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ASSESSMENT OF *OXALIS CORNICULATA* L. FOR ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS

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ABSTRACT:
Reactive oxygen species are involved in a number of chronic and degenerative diseases such as arthrosclerosis, cancer, cirrhosis and diabetes. Plant-derived antioxidants such as phenolics, tannins, flavonoids, could delay or reduce the risk for development of these diseases. Thus, the present investigation was based on assessment of total enzymatic and non-enzymatic antioxidants from the mature leaves of *Oxalis corniculata* L. Leaves were collected and the methanolic extract was prepared and the crude extract was tested for its antioxidant potential. The enzymatic parameters such as peroxidase, catalase, AAO, PPO and total phenolics, flavonoids, tannins, ascorbic acid content were estimated. The extract showed considerable free radical scavenging activity as revealed by DPPH and FRAP assay.
Oxalis corniculata L. among the smallest creation of God, but considered as one of the most significant medicinal herb. Hence, the present investigation revealed the *Oxalis corniculata* L. as potent source of natural antioxidants and could play a vital role in health, food and cosmetic industries.

Keywords : *Oxalis, antioxidant, DPPH, FRAP, hydroxyl radical DNA damage.*

INTRODUCTION:

It is well known fact that during oxidative stress, reactive oxygen species, such as superoxide (O2.), hydroxyl (.OH) and peroxyl (.OOH, ROO.) radicals are generated.[1] The occurrence of such oxygen derived free radicals may be significant causative factor in the development of many chronic and cardiovascular diseases.[2] Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. [3] Different compounds synthesized by plants show diverse metabolic activities that can be useful as medicinal tool for mankind. Antioxidant compounds like polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus prevent body from oxidative damage.

Structurally diverse components of plants can provide new ideas to face the health challenges of modern age; consequently the scientists are trying to uncover the medicinal wealth of nature by screening the herbs for curing various ailments through *in vitro* as well as *in vivo* experiments.

Oxalis corniculata L. is a well known medicinal plant and commonly called as "*Indian Sorrel*". The herb is with procumbent branches and palmately 3 foliate leaves. It is famous for its medicinal properties as a good appetizer and as a remover of *Vata*, *Kapha* and piles. It has been known to cure diarrhea, dysentery and skin diseases. [4]

Though *Oxalis corniculata* L. is widely used in traditional medicinal systems, the detailed studies on enzymatic and non-enzymatic antioxidants are not yet exploited for its use in antioxidant rich human diet. Hence, the detailed study was considered worthwhile from both fundamental and applied angles.

MATERIALS AND METHODS

Chemicals

Liquid reagents such as Ethanol, Methanol, were procured from Qualigens, a division of Glaxo India Ltd. Dr. Annie Besant Road, Mumbai 400 025. Ferric Chloride, Folin-Ciocalteau reagent and Folin-Dennis reagent, ammonium hydroxide, Potassium acetate, aluminium chloride, Sodium carbonate, were purchased from Qualigen, a division of Glaxo India Ltd. Dr. Annie Besant Road, Mumbai 400 025. DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was obtained from Hi-media.

Plant material

Fully matured leaves of *Oxalis corniculata* L. were collected from Nowrosjee Wadia College campus, Pune. It was authenticated from BSI, Western Circle, Pune. The shade-dried leaf material was extracted by boiling for two hours in methanol. Then it was centrifuged at 10,000 xg for 20 minutes and the supernatant was evaporated so as to get crude extract. It was further used as sample for reaction mixture.

Peroxidase (EC. 1.11.1.7)

The leaves of *O. corniculata* L. were cut into small pieces and 1 g of fresh material was homogenized in chilled mortar, in 5 ml of 0.1M-phosphate buffer (pH 7.0). The extract was then centrifuged in cold centrifuge at 15,000 ×g for 10 minutes and supernatant was used as enzyme source. The enzyme activity was assayed by Vaidyasekharan and Durairaj method.[5] The assay mixture of 3 ml contained 1.8 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml freshly prepared guaiacol (20 mM), 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H_2O_2. Initial optical density was read after 20 seconds and then the time (minutes) required for increasing the absorbance by 0.1 was noted at 430 nm on UV-visible spectrophotometer (Shimadzu-1700). The activity was calculated using extinction coefficient 6.39 μM^{-1}. The enzyme activity was expressed as units g^{-1} fresh weight.

Catalase (EC. 1.11.1.6)

One gram of fresh fronds of *O. corniculata* L. were cut into small pieces and was homogenized in chilled mortar with 5 ml of 0.06M phosphate buffer (pH 7.0). The extract was centrifuged at 10,000 ×g for 15 min. in cooling centrifuge at 4 ^{0}C and supernatant was used as enzyme source. The catalase activity was calculated by using Maxwell and Bateman method. [6] The reaction mixture contains 2.95 ml of 0.06 M phosphate buffer, 10% (w/v) H_2O_2 and 0.05 ml enzyme extract. The decrease in absorbance was measured at 240 nm by using UV-visible spectrophotometer (Shimadzu-1700). The residual H_2O_2 concentration was calculated using extinction coefficient 0.036 $\mu M^{-1}ml^{-1}$. The enzyme activity was expressed as units g^{-1} fresh weight.

Polyphenol oxidase (EC. 1.14.18.1)

One gram of fresh fronds of *O. corniculata* L. were cut into small pieces and was homogenized in chilled mortar, in 5 ml of 0.1M phosphate buffer (pH 6.5). The extract was centrifuged at 15,000 ×g for 20 min. in cooling centrifuge at 4 ^{0}C and supernatant was used as enzyme source. The polyphenol oxidase activity was assayed by Vaidyasekharan and Durairaj method.[5] The reaction mixture contains 2 ml phosphate buffer (pH 6.5), 0.5 ml enzyme extract and 1 ml of 0.2 mM catechol. The oxidation of catechol in the reaction mixture was measured at 412 nm after every 30 seconds interval on UV-visible spectrophotometer (Shimadzu-1700). The enzyme activity was calculated in units as $K \times (\Delta A\ min^{-1})$, where, K is 0.272 for catechol oxidase. The enzyme activity was expressed as units g^{-1} fresh weight.

Apart from that, the investigation was focused on the determination of important non-enzymatic antioxidants such as ascorbic acid contents (Vit. C), total phenolics, tannins, flavonoids.

Estimation of ascorbic acid content

Ascorbic acid content was estimated by titrimetric method suggested by Ghosh *et al.*[7] One gram of fresh leaves was extracted with 4% oxalic acid. The extract was filtered and the filtrate was transferred to 100 ml volumetric flask and the volume was made 100 ml with oxalic acid. Five ml of filtrate was added to 10 ml 4% oxalic acid and titrated against 2, 6-dichlorophenol indophenol dye (DCPIP). Colourless to faint pink colour was considered as end point. The standard ascorbic acid solution (100 µg ml^{-1}) was titrated with DCPIP dye in the same way so as to get standard values.

Estimation of total phenols

Total phenolic compounds were estimated as per the method given by Farkas and Kiraly.[8] The fresh leaves were cut into small pieces. One gram of leaves was homogenized in 10 ml of 80% hot ethanol. The extract was condensed on hot water bath to approximately 1.0 ml and then centrifuged at 10,000 ×g for 10 min. Volume of the supernatant was adjusted to 10 ml with distilled water. From this 0.2 ml aliquot was used for estimation. The blue colour developed in reaction mixture was read at 650 nm on UV-visible spectrophotometer (Shimadzu-1700). Tannic acid at the concentration of 100 µg ml^{-1} was used to prepare the standard curve.

Estimation of Tannins

Tannins were estimated by Folin-Denis reagent given by Polshettiwar *et al.* [9] Dried 0.5 gm powdered sample was boiled with 5 ml distilled water. It was then centrifuged at 5, 000 ×g for 10 minutes. Supernatant was collected and 1 ml of sample was added with 0.5 ml Folin-Denis reagent and 1 ml of sodium carbonate solution. The final volume was adjusted to 10 ml with distilled water. The reaction mixture was incubated for 30 minutes at room temperature and absorbance was read at 775 nm on UV-visible spectrophotometer (Shimadzu-1700). Standard curve was prepared by using 100 µg ml^{-1} tannic acid.

Estimation of Flavonoids

Total flavonoids were estimated by colorimetric assay by aluminium chloride method given by Chang *et al.*[10] The plant material was dried and 1 gram of powdered material was extracted with methanol (1:10 w/v). For reaction, 0.5 ml of extract was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate. The final volume of reaction mixture was adjusted to 5 ml with distilled water. It was incubated for 30 minutes at room temperature and the absorbance was read at 415 nm on UV-visible spectrophotometer (Shimadzu-1700). Quercetin solution at the concentration of 12.5 mg ml^{-1} was used for standard curve.

Assay of free radical scavenging activity by DPPH

Determination of the scavenging effect on DPPH was carried out with methanolic extracts prepared from different species. In this method a commercially available and stable free radical DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was used. It is soluble in methanol and produce violet colour. DPPH has absorption maxima at 515 nm or 517 nm when dissolved in methanol (in its radical form.) It gets disappeared on reduction by an antioxidant compound. An aliquot (100 µl) of the extract was added to 100 µl of freshly prepared DPPH solution (39.5 mg ml^{-100} of methanol) and final volume was adjusted to 3.0 ml with methanol. After 30 minutes of incubation at room temperature, the absorbance of a reaction mixture was measured at 515 nm on UV-visible spectrophotometer (Shimadzu-1700). The percent free radical scavenging activity was calculated according to Motalleb *et al.*[11] and compared with L-Ascorbic acid, which was used as standard antioxidant (Aquino *et al.,* 2001).

Radical scavenging activity (%) = [(A_B – A_A)/ A_B] x 100

Where, A_B = Absorbance of blank, A_A = Absorbance of the test solution.

Ferric Reducing Antioxidant Potential (FRAP assay)

The reducing power assay was carried out by Oyaizu method [12] with some modifications. A 0.25 ml aliquot of plant extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50^0C for 20 min. 2.5 ml of 10% TCA was added to the mixture and then it was centrifuged at 2000 g for 10 min. A 5 ml of upper layer was mixed with 5 ml of distilled water and 1 ml of 0.1 % ferric chloride was added. The absorbance was measured at 700 nm with UV-visible spectrophotometer (Shimadzu-1700). A higher absorbance indicated the higher reducing power.

RESULT & DISCUSSION:

In the present investigation the leaves of *Oxalis corniculata* L. were collected. The fresh material was taken for evaluation of enzymatic antioxidants and ascorbic acid content, whereas dry and powdered materials were used for estimation of other non enzymatic antioxidants. The results of the present investigation are tabulated in table 1 and 2. Enzyme antioxidants catalyze the breakdown of radical species usually in the intracellular environment. Preventive antioxidants bind transition metal ions such as iron and copper, preventing their interaction with hydrogen peroxide and superoxide to produce highly reactive hydroxyl radicals.

The antioxidant defense system of an organism comprises of enzymes such as: peroxidase, catalase, SOD and certain endogenous antioxidants like, alpha tocopherol, ascorbic acid and uric acid.[13] PPO and peroxidase are among the most studied enzymes in fruits and vegetables [14] and reports suggest their role in antioxidation. Polyphenol oxidase is probably present in all the plants.[15] The enzyme in the presence of oxygen catalyses the oxidation of phenolic compounds to form corresponding quinine intermediates which polymerize to form undesirable pigments. Shahanaz *et al.* [16] reported increase in ascorbic acid oxidase and polyphenol oxidase activity up to the maturity level that decreases later in *Moringa oleifera* leaves. Ascorbic acid oxidase is a specific enzyme for oxidation of ascorbic acid. Frankenthal[17] as well as Vines and Oberbacher [18] reported the presence of AAO in citrus peel and orange fruits respectively. Thus, *Oxalis corniculata* L. possess considerable amount of all the said enzymatic parameters which are mentioned in the table 1.

Non-enzymatic antioxidants include vitamin-A, C and E, glutathione, phenols, flavonoids, tannins, uric acid, bilirubin,

cysteine, ceruloplasmin etc. Vitamin 'C' (ascorbic acid) is an important water-soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. The results regarding non-enzymatic antioxidants are tabulated below in table 2.

Flavonoids are phenolic compounds, present in several plants, which inhibit lipid peroxidation and lipoxygenases in vitro. The polyphenolic antioxidants act either by trapping the initiating radical, propagating lipid peroxyl radicals, recycling α-tocopherol or deactivating the excited photosentizers etc.[19] Sawadogo *et al.* [20] estimated total phenolic and flavonoid content as well as antioxidant activities of six Acanthaceae members and recommended that three of them as a good source of antioxidants and supported their use in cardiovascular and anti-inflammatory diseases. Similarly Colkesen *et al.*[21] worked on white and red grape berries and advocated them as a potential source antioxidant and anticarcinogenic phenolic compounds. The results of this investigation completely revealed that the amount of non-enzymatic antioxidants is directly correlated with the radical scavenging activity.

Thus, there is an urgent need to identify drugs having antioxidant properties as antioxidant agents either molecules or polyherbal formulations. They have proved efficient in preventing the disturbances in normal homeostasis as well as changes in biochemical balance of the body.

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Table 1: Important enzymatic antioxidants in leaves of *Oxalis corniculata* L.

Plant material	Peroxidase Units gm FW	Polyphenol oxidase Units gm FW	Catalase Units gm FW	Ascorbic acid oxidase Units gm FW
Oxalis corniculata L.	25.80±0.26	16.25±0.11	16.40±0.29	14.75±0.65

Values of mean of five samples and three determinations

Table 2: Important non-enzymatic antioxidants in leaves of Oxalis corniculata L.

Plant material	Ascorbic acid mg/ 100gm	Total Polyphenols mg/ gm	Tannins mg/ gm	Flavonoids mg/ gm
Oxalis corniculata L.	76.35± 0.05	15.76± 0.29	12.36±0.17	79.98± 0.32

Values of mean of five samples and three determinations