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RESEARCH ARTICLE

OXALIS DEBILIS VAR. *CORYMBOSA* (DC.) LOURTEIG AND *OXALIS CORNICULATA* L: A COMPARATIVE STUDY OF NUTRACEUTICAL PROPERTIES

***Aniruddha Sarma, Pranjal Sarmah, Dhruba Kr. Dolai and Pramod Medhi**

Institutional Biotech Hub, Department of Biotechnology, Pandu College, Pandu, Guwahati – 12

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ABSTRACT

The present study was designed to make a comparative study between the antioxidant activity and nutraceutical properties of *Oxalis debilis* and *Oxalis corniculata*. The antioxidant activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and the IC_{50} value of methanolic extract of *Oxalis corniculata* and *Oxalis debilis* were found **25.82 ± 0.68** and **73.67 ± 0.91** µg/ml respectively. Among the nutraceutical properties carbohydrate, protein, crude fibre, total phenolic content and ascorbic acid were investigated. The result shows that *Oxalis debilis* have higher nutraceutical value than *Oxalis corniculata* with the exception of crude fiber content which was found to be higher in case of *O. corniculata*. The results revealed that these two plants can be used as dietary supplement as well as therapeutic agent as they would exert several beneficial effects by virtue of their antioxidant activity.

INTRODUCTION

Herbs have been used as a dietary source of nutrition and play a vital role in improving our health. Various metabolic activities results in the formation of free radicals or reactive oxygen species (ROS) that leads to the onset of many diseases such as cancer, rheumatoid arthritis, liver diseases and atherosclerosis as well as in degenerative processes associated with ageing (Halliwell and Gutteridge, 1999). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau *et al*., 2004). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur and body cell proteins, carbohydrates and lipids are attacked by the free radicals, resulting in various diseases. Natural antioxidants such as vitamin C, E, carotenoids, phenolic compounds, etc. are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress exerted by the reactive oxygen species (ROS) (Mondal *et al*., 2006). It has been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds (Cakir *et al*., 2003 and Velioglu *et al*., 1998) and ascorbic acid content. *Oxalis* is the largest genus in the wood-sorrel family Oxalidaceae having approximately 900 known species. *Oxalis debilis* var. *corymbosa* (DC.) Lourteig and *Oxalis corniculata* L are perennial herbs which flowers from March to September. *O. corniculata* is a sub-tropical herbs being native to India, are commonly known as creeping wood sorrel (Kirtikar and Basu, 1975). This herb is well known for its medicinal value as a good appetizer and as a remover of kapha, vata and piles (Ashwani Kumar *et al*., 2013). Various studies made on *Oxalis corniculata* reveal its wound healing, anticancer (Kathiriya *et al*., 2010), antioxidant and antihyperlipidemic activity (Abhilash *et al*., 2011). On the other hand, *O. debilis*, native to South America, is grown throughout India and found widely in the Brahmaputra Valley region of the North Eastern part of India. Though it is used traditionally for treatment of various ailments such as diarrhea, but there is no such report about the antioxidant activity and nutraceutical properties of *Oxalis debilis*. So, the present study was subjected to evaluate the antioxidant activity and nutraceutical properties to make a comparative study between *O. debilis* and *O. corniculata*.

Corresponding author: Aniruddha Sarma
Institutional Biotech Hub, Department of Biotechnology, Pandu College, Pandu, Guwahati – 12

MATERIALS AND METHODS

The whole plants of *O. corniculata* and *O. debilis* were collected from the foot hills of Nilachal Hill, Guwahati, India [location: 26°11′0″N 91°44′0″E with annual average rainfall 1,717mm, average temperature ranging from 18° to 38°c,

humidity 76.6%] during the month of February to March. The collected samples were washed thoroughly, sliced and oven dried at 60 ^{0}C until completely dried and get constant weight. The dried slices were then powdered and kept at 4 ^{0}C for further analysis. Chemical analysis was done on moisture free basis to estimate the carbohydrate, protein, crude fibre, total phenolic compound, ascorbic acid content and antioxidant activity of the samples.

Total Carbohydrate Estimation

The total carbohydrate content of the samples was estimated by Anthrone method (Hedge and Hofreiter, 1962). To estimate the carbohydrate, 100 mg dried samples were hydrolyzed with 2.5 N HCl for 3 hours in water bath. This acidic solution was neutralized by Sodium carbonate. The extracts were centrifuged and supernatant were collected. The residue was washed thrice with distilled water and all the supernatants were pooled and final volume was adjusted to 100ml. From this, 0.5 ml was taken and volume made up to 1ml by distilled water. 4ml of Anthrone reagent was added to the above solution. Absorbance was taken in a UV-Vis spectrophotometer at 630 nm and the amount of carbohydrate present was calculated by plotting the value in a standard curve of Glucose solution.

Total Protein Estimation

The total protein content of the samples was estimated by following the method (Lowry *et al*., 1951). Extraction was carried out with Tris-EDTA buffer (pH 7.5) used for the enzyme assay. For extraction, 500 mg of the sample was grinded well with a mortar and pestle with 5ml of buffer and centrifuged. The supernatant was used for protein estimation. The reading was taken in a UV-Vis Spectrophotometer at 660nm and the amount of protein present was calculated by plotting the value in a standard curve of Bovine Serum Albumin (BSA).

Crude Fibre Content

Crude fibre in the samples was determined by the method described by Maynard (Maynard, 1970). Defatted sample (2g) was placed in a glass crucible and attached to the extraction unit where 150 ml boiling sulphuric acid (1.25%) solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. Then, 1.25% sodium hydroxide solution (150 ml) was added and again it was digested for 30 min. Thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in desiccators and weighed (W1). Then the sample was ash at 600°C in a muffle furnace (for 2 h, cooled in desiccators and reweighed (W2). Extracted fibre was expressed as percentage of the original undefeated sample and calculated according to the following formula:

$$\%\ \text{crude fiber in ground sample} = \frac{\text{Loss in weight on ignition (W2 - W}_1\text{)}}{\text{Weight of the sample}} \times 100$$

Total Phenol Content Estimation

The total phenol content was determined by the Folin-Ciocalteau's method (Singleton and Rossi, 1965). Powdered plant samples were extracted with 70% methanol and condensed. This extracts were diluted (1 mg/ml concentration) with 70% methanol. From this, 200 µl of the plant extracts was taken and volume made up to 2 ml. 0.3 ml of Folin- Ciocalteau reagent was added. After 5 minutes, 0.8 ml of 20% Na_2CO_3 was added and the final volume was made 5 ml. Absorbance was taken by UV-Vis Spectrophotometer at 765 nm after 30 minutes incubation. The amount of phenol content was determined using Gallic acid as standard. Results were expressed as µg/mg (Gallic acid equivalent/dry weight).

Ascorbic Acid Content Estimation

The amount of ascorbic acid present in the samples was calculated by extracting the sample in 4% oxalic acid and titrating the extract against the 2, 6-dichloro phenol indophenol dye until the end point where pink colour appears that persist for a few minutes (Sadasivam and Theymoli Balasubraminan, 1987). The amount of dye consumed is equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution is used as the reference and the calculation is done by the following formula:

$$\text{Amount of ascorbic acid}\left(\frac{\text{mg}}{100\text{g}}\right)\text{sample} = \frac{0.5 \times V_1\ \text{ml} \times 100\ \text{ml}}{V_2\ \text{ml} \times 5\text{ml} \times \text{weight of the sample}} \times 100$$

Where, V_1= volume of oxalic acid, V_2= volume of the sample.

Antioxidant activity estimation

The antioxidant activities of the herb extracts along with standard were assessed on the basis of the radical scavenging effect of stable DPPH (Nooman A. Khalaf *et al*., 2008). A solution of DPPH (0.2 mM) was prepared in 70% methanol and kept overnight. Stock solution (1 mg/ml) of the extract was prepared in 70% methanol. Various concentration of the extracts *viz.*10, 20, 50,100,150,200,300,400 and 500 µl were taken in different test tubes and the volume was made up to 1000 µl. 1 ml DPPH was added to each solution and kept at dark for 30 minutes. Ascorbic acid and Gallic acid were taken as standards. Optical density of these samples was measured at 517 nm along with blank where 1 ml methanol with 1 ml DPPH solution was taken. The activities of the samples are measured in terms of percent inhibition (IC_{50}) and calculated by the following formulae:

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where, A = Optical density of the blank
B = Optical density of the sample

Statistical Analysis

The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as

Table 1. Phytochemical analysis of *Oxalis corniculata* and *Oxalis debilis**

Sample	Carbohydrate Content (µg/mg)	Protein Content (µg/mg)	Crude Fibre (%)	Ascorbic Acid Content (mg/100gm)	Total Phenol Content (µgGAE/mg)	Inhibition Concentration (IC_{50}) (µg/ml)
O. corniculata	3.81 ± 0.19	0.29±0.006	65.5 ± 0.25	92.20 ± 0.89	32.34 ± 1.36	73.67 ± 0.91
O. debilis	6.40 ± 0.20	0.31±0.005	62.00 ± 0.43	110.75 ± 0.54	52.15 ± 1.54	25.82 ± 0.68

*Values represented in the table are mean±S.D of three replicates.

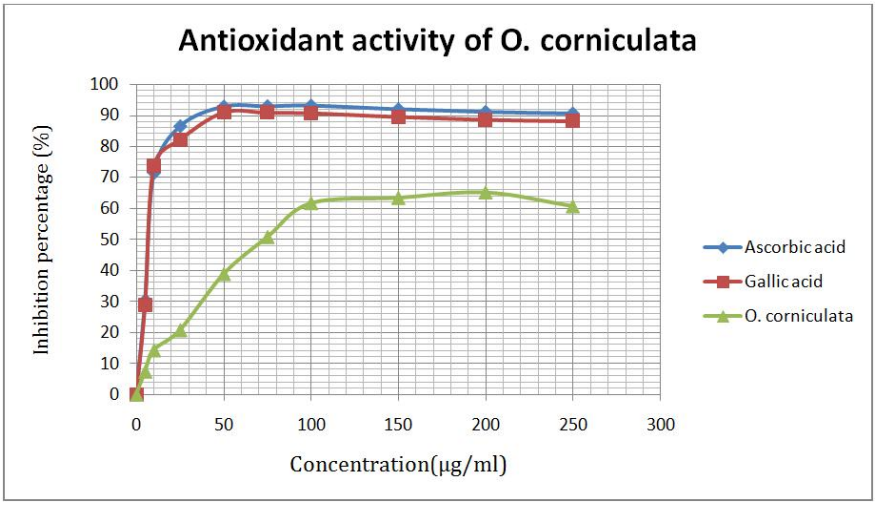

Figure 1. DPPH free radical scavenging activity of methanolic extract of *O. corniculata at* 517 nm

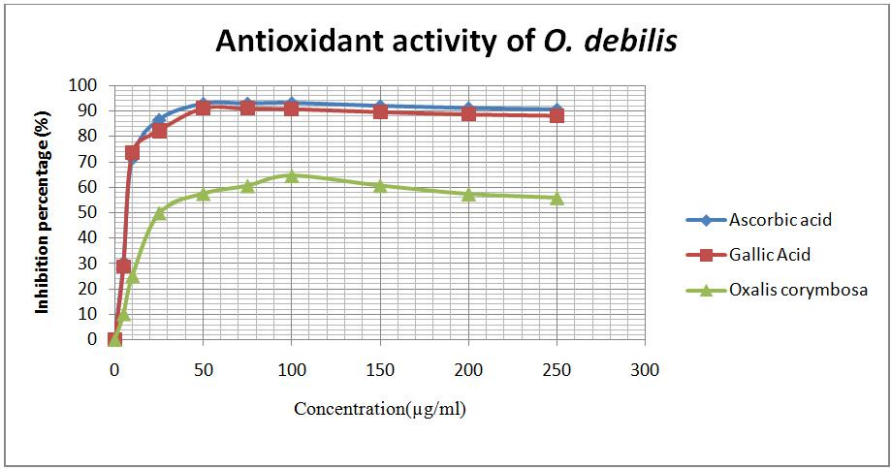

Figure 2. DPPH free radical scavenging activity of methanolic extract of *O. debilis at* 517 nm

mean ± S.D. IC_{50} value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

RESULTS AND DISCUSSION

The phyto-chemical analysis was done for total carbohydrate content, total protein content, crude fibre content, total ascorbic acid content, total phenol content in both extracts of *O. debilis* and *O. corniculata*. In *O. debilis,* the total phenol content was found to be higher (**52.15 ± 1.54** µgGAE/mg) as compared to *O. corniculata* (**32.34 ± 1.36** µgGAE/mg), and also the ascorbic acid content was higher in *O. debilis* (**110.75 ± 0.54** mg/100 gm) as compared to *O. corniculata* (**92.20 ± 0.89** mg/100 gm). The carbohydrate content was higher in *O. debilis* **6.40 ± 0.20** µg/mg than that of *O. corniculata* **3.81 ± 0.19** µg/mg. The protein content of both the herbs was quite similar and was found to be **0.29±0.006** µg/mg and **0.31±0.005** µg/mg respectively for *O. corniculata* and *O. debilis*. The crude fibre content of *O. corniculata* and *O. debilis* was found to be **65.5 ± 0.25** % and **62.00 ± 0.43**% respectively. The antioxidant activity of *O. debilis* (IC_{50}=**25.82 ± 0.68** µg/ml) was much higher as compared to *O. corniculata* (IC_{50}=**73.67 ± 0.91** µg/ml). The most common antioxidants present in herbs and fruits are vitamins C and E, carotenoids, flavonoids and

thiol (SH) compounds, etc. There were several reports that the contribution of phenolic compounds to antioxidant activity was much greater than those of vitamin C and carotenoids (Prior *et al*., 1998; Wang *et al*., 1996 and Luximon-Ramma *et al*., 2003). Phenolic compound plays an important role for enhancement of antioxidant activity of certain medicinal herbs like *Stellaria media, Persicaria chinensis* and *Olenlandia corymbosa* (Sarmah *et al*., 2014 and Sarmah *et al*., 2014) and fruits like *Garcinia cowa* (Sarma *et al*., 2014). The present investigation suggests that the major source of antioxidant capacity of *Oxalis corniculata* and *Oxalis debilis* may be not from vitamin C, but rather from phenolic compounds. The protection in the body provided by medicinal plants against oxidative damage has been attributed to the fact that these foods may provide an optimal mix of phytochemicals, such as natural antioxidants and other bioactive compounds. Therefore, the supplementation of these natural antioxidants through a balanced diet containing adequate herbs could be much more effective than the supplementation of an individual antioxidant such as vitamin C or vitamin E. However, *O. corniculata* is used for traditional medicine or consume by people but no any information available on *O. debilis* used in diet.

Conclusions

Oxalis debilis var. corymbosa (DC.) Lourteig and *Oxalis corniculata* L both species are available in Brahmaputra valley agro-climatic condition. Oxalis corniculata L is used by the people as folk medicine and diet too. But there is no information on traditional used of *Oxalis debilis* as well as in diet. However, our investigation reveals that *Oxalis debilis* is richer in nutraceutical properties. Probably there is reason like anti-nutritional factor or else which to be workout.

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