WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 12, 768-777. Research Article ISSN 2277– 7105

PHYTOCHEMICAL INVESTIGATION AND *IN VITRO* ANALYSIS OF ANTIOXIDANT ACTIVITY OF *OXALIS CORNICULATA*

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Article Received on
24 April 2018,

Revised on 14 May 2018,
Accepted on 04 June 2018

DOI: 10.20959/wjpr201812-12634

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ABSTRACT

The aim of present study is to investigate the Phytochemical investigation of the Ethanolic extract of leaves of the plant *Oxalis Corniculata* belonging to the family- *Oxalidaceae*. The present study provides physicochemical and phytochemical details of the leaves of Oxalis Corniculata which are useful in laying down standardization and pharmacopeia parameters. The present study aims to open new avenues for the improvement of medicinal uses of Oxalic Corniculata leaves are selected for anti-oxidant activity. The ethanolic extract of leaves of *Oxalis Corniculata* - *Oxalidaceae* were assessed for its antioxidant activity by Hydrogen peroxide scavenging activity, Total flavonoid content and Total Antioxidant Capacity by Phosphomolybdate method. In the H2O2 Radical Scavenging Activity, the % Scavenging Activity ethanolic extract of *Oxalis Corniculata* had shown better results with Standard α-tocopherol. Total Flavonoid Content was determined as Quercetin Equivalent dose. 1mg of the ethanolic extract of leaves of Oxalis Corniculata is Equivalent to 380 µg/ml of Quercetin Total Antioxidant capacity (%) was assayed by Phosphomolybdate method. The results were compared with standard and the extract had shown 74% while the standard had shown 59.64%.

KEYWORDS: Antioxidant, Reactive Oxygen Species (ROS), Free Radical Scavenging Capacity, Total Flavonoids.

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial

chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or the dietary antioxidants: vitamin A, vitamin C, and vitamin E.[1]

Recently, the World Health Organisation (WHO) evaluates that approximately, 80% of the world inhabitants currently depend on herbal medicine for primary health.[2,3] The total number of 2,50,000 higher plant species present on the earth, out of these more than 80,000 are medicinal.[4] Plants have been source of medicines throughout human history among ancient civilization. India is well suited for development of drugs from medicinal plants, because it is rich in unique medicinal plants, traditional knowledge and heritage of herbal medicines.[5]

Plants are potential source of natural antioxidants. Majority of the diseases or disorders are mainly linked due to excessive production of free radicals. Antioxidant constituents of plants are operating as scavenger of free radical and helps for conversion of free radicals to not as much of reactive species.[6]

Indian penny wood, creeping wood sorrel, creeping oxalis, sleeping beauty and procumbent yellow sorrel all are common names of *Oxalis corniculata*. Leaves of this plant are edible with tangy taste like taste of lemons. This herb is richest source of vitamin- C, B, Potassium and oxalic acid. Oxalis is mainly native to Hawaii and south Europe. It is found in tropical regions of America. In Asia it is found in India, Pakistan, Afghanistan, China, Indonesia, Taiwan and Japan. In India it is mostly found in open gardens, grasslands, riversides, mountains, wastelands and roadsides.[7] Among the medicinal uses reported *Oxalis corniculata* have wide medicinal importance used for curing of antihelmintic, styptic, astringent, diarrhea, dysentery, dysmenorrhoea, hepatitis, amenorrhoea and burning sensation, antimicrobial activity[8,9] recent studies reported that it possess antitumor activity, antiepileptic and anxiolytic activities.[10]

The aim of the Study is to Investigate Phytochemical components and Invitro analysis of Antioxidant Activity of Ethanolic Extract of the leaves of *Oxalis Corniculata.*

MATERIALS AND METHODS

The Study was conducted over a period of 5 months i.e., from December 2017 to April 2018 as a part of Academic research in the Department of Pharmacology, Vaageswari college of Pharmacy, Karimnagar.

Procurement of plant material

The leaves of oxalis corniculata were collected from the wild growing Weed in the botanical garden, Vaageswari College of Pharmacy, Thimmapur, Karimnagar and Telangana, India. A specimen was deposited in the institutional herbarium. The collected plant material was made thoroughly free from any foreign organic matter. Leaves were separated, shade dried and powdered with laboratory mixer and sieved. Phytochemical studies were conducted with fresh leaves and leaf powder.

Drugs and chemicals

α-tocopherol, Ethanol, Sulphuric acid, Sodium phosphate, Ammoniummolybdate, Aluminum nitrate, Quercetin, potassium acetate, Hydrogen peroxide solution.

Preparation of extracts

Ethanolic extracts of oxalis Corniculata leaves were prepared by both Soxhlation and maceration methods at suitable temperature. 50gms of powder of leaves was packed in a Thimble and placed in Soxhlet Apparatus against 200 ml of solvent. The Soxhlation process was carried out for about 7hrs. The extracts obtained were evaporated and dried in desiccators.

Preliminary phytochemical screening

The ethanolic extract of the Leaves of *Oxalis Corniculata* was subjected to different qualitative phytochemical screening tests for detection and establishment of the nature of chemical composition and presented in Table no 1.

Method of Antioxidant Activity

Phosphomolybdate method

The total antioxidant capacity of the extract was determined by procedure followed by Kumar *et al*., (2008) with phosphomolybdenum using α-tocopherol as the standard. An aliquot of 0.1 mL of GOEE (100 µg) solution was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and

incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in a UV spectrophotometer. The blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the sample. The total antioxidant capacity was expressed as;

$$\text{Total Antioxidant Capacity (\%)} = \frac{\text{Absorbance of the control- Absorbance of test Sample}}{\text{Absorbance of the control}}$$

Total flavonoid content

Total soluble flavonoid of the extract was determined by following the method reported by Park *et al.* (2008) with aluminum nitrate using quercetin as standard. Plant extract (1mg) was added to 1 mL of 80% ethanol. An aliquot of 0.5 mL was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 4.3 mL of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after 40 min at room temperature. Total flavonoid content is expressed as quercetin equivalent (QE).

Hydrogen peroxide scavenging activity

This assay was carried out following the protocol given by Kumar *et al*., (2008). Hydrogen peroxide solution (2 mM) was prepared with standard phosphate buffer (pH, 7.4). Extract samples (25-400 µg/mL) in distilled water were added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined spectrophotometrically after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of both plant extract and standard compound (α-tocopherol) was determined by using formula,

$$\text{Total Scavenging Capacity (\%)} = \frac{\text{Absorbance of the control- Absorbance of test Sample}}{\text{Absorbance of the control}}$$

RESULTS

Extract yield

Total ash value of the sample=100(z-x)/y%

100(62-61)/2=50%

Organoleptic properties

Leaf shape: heart shaped.

Size: 0.3-1.8cm long and 0.4-2.3cm.

Color: green.

Taste: sour, astringent.

Phytochemical screening

The ethanolic extract of Leaves of *Oxalis Corniculata* was subjected to phytochemical test for detection of various chemical constituents like alkaloids, glycosides, carbohydrates, proteins and amino acids, steroids, terpenoids, flavonoids and tannins. The results were furnished in the Table no 1.

Table No 1: Evaluation of Phytochemical Constituents.

Detection of Alkaloids	
Mayer's test	+
Wagner's test	+
Dragendorff's test	+
Detection Of Carbohydrates	
Fehling's test	+
Benedict's test	+
Detection Of Glycosides	
Libermann's test	+
Legals test	-
Keller-killani test	+
Detection Of Proteins And Amino Acids	
Millon's test	-
Biuret test	-
Ninhydrin test	-
Detection Of Phytosterols	
Leibermann-burchards test	+
Salkowski test	+
Detection Of Tannins	
Lead acetate test	-
Potassium dichromate test	-
Gelatin test	-

Note: **"+" - Present "–" – Absent**

The ethanolic extract of the powder leaves of *Oxalis corniculata* showed the presence of carbohydrates, glycosides and steroids.

Antioxidant activity

1) H_2O_2 radical scavenging activity

Table No 2: H_2O_2 Radical Scavenging Activity.

Concentration (µg/ml)	Absorbance			% Scavenged	
	Standard	Extract	Control	standard	extract
25	0.457	0.441	0.589	22.41	25.12
50	0.384	0.351	0.645	39.29	45.58
100	0.346	0.286	0.724	52.2	60.49
300	0.204	0.159	0.825	75.27	80.72
500	0.123	0.984	0.984	87.5	90.04

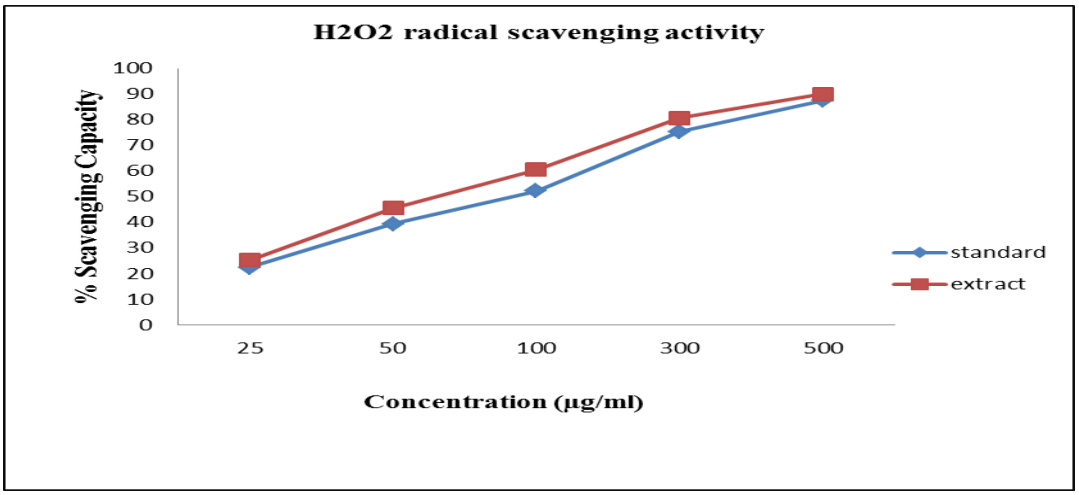


Figure 1: Graph of H_2O_2 Radical Scavenging Activity.

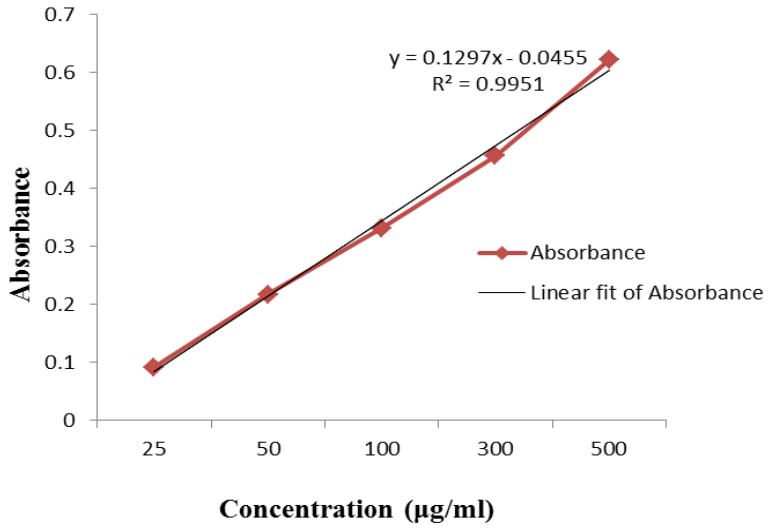
2) Total flavonoid content

Table no 3: Total Flavonoid content.

Concentration(µg/ml)	Absorbance
25	0.092
50	0.217
100	0.332
300	0.456
500	0.621
1 mg/ml extract	0.57

Quercetin Equivalent (QE) Dose of the Extract was found to be (µg/ml) = 380 µg /ml i.e., 1mg of extract equivalent to 380 µg of Quercetin.

'igure 2: Graph Of Standard Graph of Quercetin



3) Total antioxidant capacity phosphomolybdate assay

Table No 4: Total antioxidant capacity (%).

Absorbance			Total Antioxidant Capacity (%)	
Control	**Standard**	**Extract**	**standard**	**Extract**
0.57	0.23	0.148	59.64	74.03

DISCUSSION

Oxalis corniculata is a well-known plant in India and is one of the most versatile medicinal plants having a wide spectrum of biological activity. Oxalis species mainly corniculata have been documented to posses plethora of activities including anti-inflammatory, antiseptic, anti-diabetic and anti-helminthic. It is used in traditional medicines for the treatment of diarrhea, influenza and enteritis. It showed the presence of glyoxylic acid, oxalic acid, pyruvic acid, isovitexin, vitexin-2-O-beta-D-glucopyranoside, glycolipids; vitamin C; phospholipids; fatty acids, alpha and beta tocopherols. Plethora of studies has been focused on Oxalis corniculata, whereas multiple medicinally important species remain to be explored for bioactive agents.

In a study conducted by Archana R *et al.*, 2009 the Methanol extract of whole plant of Oxalis corniculata Linn (Family: Oxalidaceae) was assessed for its antioxidant and anti-inflammatory activity by in-vitro methods. Antioxidant activity was studied using 1,1-Diphenyl-2- Picrylhydrazyl (DPPH) and nitric oxide radical scavenging activity. Inhibition of lipid peroxidation was studied by thiobarbituric acid reactive substances (TBARS) method on isolated rat liver tissues. Quantitative analysis of antioxidative components like total amount of phenolics, flavonoids and flavonols were estimated using spectrophotometric method. In-

vitro anti-inflammatory activity was evaluated using albumin denaturation assay, membrane stabilization assay and proteinase inhibitory activity at different concentrations. Aspirin was used as a standard drug for the study of anti-inflammatory activity. Linear regression analysis was used to calculate IC50 value. Results showed that, the extract exhibited significant DPPH and nitric oxide radical scavenging activity with IC50 value of 302.93±4.17 and 73.07±8.28µg/ml respectively. Lipid peroxidation induced by the Fe2+, was inhibited by the extract with IC50 value 58.71±2.55µg/ml. Total phenol content was estimated as 25.62±0.10mg of gallic acid equivalents of dry extract. Total flavonoids and flavonols were found to be 150.88±12.61 and 150.16±2.16 mg of rutin equivalents per gram of dry extract respectively. Extract also showed in-vitro anti-inflammatory activity by inhibiting the heat induced albumin denaturation and Red Blood Cells membrane stabilization with the IC50 values of 288.04±2.78 and 467.14±9.56mu;g/ml respectively. Proteinase activity was also significantly inhibited by the extract (IC50=435.28±5.82µg/ml). From the results, it is concluded that flavonoids and related polyphenols present in the O. corniculata extract may be responsible for the activity. In the present study we found that Ethanolic extract of the same plant had shown better results with Standard α-tocopherol. Total Flavonoid Content was determined as Quercetin Equivalent dose. 1mg of the ethanolic extract of leaves of Oxalis Corniculata is Equivalent to 380 µg /ml of Quercetin. Total Antioxidant capacity (%) was assayed by Phosphomolybbdate method. The results were compared with standard and the extract had shown 74% while the standard had shown 59.64%.

CONCLUSION

The Preliminary Phytochemical screening of ethanolic extract of *Oxalis Corniculata* leaves identified the presence of Alkaloids, Carbohydrates, Glycosides and Phytosterols These results indicated that this plant should have an antioxidant property Antioxidant Activity was performed using three methods i.e., H2O2 Radical Scavenging Activity, Determination of Total Flavonoid Content and Total Antioxidant capacity by Phosphomolybdate Assay. The results were compared with the standard α-tocopherol In the H2O2 Radical Scavenging Activity, the % Scavenging Activity ethanolic extract of *Oxalis Corniculata* had shown better results with Standard α-tocopherol. Total Flavonoid Content was determined as Quercetin Equivalent dose. 1mg of the ethanolic extract of leaves of *Oxalis Corniculata* is Equivalent *to* 380 µg /ml of Quercetin. Total Antioxidant capacity (%) was assayed by Phosphomolybdate method. The results were compared with standard and the extract had shown 74% while the standard had shown 59.64%.

In conclusion, the study provides a detailed insight of the antioxidant activity that may be useful to combat diseases involving production of free radicals. Our findings may help in identifying *Oxalis corniculata* as a storehouse of potent bioactive compounds of medicinal value in *Oxalidaceae* family. The finding further warrants investigation to decipher the active ingredient underlying the antioxidant capability. Although the plant has already been investigated for its phytochemicals, there is a need for more extensive studies. Assay-guided isolation of natural products from the plant may result into the discovery of new and cheaper therapeutic agents.

REFERENCES

1. Archana Borah, Yadav RNS, Unni BG. Evaluation of antioxidant activity of different solvent extracts of Oxalis corniculata. Journal of Pharmacy Research, 2012; 5(1): 91-93.
2. Eloff J.N. which extraction should be used for screening and isolation of antimicrobial componenets from plants?. J. Ethnopharmacol., 1998; 60: 1-8.
3. Javed Hina, Erum Shazia, Tabassum Sobia and Ameen Farhana. An overview on medicinal plant importance of Thymus vulgaris. JASR., 2013; 3(10): 974-982.
4. Edeega H.O., Okwu D.E. and Mbaebie B.O. Phytochemical constituents of some Nigerian medicinal plant. Afr. J. biotechnol., 2005; 4(7): 685-688.
5. Ncube B., Finnie J.F. and Staden J.V. Quality from thefield: The impact of environmental factors as quality determinants in medicinal plants. S.Afr. J. Bot., 2012; 82: 11-20.
6. Devasagayam TPA, Tilak JC, Boloor KK, Ketaki Sane S, Ghaskadbi Saroj S, Lele RD. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. JAPI, 2004; 52: 794-804.
7. Hall David W, Vandiver Vernon V, Sellers Brent A. Creeping Wood Sorrel, Oxalis corniculata. Southern Yellow Wood Sorrel, Oxalis florida' Salisb Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, 1996; 37: 01- 02.
8. Kathiriya A, Das K, Kumar EP, Mathai KB. Evaluation of antitumor and antioxidant activity of oxalis corniculata Linn. against Ehrich Ascites Carcinoma on Mice. Iran J Cancer Prev., 2010; 3(4): 157-165.
9. Kumar Ashwani, Rani Niketa Sapna, Somiya Sagwal An Absolute Review on Oxalis corniculata. Int J Pharma Bio Sci., 2012; 3(3): 1173-1188.

10. Sampath Sai T, Santosh P, Lahkar M, Ajaygodwin P, Kumar Pavan S, Lingesh A. Anxiolytic Effect of Ethanolic Extract of Oxalis corniculata in Mice. Inter J Pharm Bio Sci, 2011; 2(3): 281-90.
11. Kumar S, Kumar D, Prakash O. Evaluation of antioxidant potential, phenolic and flavonoid contents of Hibiscus tiliaceus flowers. Electronic Journal of environmental, Agricultural and Food chemistry, 2008; 7(4): 2863-71.
12. Park, YS, Jung ST, Kang SG, Heo BK, Arancibia Avila P, Toledo F. Antioxidants and proteins in ethylene treated kiwifruits. Food Chemistry, 2008; 107: 640-648.
13. Sakat SS, Juvekar AR. Gambhire MN. In vitro antioxidant and anti-infammatory activity of methanol extract of oxalis corniculata Linn. Int. J. Pharm. and Pharmaceut. Sci, 2010; 2: 146-155.
14. Umamaheswari M, Chatterjee TK. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. African J. Trad. Compl. Altern. Medicines, 2008; 5: 61-73.
15. Reddy KY, Kumar AS, Lakshmi SM, Angothu S. Antioxidasnt properties of methanolic extract of Oxalis corniculata. International Journal of Phytopharmacology, 2010; 1: 43-46.
16. Kathiriya A, Das K, Kumar EP, Mathai KB .Evaluation of antitumor and antioxidant activity of oxalis corniculata Linn. against Ehrich Ascites Carcinoma on Mice. Iran J Cancer Prev, 2010; 3(4): 157-165.
17. Rangari. Pharmacognosy And Phytochemistry part-1. 1st edition., career publications, 2004; 129-150.
18. K.R.Kandelwal. Text Book of Practical Pharmacognosy. 10th edition., Nirali Prakashan, 2003; 38-161.