IN VITRO ANTIOXIDANT ACTIVITY AND QUANTITATIVE ESTIMATION OF PHENOLIC CONTENT OF ANTIDIABETIC PLANTS

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Summary

Present paper deals with in vitro antioxidant activity as well as quantitative estimation of phenolic content of two very important plants i.e. *Salacia chinesis* (roots) and *Coccinia indica* (leaves). The antioxidant activities of different concentrations of ethanol and aqueous extracts of the selected plant part were determined by the three assay techniques i.e. DPPH radical scavenging assay, Reducing power ability, and ferric thiocyanate method. Both plant's extract have shown promising antioxidant activity with 89.23 and 71.98 % of phenolic content respectively.

Key Words: *Salacia chinesis, Coccinia indica,* Antioxidant activity, DDPH, reducing power.

Introduction

Reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer[1]. In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease. Therefore, research for the determination of the natural antioxidants source is important. Hence in this study very popular hypoglycemic plants were selected to find out its antioxidant potential and thus constituents responsible for antidiabetic activity. *Salacia chinesis* (Celastraceae) *and Coccinia indica* (Cucurbitaceae), are both very popular hypoglycemic, antidiabetic agents[2, 3, 4, 5] As there is no data available on its free radical scavenging effects so present studies has undertaken to carry out its antioxidant activity.

Materials and Methods

Collection of Plant Material

Salacia chinesis (roots) and *Coccinia indica* (leaves) both plants were collected in the months of July-August 2008 from the local market of Amaravati, Maharashtra state, India, and authenticated by the authority of the botany department, VMV, Amaravati. A voucher specimen was submitted at Institute's herbarium for future reference.

Extraction of Plant Material

Dried material of both plants was ground to coarse powder. Each powder was first defatted with pet. Ether and then extracted with 70 % ethanol. Extract of *Salacia chinesis* (SC) and *Coccinia indica* (CI) were vacuum dried.

DPPH radical scavenging assay[6]

The free radical scavenging activity of the fractions was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay method. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a Shimandzu spectrophotometer. The percentage scavenging activity at different concentrations was determined and was compared with that of ascorbic acid (vit. C), which was used as the standard.

Reducing power ability[6]

Different concentrations of each extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ^{o}C for 20 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power.

Ferric Thiocyanate method (FTC assay) [6]

The peroxy radical scavenging activity was determined by thiocyanate method using vit. C as standard. Increasing concentration of the extracts in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04M, pH 7) in a test tube and incubated in darkness at 37^{o}C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentage scavenging activity was calculated and compared with the standard, Vit. C.

Estimation of total phenolic content[6]

The assay used for the determination of total phenolics content employs Folin and Ciocalteu's phenol reagent which response depending on the chemical structure of phenolics. Total soluble phenolic compounds in the ethanolic extracts were expressed as gallic acid equivalents.

A sample of the ethanolic extract was added to distilled water for a final volume of 2 ml. After, it was mixed with 0.3 ml of a saturated sodium carbonate (Na_2CO_3) solution and 0.1 ml of 1 N Folin–Ciocalteu's phenol reagent. The mixture was placed for 1 h at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of gallic acid equivalents.

Results and Discussion

Total phenolic content of *Salacia chinesis and Coccinia indica* are 89.23 and 71.98 % respectively.

In case of DPPH method, the antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1, 1-diphenyl-2-picryl hydrazine with decoloration. The scavenging effects of extract increased with their concentrations to similar extents. The percentage inhibitions of concentration 20, 40, 60 mg/ml of CI are about 74.03, 82.20 and 96.19 % respectively while that of SC are 84.82, 91.22 and 97.10 % which are shown in figure 1.

The reducing power of the both extracts CI and SC found to be concentration dependent. At 20, 40 and 60 mg/ml, reducing powers of both extracts were 82.02 and 93.80 respectively (Table 1).

Results obtained from FTC assay revealed that extracts of CI and SC carry the antioxidative potential for chain-breaking inhibition of lipid peroxidation and for free radical scavenging as extract has shown 65.01, 72.12 and 86.02% inhibition (Figure 2)

Figure 1: Results of DPPH radical scavenging assay

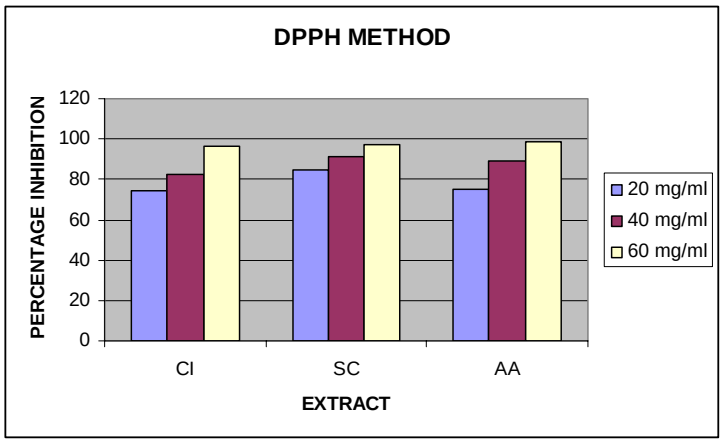
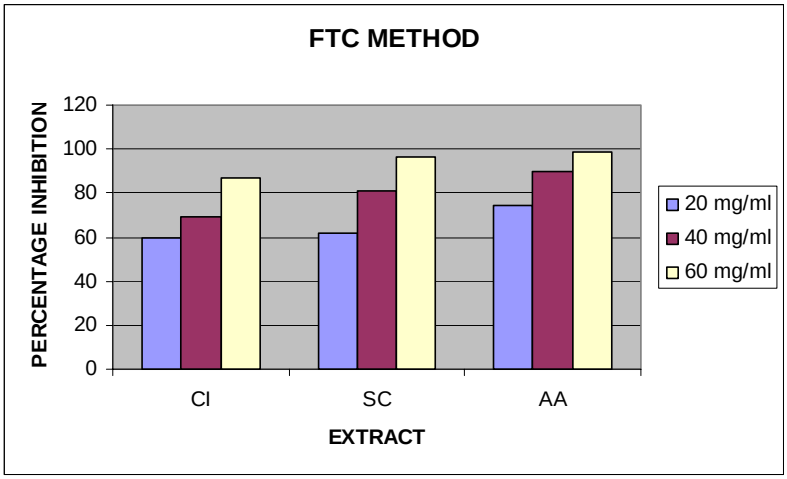


Table 1. Results of reducing power ability assay

DRUG	CONCENTRATION	ABSORBANCE	PERCENTAGE INHIBITION
CI	20	0.08237	64.43
	40	0.11338	71.5
	60	0.20078	82.02
SC	20	0.23508	74.81
	40	0.28879	88.04
	60	0.33213	93.80
AA	20	0.27019	84.9
	40	0.35986	92.71
	60	0.55249	98.13

Figure 2: Results of FTC radical scavenging assay



Conclusions

Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and plays critical role in disease prevention. Oxidative stress has been shown to have a role in the elevation of diabetes and related problems. In diabetes, protein glycation and glucose auto oxidation may generate free radical in the body, which in turn catalyzes lipid peroxidation[7]. Flavonoides and Phenolic constituents are known to be responsible for scavenging of such free radicals and thus in control of diabetes[8.] In this study, *Salacia chinesis and Coccinia indica* which are found to be effective antioxidants and hence this role might be responsible for its already reported antidiabetic activity.

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