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An Estimation of *In Vitro* Antioxidant Activity of Ethyl Acetate Extract of *Oxalis corniculata* Linn and Quantitative Estimation of Phenolic Content

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Tasfi Shafi*, Suchitra Banerjee, Mukta Shrivastava

Department of zoology, Govt. MLB Girls P.G. College Bhopal-462 003 MP. (India)

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ABSTRACT

Objective and background: To study the in vitro antioxidant activity of ethyl acetate extract of *Oxalis corniculata* Linn. The *Oxalis corniculata* is an edible and a medicinal plant which is important to the food industry and may also have a significant role to play in health care. The chemical constituents, including flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins, vitamins, and minerals are rich sources found in this plant. Material and Methods: *Oxalis corniculata* fractions obtained from the ethyl acetate extract of the plant by reversed-phase separation were investigated for their in vitro antioxidant activities and phenolic compounds content. Five fractions were classified based on optical absorption between the wavelength ranges of 200–400 nm. DPPH radical scavenging assay was carried out. The total phenolic content was estimated and analyses of the Folin Catechu Method for the Determination of the Total Phenolic Content from Ethyl acetate extract *Oxalis corniculata*. Observation, findings, and Conclusion. DPPH radical scavenging activity of ethyl acetate extracts of *Oxalis corniculata Linn*. (EaEOC) added to the methanol solution of DPPH and total phenol content of the *Oxalis corniculata* Linn. Antioxidant activity and may prove to be effective for the treatment of various diseases caused by free radicals.

INTRODUCTION

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress[1]. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process[2]. This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease. Antioxidants are the substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance[3]. It is a characteristic molecule having the feature of inhibiting the oxidation of other molecules present within it. The free radicals are produced during the oxidation reaction by transferring the electrons from the substance to the oxidizing agents; these free radicals undergo the chain reaction and damage the surrounding tissues by deteriorating the structure and function. For this dangerous reaction, the solution is the antioxidants, by arresting this chain reaction by terminating the free radicals from the series reaction of oxidation. These antioxidants are being itself oxidized and are often reducing agents for example polyphenols, ascorbic acid, or thiols[4]. Phenols constitute many types of simple phenols as well as derivatives of benzoic and cinnamic acid in the form of phenolic acid. Coumarins, stilbenes, hydrolysable and condensed tannins, lignans, and lignins are the phenolic compounds in the form of secondary metabolites present in the plant as its pigments, antioxidants, and some shielding agents from UV radiations [5]. *Oxalis corniculata* Linn belongs to family Oxalidaceae is the annual creeper, stolen, grown in moist places, lawns, vegetable gardens with the cosmopolitan distribution. It grows in well-drained and loamy soil but is acidic in nature, preferring no shade, with wide geographical distribution, also called Procumbent Yellow sorrel or sleeping beauty resembles the common yellow wood sorrel having perennial/ annual life cycle. In the warmer places the plant shows perennial life cycle but in the cold place, its life cycle is annual due to the overwinter temperature. It reproduces by the dispersion of seeds whether the corolla is removed or not, seed set occurred when flowers were left to self-pollinate. The seeds are enclosed in smooth turgid arid which breakdown suddenly along the abaxial axis and turns inside out. This makes the dispersal of seeds to happen at a considerable distance from the parent plant up to 2m **[6].**

MATERIALS AND METHODOLOGY

For this estimation of phenol in the ethyl extract; Gallic acid, Methanol, Folin- Ciocalteu Reagent 1:10 in deionized water, Sodium Carbonate (7.5%w/v in sodium carbonate solution), Graduated pipette, Volumetric flask (5ml/10ml) or test tubes, Beaker (25/50ml) The UV/VIS spectrophotometric technique for various colorimetric reactions is practical in the laboratories which are simple and rapid to execute at low cost [7]. The Folin Catechu measures the total concentration of phenolic hydroxyl groups. Folin Catechu reagent reacts with polyphenols present in the plant extract reacts which reacts to make blue complex compounds that can be quantified by visible- light spectroscopy technique, this blue complex is absorbed by the alkaline solution and concentration of the phenolic compounds [8]. The reagent Folin Catechu prevents the turbidity in the redox reaction when excess precipitation can occur making the spectrophotometric technique unsuccessful [9]. The colorimetric reactions change the color of many phenolic compounds differently due to the difference in the unit mass which gives accurate and specific data of the phenolic estimation and the kinetics of reaction [10]. The total amount of phenol in the extracts was estimated with Folin Catechu reagent. Gallic acid was used as a standard and the total phenol was expressed as mg/g gallic acid equivalent (GAE). A series of concentration 0.01, 0.02, 0.03, 0.04 and 0.05mg/ml was prepared in methanol. The concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold diluted Folin Catechu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and incubated for 30 minutes at room temperature before the absorbance was measured at 765nm spectrophotometrically. The estimation was determined in triplicate. The Folin Catechu reagent is sensitive to reducing compounds and polyphenols and produces blue color upon the reaction. Then this blue color is measured spectrophotometrically[10, 11]. Anti-Oxidant Activity was carried out by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity The DPPH is scavenged by the antioxidants, gets reduced after acquiring the proton. When the DPPH undergoes reduction reaction it changes its color from purple to yellow. The radical scavenging is proportional to the quantity of the free radicals inhibition. The extent of discoloration of the DPPH confirms the free radical scavenging potentials of extract with their proton donating ability. The pairing off of electrons makes the discolouration of solutions stoichiometrically depending upon the number of electron uptake[12]. 0.1mM of DPPH was prepared with a concentration of 4mg/100ml in methanol. Then different concentrations of test samples were prepared in

methanol (20, 40, 60, 80, 100) µg/ml. A volume of 2ml of ethyl acetate extract of oxalis corniculata and 1 ml of DPPH incubate at room temperature for 30 minutes. At 515 nm the absorbance was recorded on the UV Spectrophotometer [13]. Calculation is measured by the formula [(Ab Ctrl 515 nm- Ab Sample 515 nm/ AC 515 nm) x 100]. Then a curve is being plotted % Inhibition and concentration and using the line of regression estimate IC_{50} [14].

RESULTS AND DISCUSSION

In this experiment, the total phenolic content of the ethyl acetate extract of the *Oxalis* corniculata was estimated with reference to the gallic acid equivalent. The absorbance values of gallic acid in different concentration are shown in the (table 1) and the graphical representation is expressed in the (graph 1). Total phenolic content of the ethyl acetate extract of *Oxalis corniculata* was estimated using the spectral analysis. The total phenolic content (TFC) estimation at different concentrations is shown in (table 2). The mean and standard deviation of the Ethyl Acetate extract were measured as 376.6667 and 7.023769 respectively. Total phenolic content increased in the ethyl acetate extract significantly as the concentration of the extract was enhanced similarly like in the standard Gallic acid as the concentration was increased in it.

In the antioxidant activity applying the DPPH assay of the Ethyl acetate showed the percentage of inhibition increased significantly from the concentration of 20µg/ml to 100µg/ml which was 25.85 to 47.55. The IC50 value of this extract observed was 105.13. The representation of the DPPH data of the ethyl acetate extract of the *Oxalis corniculata* is shown in the Table (3) and in a graph (2).

Table (1) Total Phenolic standard estimation of the gallic acid

Sr. No.	Concentration	Absorbance
1.	10	0.1042
2.	20	0.1703
3.	30	0.2426
4.	40	0.2911
5.	50	0.3187

Graph (1) Standard curve of the total phenolic content of the gallic acid

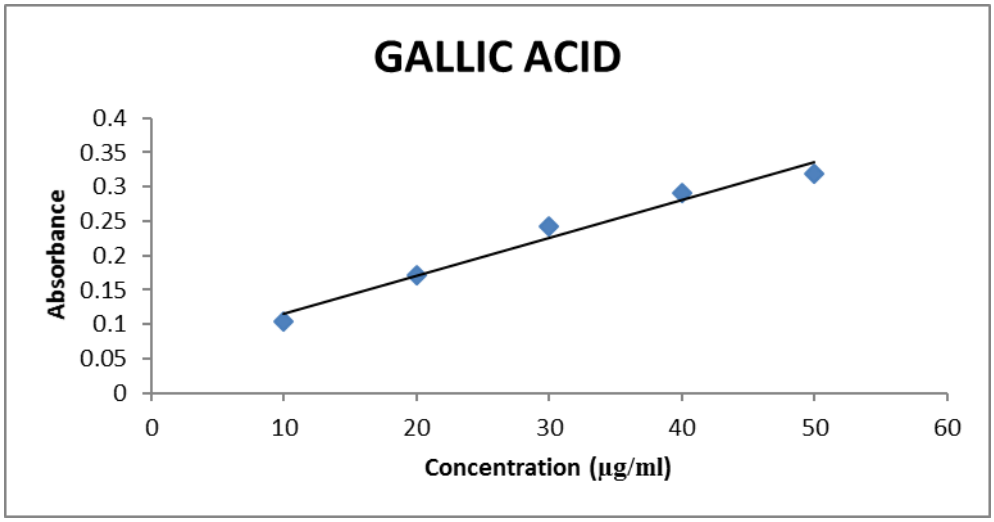


Table (2) Total Phenolic content estimation of Ethyl Acetate extract of *Oxalis corniculata*

Sr. No.	Absorbance	concentration	TPC (mg/g)
1	1.94	1mg/ml	376
2	1.98	1mg/ml	484
3	1.91	1mg/ml	370

Mean = 376.6667

Standard Deviation = 7.023769

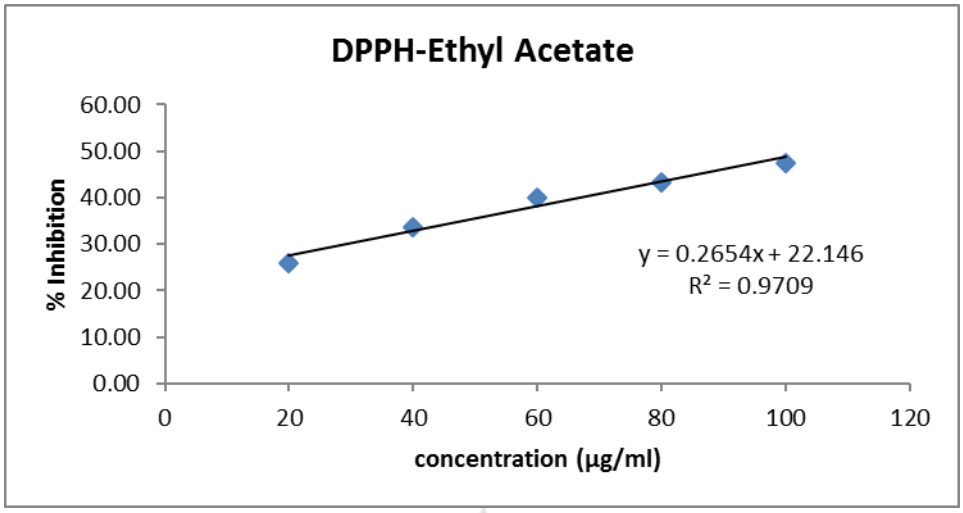
Table 4.8.3.1 (c) DPPH of the Ethyl acetate extract of Oxalis corniculata

Sr. No.	Concentration (ug/ml)	Absorbance sample	Absorbance of control	Ctrl-sample/ctrl	% inhibition
1	20	0.591	0.797	0.25847	25.85
2	40	0.529	0.797	0.33626	33.63
3	60	0.478	0.797	0.40025	40.03
4	80	0.452	0.797	0.43287	43.29
5	100	0.418	0.797	0.47553	47.55

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

IC_{50}=105.13

Graph (2) DPPH of the Ethyl acetate extract of Oxalis corniculata



CONCLUSION

In this study, it was observed that the *Oxalis corniculata* Linn, possess the antioxidant activity up to great extent. Furthermore, plant has the goodness of phenols as well. Being this plant rich in antioxidants can be used for the human welfare. This plant has also antimicrobial, antibacterial, Hepatoprotective property reported earlier. This plant can be useful to cure many diseases due to its rich antioxidant activity. It has also the goodness of phenols also, as the phenols are also useful for the human health. However, this plant needs further observation to elucidate more beneficial properties of the *Oxalis corniculata* Linn.

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