Evaluation of environment on polyphenols and flavonoids in oxalis corymbosa extracts as a potential source of antioxidants

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Abstract. Oxalis corymbosa is an important medicinal and edible perennial herb that distributes widely in the world. To evaluate the effect of the environment on the antioxidant components in Oxalis corymbosa, this study was performed to evaluate phenolic compounds contents and antioxidant activities of O. corymbosa in different parts and seasons. Total phenolic content (TPC) and total flavonoid content (TFC) of different parts were decreased in four seasons in the following order: shoots > leaves > stems. The highest TPC and TFC were 76.63 ± 4.32 mg GAE/g and 71.22±3.61 mg RE/g in shoots in winter. Notably, the decreased trend of DPPH and FRAP was also in the order of shoots > leaves > stems in four seasons. The supreme antioxidant activities (IC50) of DPPH and FRAP were 0.07±0.00 mg/mL and 0.08±0.00 mg/mL in shoots respectively. A good antioxidant potential of neutralization OH radical was gained in shoots and stems, the averages of IC50 were 2.89 mg/mL and 2.59 mg/mL respectively. The highest protection factor of Ranchman was 1.43±0.01 in leaves, which was first reported in the extracts of Oxalidaceae. Moreover, a strong correlation between TPC, TFC and antioxidant activities indicated that phenolic compounds were the main components responsible for the antioxidant behavior of O. corymbosa. Consequently, the shoots of O. Corymbosa may have good oxidation resistance in winter due to its high contents of TPC and TFC. This study demonstrates that O. corymbosa possessed potent antioxidant properties, and could be a valuable natural source of antioxidants used in both medical and food industries.

1. Introduction

Abundant evidences now suggest that reactive oxygen species (ROS) and free radicals may be a major cause of oxidative damage to biological molecules in the human body, such as DNA, proteins and membrane lipids)[1,2]. To control these reactive free radicals, a number of synthetic antioxidants are available, but they have harmful side effects [3]. Consequently, natural antioxidants from plant extracts are now receiving much attention [4].

The genus Oxalis, which belongs to the wood-sorrel family Oxalidaceae, is a cosmopolitan genus comprising at least 500 species, most of them are native in South America and Southern Africa [5]. Oxalis corniculata has been reported highlighting its diverse ethno medicinal applications like anti-inflammatory, digestive, diuretic, antibacterial, antiseptic etc. [6]. Rats pre-treated with ethanol extract of O. corniculata indicated hepatoprotective and antioxidant potential [6]. O. corniculata fractions in
various solvents showed remarkable ability to scavenge free radicals generated by \( \text{CCl}_4 \) and thus protected the lungs [7]. \( \text{O. corniculata} \) also showed excellent activities against \( \text{Escherichia coli, Sheila dysenteries, Salmonella tophi, and Bacillus subtiles} \) [8]. \( \text{Oxalis tuberose} \) contains dietary fiber and phenolic compounds and therefore produces effects that contribute to the intestinal health of the experimental animals [9]. Token et al. [10] reported that the extracts of \( \text{Oxalis corymbs} \) except root possessed relatively higher antioxidant capacity and total phenolic content (TPC) in the study of fifteen plants. However, no studies have been conducted to investigate phenolic compounds and antioxidant activities of genus \( \text{Oxalis} \) in different parts and seasons.

\( \text{O. corymbs, a medicinal and edible perennial herb} \) [10], which was introduced into China as an ornamental plant in the middle of 19th century [11], is now widely distributed and abundantly found in agricultural farms, gardens and lawns. The aim of this study was to evaluate the potential antioxidant effectiveness of \( \text{O. corymbs} \) in different parts and seasons by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical (OH) scavenging activity tests, ferric reducing antioxidant power (FRAP) and oxidative stability (Ranchman). Oxidative stability experiments are first applied to analyze antioxidant activities of the extracts of oxalidaceae. This study can help us know the harvest part and season to maximize TPC and total flavonoid assay (TFC) and thus make a make an optimal application of these potential natural antioxidants. Consequently, this paper has great significances for the development and utilization of \( \text{O. corymbs} \) as potential natural antioxidants and research on phenolic compounds of the plants.

2. Methods and Materials

2.1. Chemicals and Reagents
\( \text{Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH)} \) were purchased from China Reagent Co. (Shanghai, China). \( \text{Gallic acid, ruin and Buty lated Hydroxytoluene (BHT)} \) were purchased from Sigma-Aldrich (Shanghai, China). All other solvents and chemicals used were of analytical grade.

2.2. Sample collection and extraction
The plant was collected in four seasons (in January, April, July and October) in the year of 2014 from Baoshan Campus of Shanghai University, China. Each sample was collected about 1kg one time from three places. The plant was manually separated by the shoot, stem and leaf, and then cleaned, dried in an oven at 50°C until the water was evaporated. After that the dried plant specimen material was ground using grinder (Q-100A3, China) to obtain a fine powder (about 150 µm).

The powdered material (1 g) was soaked in 50 mL of 70% ethanol (v/v) at 70°C for 1.5 h, and shaken thoroughly [12]. After centrifuging at 5000 g for 20 min, and the extract solution was evaporated under reduced pressure with rotary evaporator to obtain crude extract. The crude extract was resuspended in 10 mL methanol for further analysis.

2.3. Total phenolic assay
Total phenolic content (TPC) was determined using the Fooling Ciocalteau spectrophotometric method described by Singleton, et al.[13]and Moraes-De-Souza et al. [14]. Briefly, 0.1 mL sample appropriately diluted was transferred to a tube with 1.5 mL of Fooling Ciocalteau reagent. The mixture was left standing for 3 min, then 2 mL of 10 % (w/v) sodium carbonate was added, and kept in the dark for 1 h. afterwards, the absorbance was measured at 765 nm using a MAPADA UV-1600PC ultraviolet-visible spectrophotometer (Shanghai, China). The standard curve was prepared with Gal lic acid as a standard following the same method. The results were expressed as mg of Gallic acid per g of dried mass obtained from the following equation (1).

Gallic Acid Equivalent (GAE) \( = \frac{\text{[Absorbance (765 nm) / 0.1194] - 0.3183}}{R^2 = 0.9968. \quad (1)} \)
2.4. Total flavonoid assay
Total flavonoid assay (TFC) in the samples was determined by modifying the method of Park et al. [15]. In each experiment, 0.1 mL sample appropriately diluted was transferred to a tube with 0.3 mL of 5% (w/v) sodium nitrite and 0.3 mL of 10% (w/v) aluminium chloride, and then mixed thoroughly. After 5 min, 4 mL of 4% (w/v) sodium hydroxide was also added. The absorbance was determined 10 min later at 510 nm against a blank. The standard curve was plotted using ruin as a standard following the same method. The results were expressed as mg of ruin per g of dried mass as obtained from the following equation (2).

Ruin Equivalent (RE) = \[ \frac{\text{Absorbance (510 nm)}}{0.0109} \] + 0.6881, \( R^2 = 0.998. \) (2)

2.5. DPPH free radical scavenging assay
DPPH (2, 2-diphenyl-1-picryl-hydrazine) assay was performed as described by Shi et al. [16] and Sreeramulu et al. [17]. In this method, 2.5 mL of 100 µM DPPH/ethanol solution and 0.5 mL sample appropriately diluted were mixed. The mixture was incubated for 30 min in the dark and absorbance was measured at 517 nm. The blank contained all reagents except the sample. The synthetic antioxidants mutilated hydroxytoluene (BHT) was used as a positive control. Antioxidant activity was expressed as an inhibition percent of DPPH radical and calculated from the following equation (3).

The inhibition (%) = \[ \frac{\text{Abscontrol}-\text{Abssample}}{\text{Abscontrol}} \] ×100. (3)

2.6. OH free radical scavenging assay
Scavenging capacity of the extract for hydroxyl radical was determined by modifying the method of Cos et al. [18]. 1 mL of 9 mm ferrous sulfate, 1 mL of 9 mm salicylic acid/ethanol solution and 0.5 mL sample appropriately diluted were mixed. Then, 1 mL of 8.8 mm hydrogen peroxide solution was added and the mixture was incubated for 30 min at room temperature. The absorbance was measured at 510 nm. The synthetic antioxidants mutilated hydroxytoluene (BHT) was used as a positive control. Antioxidant activity was expressed as an inhibition percent of OH radical and calculated from the equation (4).

The inhibition (%) = \[ \frac{\text{Abscontrol}-\text{Abssample}}{\text{Abscontrol}} \] ×100. (4)

2.7. Ferric reducing antioxidant power (FRAP) assay
To determine the antioxidant activity by iron reduction, using the ferric reducing antioxidant power (FRAP) assay, was followed the methodology described by Benzie et al., [19] with some modifications. FRAP measures the ferric (Fe3+) form is reduced to the ferrous (Fe2+) form. FRAP reagent was prepared immediately before analysis by mixing 2.5 mL phosphate buffer (0.2 M, pH 6.6), 2.5 mL potassium ferricyanide (1 %, w/v), 2.5 mL trichloroacetic acid (10 %, w/v) and 0.5 mL ferric chloride (0.1 %, w/v). 0.5 ML sample appropriately diluted was added to 2.5 mL FRAP reagent and incubated in a water bath at 50°C for 20 min. After that, the absorbance was measured at 700 nm. The concentration of samples required to reduce the ferric to ferrous by 50% is expressed as mg/ milk

2.8. Oxidative stability—Ranchman
The Ranchman was performed using a modified and validated method [20]. 3 g pure lard oil (without added antioxidants) was mixed with the solid extract obtained by freeze drying (Free Zone 6L, USA) at a final concentration of 0.1% (w/v). The oxidative stability index of this mixture was measured by the Ranchman method at 100±1°C and a flow rate of 20 L/h of dry air, using 743 Ranchman (Met Rohm AG, CH-9100 Hernias, and Switzerland). The conductivity increase due to the accumulation of oxidized compounds and allowed the construction of the curve, calculation of the induction period (IP). A blank control was prepared with pure lard oil without added antioxidants. Synthetic antioxidant BHT (0.1%, w/v) was added to the sample as a positive control. The protection factor (PF) was calculated as: \( \text{PF} = \frac{\text{Pla}}{\text{Plc}} \), where \( \text{PF} \) = protection factor; \( \text{Pla} \) = IP of the oil with the extracts or BHT; \( \text{Plc} \) = IP of the blank control.
2.9. Statistical analysis
All experiments were conducted in triplicate and the mean values were calculated. The results obtained were statistically analyzed and correlated using SPSS for Windows (version rel. 10.0.5, 1999, SPSS Inc., Chicago, IL, USA). Statistical significance is declared at P < 0.05.

3. Results and discussion

3.1. Determination of TPC and TFC
TPC and TFC varied greatly depending on plant parts and seasons (Figure 1). TPC and TFC in shoots were remarkably higher than those in stems and leaves in four seasons. The decreased trend was in the order of shoots > leaves > stems. The reason may lie in several factors such as temperature, water availability, soil nutrients, and pollution, and pathogen attack can affect the content of secondary metabolites in plants, such as phenolic compounds [21].

TPC and TFC were maximum in shoots in winter. The levels were 76.63±4.32 mg GAE/g and 71.22±3.61 mg RE/g respectively, which were 2.44 and 1.45 times of those in shoots in spring. TPC in shoots were varied with seasons in the range of 31.45 and 76.63 mg GAE/g, which was as much as that reported for O. corniculata extracted by hexane (28.23 mg GAE/g) or n-Bough (70.05 mg GAE/g) [22]. The average TFC in shoots were 61.18 mg RE/g, which was within 53.52 mg RE/g (extracted by H2O) and 98.82 mg RE/g (extracted by n-Bough) reported for O. corniculata [22].

TPC was almost unchanged in stems and leaves during four seasons, and their means were 4.20 mg GAE/g and 8.74 mg GAE/g, respectively. TFC in stems changed a little, while it increased significantly in leaves in winter.

![Figure 1 A](image1.png)

![Figure 1 B](image2.png)

**Figure 1.** TPC (A) and TFC (B) of O. corymb extract in different parts and seasons. A: TPC (mg GAE/g), B: TFC (mg RE/g)

3.2. Antioxidant activity assays
Although in recent years the antioxidant analysis of plants has been extensively studied worldwide [23-27], very few studies have been carried out to assess antioxidant activities of O. corymb in different parts. The antioxidant activities of O. corymb extracts have been evaluated in a series of in vitro tests (Figure 2).

All of the assessed extracts of O. corymb were able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H form with IC50 (50% of reduction) values determined. No matter in which season, DPPH radical scavenging activity of plant parts was decreased in the order of shoots > leaves > stems (Figure 2A). Compared with 0.02% BHT (IC50=0.04 mg/mL), the DPPH radical scavenging activity of O. corymb showed that the lowest IC50 value was 0.07 mg/mL in
shoots, which was in agreement with the data reported by Sonja et al. [28] Trifolium pretense (IC50=0.07 mg/mL), but less than 0.30 mg/mL that for methanol extracts of O. pes-caprae[29]. Additionally, DPPH radical scavenging activity of stems and leaves showed a tendency to rise depending on seasons.

The ferric reducing antioxidant power (FRAP) of O. corymbs was comparable to that of standard 0.02% BHT (IC50=0.01 mg/mL). Figure 2B showed that the highest ability to reduce Fe3+ to Fe2+ was found in shoots in winter (IC50=0.08 mg/mL), which was consistent with the study of Fernando and Soya [30] for Atlanta cyanic (IC50=87.70 µg/mL) and O. corniculata (IC50=71.98 µg/mL). The ferric reducing activity of shoots (the average of IC50=0.12 mg/mL) was 13 folds of that for compared to stems (the average IC50=1.58 mg/mL). Interestingly, the FRAP trend among different parts was similar to that of DPPH radical scavenging activity, which decreased in the following order: shoots > leaves > stems. Figure 1 showed that TPC and TFC were decreased in the order of shoots > leaves > stems, which was consistent with the order of DPPH and FRAP assays. In general, the DPPH and FRAP values showed parallel behavior with the TPC and TFC, suggesting that phenolic and flavonoid may play a possible role as potential antioxidants, which were also reported by Biljana et al.[31] .

The cellular damage resulting from hydroxyl radical is the strongest among free radicals. Hydroxyl radical can be generated by biochemical reaction. The OH radical scavenging activity of O. corymbs was compared with that of standard 0.02% BHT (IC50=0.10 mg/mL). Figure 2C showed that a good antioxidant potential by neutralizing OH radical were achieved by shoots and stems. The average IC50 in shoots and stems are 2.89 mg/mL and 2.59 mg/mL respectively. The smallest effect on the OH radical neutralization was shown by leaves in winter (IC50=8.45 mg/mL), which was higher than that reported by Biljana et al.[31] for Trifolium pretense (IC50=231.49 µg/mL).

The Ranchman method is widely used for the determination of the oxidative stability of natural fats and oils. The induction period is characterized by the change in conductivity of deionized water due to oxidation-generated products. The process is carried out under high temperatures and constant aeration. The protection factor (PF) of O. corymbs was compared with that of standard 0.02% BHT (PF=7.21) and all O. corymbs samples had a longer induction period (PF between 1.2 and 1.6), with a protection factor and consequent antioxidant activity (Figure 2D). The highest PF was obtained in leaf in summer (PF=1.43), which was higher than that reported by Tiveron et al.[20] for watercress (PF=1.29), broccoli (PF=1.29) and chives (PF=1.24). Besides, the lowest PF was detected in shoot, which was opposite to the results of DPPH and FRAP assays. Compared with other three antioxidant activity assays, the Ranchman method for the same plant parts in four seasons showed no huge changes, this may be due to some fixed substances.

In summary, this study suggests that the harvest part and season are crucial to determine the antioxidant potential of O. corymbs.
3.3. Relationships between phenolic compounds contents and different antioxidant activities

As shown in Table 1, there was a good correlation between DPPH and FRAP values, while DPPH with other antioxidant assays did not present the same correlation. The overall trend was the same as reported by Tiveron et al. [20]. The similar variation trend in Figure 2A (DPPH) and 2B (FRAP) also predicated the best correlations existed between DPPH and FRAP.

Significant negative correlations were observed between TPC, TFC and IC50 values for DPPH and FRAP, indicating significant contribution of phenolic acids and flavonoid to these antioxidant assays (Table 2). Erkan et al. [32] Reported a strong correlation between radical scavenging activity and TPC of extract from various natural sources. Besides, the protection factor in Ranchman demonstrated a remarkable positive correlation with both TPC and TFC.
Table 1. Pearson’s correlation coefficient between different antioxidant activity assays. One asterisk represent statistical significance at P<0.05.

| Parameters | Correlation (R) |
|------------|-----------------|
|            | DPPH  | OH  | FRAP | Ranchman |
| DPPH       | 1     | -0.309 | 0.989* | 0.364    |
| OH         | 1     | -0.246 | 0.671 |
| FRAP       | 1     | 0.416   |
| Ranchman   | 1     |

Table 2. Pearson’s correlation coefficient between different parameters. One asterisk represent statistical significance at P<0.05.

| Parameters | Correlation (R) |
|------------|-----------------|
|            | TPC  | TFC |
| DPPH (IC50) | -0.687* | -0.847* |
| OH (IC50)  | -0.332 | -0.153 |
| FRAP (IC50) | -0.735* | -0.883* |
| Ranchman   | 0.742* | 0.717* |

4. Conclusions
Phenolic compounds contents and antioxidant activities of O. corymba in different plant part and season have been documented. TPC and TFC were decreased in four seasons in the following order: shoots > leaves > stems. The ethanol extracts of shoots exhibited the supreme antioxidant activities of DPPH and FRAP compared with that of stems and leaves. A good antioxidant potential of neutralizing OH radical was shown by the shoots and stems. On the contrary, the highest protection factor of Rancimat was shown by the leaves. A strong correlation between TPC, TFC and antioxidant activities indicates that phenolic compounds are probably the main components responsible for the antioxidant behavior of O. corymbosa. The results imply that the shoots of O. corymbosa are a potential natural source of phenolic compounds and antioxidants. The extracts of O. corymbosa could be used in both medical and food industries. Methods of analyzing seasonal and plant parts variation could be used in investigating choosing other natural source of antioxidants.

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