *Sophora*. They were investigated to pocess abortifacient, antibacterial, anticholesterolemic, antiinflammatory, antispasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic, and tonic properties (Poretz *eta al.* 1976).

Sophora Interrupta is one of such plants which is traditionally used in treatment of cancer. Assuming the anticancer compounds pocess the antioxidant activity and its family members investigated for presence of antioxidants (Ren-You Gan *et al.*, 2010), the plant has been investigated for the same.

MATERIALS AND METHODS

Plant materials

The fresh plant *Sophora interrupta* Bedd had been collected from the hills of Tirumala, Andhra Pradesh, India. The collected material was duly authentified by Dr. Jayaraman, PARC, Chennai and The voucher specimen (PARC/2011/688) of the plant was deposited at the college, for further reference. The plant material was dried under shade and ground properly. The powdered plant was extracted with methanol using a soxhlet apparatus (MESI).

Chemicals

All the chemicals, reagents used in the research have been procured from S.D.Fine Chem. Ltd. Mumbai. Quercetin had been procured from Sigma Aldrich, USA.

Phytochemical Screening

On preliminary screening of *Sophora interrupta* the methanol extract has been tested For the Chemical Constituents Present.

Total Phenolic Content

Following the literature and the results of priliminary phyto chemical screening for plant to contain poly phenols, it was tested to estimate the total phenolic content. It was measured colorimetrically using quercetin and FC (Folin Ciocault's) reagent (Mariela González *et al.*, 2003).

Standard curve of Quercetin

1mg of quercetin was weighed and dissolved in 100ml of distilled water and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 µg/ml. A volume from above aliquots was taken and mixed with 1.25ml of FC reagent. It was left for 5 mins. Then 2.5ml of sodium carbonate was added and it was let to react for 30 min then the volume was made upto 10ml. Then the absorbance was measured at 765 nm. The calibration curve was drawn plotting the absobance and concentrations.

Sample preparation

0.5g of Methanolic extract was weighed and dissolved in 100ml of water. From this 0.1ml was taken into 10ml standard flask and 1.25ml of FC reagent was added and let to react for 5 min. then 2.5ml of sodium carbonate was added and the colume was made upto 10ml. it was kept for 30 min for complete reaction. Now the absorbance was measured at 765 nm. Total phenolic content was calculated from the calibraition curve of quercetin and the value was expressed in quercetin equivalents.

IN-VITRO MODELS FOR THE EVALUATION OF ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Test

The free radical scavenging activity of the methanol extracts of *Sophora interrupta* (MESI) was determined by using 2, 2 Diphenyl-l-picryl hydrazyl radical (DPPH) using UV-Spectrometry (Mathiesen *et al.*, 1995) at 517 nm. The DPPH solution was prepared in 95% methanol. The MESI was mixed with 95% methanol to prepare the stock solution (10mg/100ml or 100µg/ml). From the stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes and by serial solution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of there test tubes. Containing MESI (20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) and after 10 min, the absorbance was taken at 517nm, using a spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as a reference standard. It is dissolved in distilled water to make stock solution with the same concentration of MESI control sample was prepared without extract and reference ascorbic acid. 95% methanol was used as blank % scavenging of the DPPH free radical was measured using following equation.

%DPPH radical-

$$\text{Scavenging} = \frac{\text{Absorbance control} - \text{Absorbance of test sample}}{\text{(Asorbance of control)}} \times 100$$

Reducing Power Method

The assay of reducing power method (Makari *et al.*, 2008) is one to determine the antioxidant activity. In this 1 ml of plant extract of MESI solution mixed with 2.5 ml

phosphate buffer (0.2M, pH 6.6) and 2.5 ml Potassium Ferricyanide [K_3Fe (CN6)] (10g/l), the mixture was incubated at 50^0 C for 20 minutes. 2.5ml of Trichloroacetic acid (100g/l) was added to mixture. This was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the supernantant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (lg/L) and absorbance measured at 700nm in UV-visible spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotomerer). Ascorbic acid was used as standard and phosphate buffer used as blank.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis of Methanolic extract of *Sophora interrupta* plant revealed the presence of Poly phenols, alkaloids, glycosides, carbohydrates and proteins.

Total phenolic content

The standard graphs of quercetin yielded curve with regression coefficient, r^2=0.998. The total phenolic content in the methanolic extract of *Sophora interrupta* was estimated by Quercetin equivalents. The total phenolic content was found to be 172 mg quercetin per g weight of extract. The phenols present in the extract might counter act the oxidative free radicals and the respective activity was estimated.

In this present study the methanolic extract of whole plant *Sophora interrupta* were investigated by using DPPH scavenging test and reducing power method. The whole plant MESI showed by there two methods effectively when compared with reference standard ascorbic acid. In the DPPH scavenging method is based on the capability of DPPH radical to decolorize in the presence of antioxidants. The DPPH radical is considered to be model of a stable lipophilic radical a chain reaction in lipophilic radicals was initiated by the lipid autooxidation antioxidants react with DPPH reducing a number of DPPH molecules equal to number of their hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH. In figure-2. The MESI exhibited a significant dose dependent inhibition of DPPH activity. The IC50 values of the MESI and reference standard ascorbic acid were found to be 30 µg/mL and 37 µg/mL respectively. The reducing power method based on the capability of a reducing the compound due to presence of reductants which are breaking the free radical chain by donating hydrogen atom. The whole plant of MESI exhibited the antioxidant activity due to presence of reductants (i.e., antioxidants). The reduction of Fe3+/Ferricyanide complex to ferrous form. In this main principle is increasing the absorbance of the reaction mixture indicates the antioxidant activity that leads to reducing power of the samples. In Figure-3 MESI was very potent and the power of extract was increased with quantity of sample. By comparing the reference standard ascorbic acid the MESI showed potent antioxidant activity.

Antioxidant activity

Table no. 1. Antioxidant activity by DPPH method

S. No	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of MESI	% scavenging DPPH of Ascorbic acid	% scavenging DPPH of MESI
1	20µg/ml	0.142	0.128	35.15	41.55
2	40 µg/ml	0.102	0.089	53.42	59.36
3	60 µg/ml	0.086	0.067	60.73	69.40
4	80 µg/ml	0.058	0.039	76.51	82.19
5	100 µg/ml	0.032	0.011	85.38	94.97

Table no. 2. Antioxidant activity by reducing power method

S. No	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of MESI
1	0.1	0.16	0.11
2	0.2	0.22	0.20
3	0.3	0.31	0.28
4	0.4	0.39	0.35
5	0.5	0.48	0.41

Fig: 1. Calibration curve of Quercetin

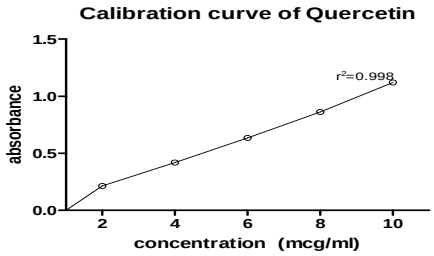


Fig: 2. Graphical representation of the Antioxidant activity by DPPH method

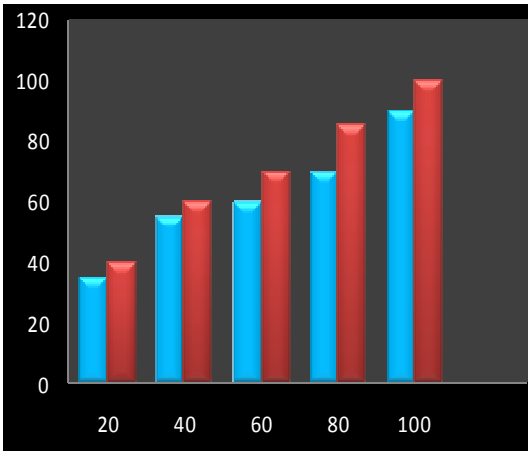
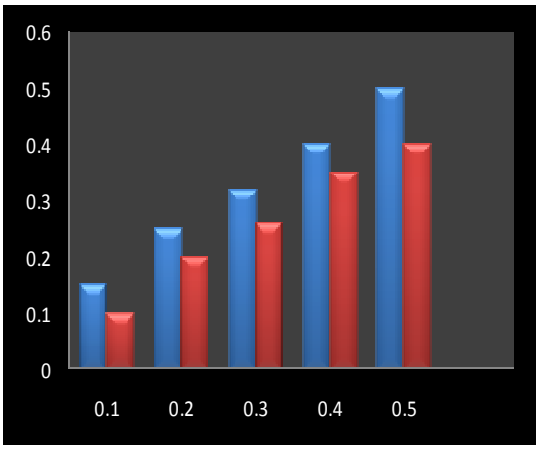


Fig: 3. Graphical representation of the Antioxidant activity by reducing power method



CONCLUSION

Form the past plants had served us with food, shelter and medicine. Investigating the natural sources for the medicines and treatment of diseases has become and advent ion the field of pharmacy these days. Concerning the side effects of synthetic drugs, natural drugs had been given utmost importance as antioxidants. This research might hopefully bring further investigations on plant to treat oxidative degeneration based diseases like cancer, neurodegenerative diseases, cardiovascular diseases etc.

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