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Antioxidant activity and Estimation of Total Phenols and Flavonoids in Extracts of *Smilax ovalifolia* leaves

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ABSTRACT

Antioxidants are those substances that protect the body cells and tissues from the damages caused by free radical and reactive oxygen species which are normally generated during oxygen metabolism. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress. The aim of the present study is to determine the antioxidant activity and to estimate the total phenol and flavonoid content of Smilax ovalifolia leave extracts. Methanolic and ethanolic leave extract of Smilax ovalifolia were evaluated for the antioxidant property by using scavenging activity of DPPH radical method. Phenol content was determined by Folin-Ciocalteu reagent method with slight modifications and the flavonoid content was determined by spectrophotometric method. In DPPH radical scavenging assay the IC_{50} value of methanolic extract and ethanolic extract were found to be 26.12 and 17.43µg/ml respectively. The same for the standard i.e. ascorbic acid was 02.98 µg/ml. The total phenol and flavanoid content in the methanolic extract was found to be 8.34±0.022 mg of GAE/g of extract and 5.16±0.033 mg of quercetin /g of extract respectively and in the ethanolic extract it was found to be 10.87±0.043 mg of GAE/g of extract and 7.65±0.011 mg of quercetin/g of extract respectively This study thus indicates that Smilax ovalifolia is a potential source of natural antioxidant

Key words: *Antioxidant, DPPH, Total Phenol, Total Flavonoid, Smilax ovalifolia.*

INTRODUCTION

Antioxidants are those substances that protect the body cells and tissues from the damages caused by free radical and reactive oxygen species which are normally generated during oxygen metabolism[1]. Free radicals contribute to more than one hundred disorders in humans[2,3]. Antioxidants stabilize free radicals or deactivate them, often before they attack targets in biological cells[4]. In recent years there has been an increased interest in the application of antioxidants to medical treatment[5]. Antioxidants components include carotenoids, vitamins, flavonoids and phenols that are micro-constituents capable to prevent the destructive process caused by oxidative stress[6]. Natural food usually contains natural antioxidants that can scavenge free radicals. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress[7]. *Smilax ovalifolia* is a multipotential medicinal plant used by different tribes for treating various diseases. The present study was carried out to determine the antioxidant activity and to estimate the total phenol and total flavonoid in the leaf extract of *Smilax ovalifolia* .

MATERIAL AND METHODS

DPPH Radical Scavenging Activity

Antioxidant activity was carried out by following standard method[8]. 3 ml of freshly prepared DPPH solution (0.1mM in methanol) was added to 3 ml of methanol and incubated for 30 mins in dark at room temperature. After 30 min, the absorbance was recorded at 517 nm using UV/Vis spectrophotometer against methanol as blank. Ascorbic acid was taken as standerd and 100 µg/ml of stock solution was prepared. From the stock solution, lower concentrations (2, 4, 6, 8 or 10 µg/ml) were prepared in methanol. 2ml of different concentrations of standard were added to 2ml of methanolic solution of DPPH. The mixture was incubated for 30 mins in dark at room temperature. After 30 min, the absorbance was recorded at 517 nm using UV/Vis spectrophotometer against methanol as blank. 500 µg/ml stock solution of the methanolic and ethanolic extract were prepared in methanol. Lower concentrations (20, 40, 60, 80 or 100 µg/ml) were prepared by diluting with methanol. 3ml of different concentrations of methanol extract and ethanol extract were added to 3ml of 0.1 mM methanolic solution of DPPH, and kept for 30 min at room temperature in dark. After 30 min, the absorbance was recorded at 517 nm using UV/Vis spectrophotometer against methanol as blank. Percentage radical scavenging activity was calculated by the following formula:

% Radical Scavenging Power = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

Where,

$A_{control}$ = Absorbance of control (DPPH);

A_{sample} = Absorbance of sample/standard + DPPH.

The measurements were taken thrice, and scavenging effect was calculated based on the percentage of DPPH scavenged. The inhibition curve was plotted and IC_{50} values were calculated.

Estimation of Total Phenol

Phenol content was determined by Folin-Ciocalteu reagent method with slight modifications[9-12]. Quantification was done on the basis of a standard curve of gallic acid. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract). Total phenol content = GAE x V x D /m, where GAE is the gallic acid equivalence (mg/ml); V is the volume extract (ml), D is dilution factor and m is the weight (g) of the pure plant extract.

Estimation of Total Flavonoid

Flavonoid content was determined by spectrophotometric method[13]. The same procedure was repeated for the standard solution of Quercetin of different concentration and the standard curve was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms quercetin equivalent (mg of quercetin /g of extract). Flavonoids content = QE×V×D/W, where QE – quercetin equivalent (µg/ml), V - total volume of sample (ml), D - dilution factor, W - sample weight (g).

RESULT AND DISCUSSION

Free radicals are associated with various diseases and are naturally produced in our body. DPPH radical Scavenging method is a very useful way to determine the antioxidant activity of any substance. DPPH is purple in colour having maximum absorption at 517 nm. In the presence of an antioxidant, the purple colour of DPPH decays that can be measured spectrophotometrically. The % inhibition vs. concentration graph showed concentration dependent increase in the % inhibition. The antioxidant potential of the sample is expressed in IC_{50}. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. The IC_{50} values of methanolic and ethanolic extract were found to be 26.12 and 17.43µg/ml respectively. The same for the standard i.e. ascorbic acid was 02.98 µg/ml. For the gallic acid, the curve of absorbance versus concentration was described by the equation $y = 0.016x - 0.135$ ($R^2 = 0.986$) and for flavonoids, the curve of absorbance versus concentration was described by the equation $y= 0.002x + 0.055$, $R^2 = 0.966$, where, y = absorbance and x = concentration.

The total phenol and flavonoid content in the methanolic extract was found to be 8.34±0.022 mg of GAE/g of extract and 5.16±0.033 mg of quercetin /g of extract respectively and in the ethanolic extract it was found to be 10.87±0.043 mg of GAE/g of extract and 7.65±0.011 mg of quercetin /g of extract respectively (Table 1).

Table I: IC $_{50}$ and Total phenol and flavonoid content in the leaf extract of *Smilax ovalifolia*

Extract/Reference*	IC_{50} µg/ml	Total Phenol [mg of GAE/g of extract] (Mean ± S.D.)	Total Flavonoid [mg of quercetin /g of extract] (Mean ± S.D.)
Methanol	26.12	8.34 ± 0.022	5.16±0.033
Ethanol	17.43	10.87±0.43	7.65±0.011
Ascorbic acid*	02.98	-	-

The antioxidant activity found in plants is correlated with the polyphenolic compounds, like flavonoids and phenolic acids[14-17]. Antioxidants with free radical scavenging activities are considered to have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated[18]. The leave extract of *Smilax ovalifolia* is found to have a significant antioxidant activity.

CONCLUSION

From the present study it can be concluded that the leaves of *Smilax ovalifolia* could be a good source of antioxidant. The free radical scavenging activity of the leave extract can thus be correlated with its use in traditional medicinal systems for treating various diseases.

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