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PHYTOCHEMICAL STUDIES AND INVITRO ANTI OXIDANT ACTIVITY OF OXALIS CARNICULATA.L

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ABSTRACT

The methanolic cured extract of *oxalis corniculata L*. was screened for their free radical scavenging property using ascorbic acid by standard antioxidant. Free radical can cause disruption of cell metabolism. A single free radical can damage so many molecules in our body, preventing our body from functioning properly. This molecular destruction is continually occurring in our body. Therefore, it is essential to maintain the equilibrium, either by reducing the unnecessary oxidative stress or decreasing needless oxidative stress, or by doing both. Normally, sufficient amounts of antioxidants are present in our foods, but under adverse conditions (poor quality diet, limited food supply, environmental pollution etc.) the balance can be disturbed. Certain foods contain significant quantities of potentially therapeutic antioxidants to help sustain human health and well-being.

KEYWORDS: Antioxidant, methanol, chronic disease, DPPH, *oxalis*

corniculata L.

INTRODUCTION

Plants are indispensable to man for his life. The three important necessities of life food, clothing and shelter and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a store house of remedies to cure ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive

nature, so that today we possess many effective means of ensuring healthcare. Today a vast store of knowledge concerning therapeutic properties of different plants has accumulated.

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Throughout the history of mankind, many infectious diseases have been treated with herbals. A number of scientific investigations have highlighted the importance and the contribution of many plant families.

Medicinal plants play a vital role for the development of new drugs. The bioactive extract should be standardized on the basis of active compound. The bioactive extract should undergo safety studies. Almost, 70% modern medicines in India are derived from natural products. India has a very small share (1.6%) of this ever-growing global market. To compete with the growing market, there is urgency to expeditiously utilize and scientifically validate more medicinally useful plants.

Antioxidants may be defined as radical scavengers, which are protecting the human body against free radicals. Free radicals may cause pathological conditions like anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, mongolism, ageing and dementia. Free radicals are generated as part of the body's normal metabolic process, and free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, atmospheric pollutant, drugs and metal catalysts. Free radicals cause oxidative damage to lipids, nucleic acids.

In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells.

Normally, antioxidant system occurring in human body can scavenge these radicals, which would keep the balance between oxidation and anti-oxidation. Nonetheless, the exposure of cigarette smoking, alcohol, radiation, or environmental toxins induces the production of excessive ROS and RNS, which disrupt the balance between oxidation and anti-oxidation and result in some chronic and degenerative diseases.

By preventing the start or continuation of an oxidative chain reaction, increasing the intake of exogenous antioxidants can reduce the damage produced by oxidative stress. These antioxidants also serve as reducing agents, free radical scavengers, and singlet oxygen quenchers.

The exogenous antioxidants are mainly derived from food and medicinal plants, such as fruits, vegetables, cereals, mushrooms, beverages, flowers, spices and traditional medicinal herbs. Furthermore, the sectors that process agricultural by products have the potential to be significant suppliers of free radicals.

These natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C).

Generally, these natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, such as antiinflammatory, antibacterial, antiviral, anti-aging, and anticancer.

The plants belonging to family Oxalidaceae, these plants are herbs, shrubs, or rarely trees. This family comprises 7 or 8 genera and about 800 species in the genus *Oxalis* (wood sorrels). Members of this family typically have divided leaves, the leaflets showing "sleep movements", spreading open in light and closing in darkness.

The leaves are alternate and pinnately or palmately compound or rarely simple by suppression of leaflets; stipules are absent. The flowers are bisexual and actinomorphic. The perianth consists of a calyx of 5 distinct sepals and a corolla of 5 distinct or sometimes basally connate petals.

The stamens are basally connate and diplostemonous, that is, of two series with the outer series opposite the petals; occasionally 5 stamens are reduced to staminodes. The gynoecium consists of a single compound pistil of 5 carpels, 5 distinct styles, and a superior ovary with 5 locules, each containing one or more axile ovules. The fruit is a loculicidal, sometimes explosively dehiscent capsule or berry.

The genus *Averrhoa* of which starfruit is a member, is often included in this family, is treated by some botanists in a separate family Averrhoaceae.

Hence, the medicinally and nutritionally important *oxalis corniculata L.* plant part were used for the antioxidant activities in the present study.

MATERIALS AND METHODS

- ➤ Plant material: Oxalis corniculata L. (Oxalidaceae) whole plant were collected, washed, cleaned, dried in shade, and pulverized in a grinder-mixer to obtain a coarse powder and then passed through a 40- mesh sieve.
- ➤ **Apparatus:** Test tubes, Test tube holder, Test tube stand, Glass rods, Spatula, Burners, Measuring cylinder, Digital weighing balance, Grinder mixer, Sieve no. 40, Soxhlet apparatus, heating mantle.
- ➤ Chemicals: Alcohol, Alpha napthol, Conc.H₂SO₄, Ferric chloride, 5% HgCl₂ solution, 5% Lead acetate solution, Acetic acid solution, Potassium dichromate, 95% Ethanol, Magnesium turnings, Tannic acid, 1% Copper sulphate, Sodium nitroprusside, 10% NH₄OH, Iodine solution, 5% Ammonium sulphate, 5% NaOH, Nitric acid, Acetic anhydride, Picric acid, etc
- ➤ **Reagents:** Millons reagent, Barfoed's reagent, Ninhydin reagent, Mayer's reagent, Dragendorffs reagent, Wagner's reagent, Hager's reagent, Fehling's solution A & B, Benedict's reagent, etc.
- ➤ Methanol extract: About 1000 gm of powdered drug was successively extracted with methanol, by using soxhlet apparatus. The extraction was carried out until the extract becomes colorless. The solvent is removed from extract by distillation under reduced pressure. The concentrated extract were kept in a desiccator and used for further experiment.

EVALUATION OF ANTIOXIDANT ACTIVITY

DPPH Radical scavenging test

The free radical scavenging activity of the methanol extracts of *Oxalis corniculata* L. (MEOC) was determined by using 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) using UV-Spectrometry (Mathiesen *et al.*, 1995) at 517nm. The DPPH solution was prepared in 95% methanol. The MEOC 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 dilution with same solvent were made the final volume of each test tube up to 10ml whose concentration was then 20μg/ml, 40μg/ml,

60μg/ml, 80μg/ml and 100μg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of their test tubes. Containing MEOC (20μg/ml, 40μg/ml, 60μg/ml, 80μg/ml and 100μg/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 MEOC 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 radicals-scavenging = [(Absorbance of control – Absorbance of test Sample) / (Absorbance of control)] x 100.

Reducing Power Method

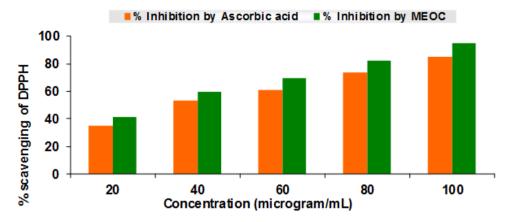
The assay of reducing power method (Koleva *et al.*, 2002, Makari *et al.*, 2008) is one to determine the antioxidant activity. In this 1 ml of plant extract of MEOC solution mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml Potassium Ferricyanide [K₃Fe (CN6)] (10g/l), the mixture was incubated at 50°C for 20 minutes. 2.5 ml of Tri chloroacetic acid (100g/l) was added to mixture. This was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl₃ (lg/L) and absorbance measured at 700nm in UV-visible spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as standard and phosphate buffer used as blank.

RESULTS: EVALUATION OF ANTIOXIDANT ACTIVITY

DPPH Radical scavenging test

Table 1: Antioxidant activity by DPPH method.

S. No.	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of MEOC	% scavenging DPPH of Ascorbic acid	%scavenging DPPH of MEOC
1	20μg/ml	0.142	0.128	35.15	41.55
2	40μg/ml	0.102	0.089	53.42	59.36
3	60μg/ml	0.086	0.067	60.73	69.40
4	80µg/ml	0.058	0.039	73.51	82.19
5	100μg/ml	0.032	0.011	85.38	94.97

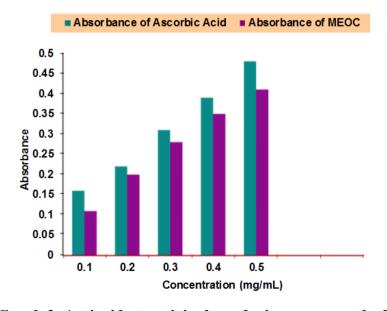


Graph 1: Antioxidant activity by DPPH method.

Graph 1: DPPH radical scavenging activity of methanol extracts of *Oxalis corniculata* L. (MEOC) added to methanol solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard Ascorbic acid. Values are the average of triplicate experiments.

Table 2: Antioxidant activity by reducing power method.

S. No.	Concentration (mg/ml)	Absorbance of Ascorbic acid	Absorbance of MEOC
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



Graph 2: Antioxidant activity by reducing power method.

Graph 2: Reducing power of methanol extract of *Oxalis corniculata* L. (MEOC) of as compared to Ascorbic acid. Values are the average of triplicate experiments.

The percentage yield of various extracts of the whole plant of *Oxalis corniculata* L. (Oxalidaceae) were presented in the Table 8. The methanol extract gives the high percentage yield and it was found to be 24.94% w/w.

Table 3: Percentage yield of *Oxalis corniculata* L. (Oxalidaceae).

Plant name	Part used	% yield of extractive (%w/w)				
Onalia comiculta I	Whole plant	Methanol	Ethanol	Pet. ether	Water	
Oxalis corniculta L.		24.94	8.3	12.34	10.4	

DISCUSSION

The pharmacognostical studies of *Oxalis corniculata* L. belongs to family Oxalidaceae was done. In macroscopic studies, it is observed that the leaves are green and flowers are yellow color, pseudo umbels, axially, 1-6 flowered bracts two, linear, bracteole, sepals five lanceolate, petals are oblongata in nature apex and emarginated. Leaves are 3-foliate, leaflets obcordate, Chartaceous, pilose base cunate, margin entire. In this plant fruits are capsule in nature, oblong, abrupty tapering above; puberulous seeds are numerous per locule, ovoid transversely.

In transverse section of the lamina, the lamina part is about $100\mu m$ wide. Both adaxial and abaxial epidermal layers are wide, large, thin walled circular cells, measuring $25\mu m$ in thickness. The leaf margin is slightly narrow leaflet and posse's thin walled cells are $25\mu m$ in diameter.

Physicochemical properties are an important parameter in detecting adulteration on improper handling of the drug. In the evaluation of crude drug, ash values, extractive values are important parameters. The estimation of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy matter. The determination of extractive values with a range of solvents gives information about extractable non-polar and polar as well as total extractable plant constituents.

The pharmacognostical studies of the plant were carried out with a focus on bringing out diagnostic characters will be of immense help in the proper identification and standardization

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of botanical species of the plant drugs. Which play a major role to establish the particular standards and helps to minimize the adulteration of the plant *Oxalis corniculata* L.

In this present study the methanol extract of whole plant *Oxalis corniculata* L. (Oxalidaceae) were investigated by using DPPH scavenging test and reducing power method. The whole plant of MEOC showed by their 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 autoxidation 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 (Xu *et al.*, 2005). In Graph-1, The MEOC exhibited a significant dose dependent inhibition of DPPH activity, the IC50 value of the MEOC 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 MEOC 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 Graph-2 MEOC was very potent and the power of extract was increased with quantity of sample. By comparing the reference standard Ascorbic acid, the MEOC showed potent antioxidant activity.

CONCLUSION

It is concluded from the data, that the methanol extract of *Oxalis corniculata* L. possess significant Antioxidant activity and may prove to be effective for the treatment of various diseases caused by free radicals. The antioxidant activity may be rich in Vitamin C and C-glycosyl flavones in this plant. However further studies required to elucidate the exact mechanism of action for develop its as potent antioxidant.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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