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Permanganate Reducing Antioxidant Capacity Assay of Methanolic Extract *Oxalis corniculata*

Pushpa Prakash Durgawale[1]

Rohan Sharadanand Phatak[2*]

Anup Subhash Hendre[1]

[1]Department of Biochemistry, Krishna Institute of Medical Sciences, Karad-415110, Maharashtra, India
[2]Directorate of Research, Krishna Institute of Medical Sciences University, Karad-415110, Maharashtra, India

Corresponding Authors:
Mr. Rohan Sharadanand Phatak,
Junior Research Officer, Directorate of Research, Krishna Institute of Medical Sciences University, Karad-415110, Maharashtra, India.
Email: phatak.rohan1983@gmail.com

Abstract:
Oxalis corniculata L. is well known as procumbent yellow sorrel or creeping wood sorrel grown as weeds in the tropical countries. A wide range of active constituents with potent medicinal properties present in the plant. The main objective was to determine oxalic acid content by using standard synthetic oxalic acid and to overview the total antioxidant capacity of the *Oxalis corniculata.* In PRAC assay, antioxidant activity of *Oxalis corniculata* has been increased with increasing concentration and expressed in terms of percent inhibition. It was found to be significant and valuable.

Keywords: Antioxidant activity, PRAC, *Oxalis corniculata*

Introduction:

In most of plants, oxalic acid is biosynthesized from ascorbic acid, glycolate and glyoxylate. Oxalic acid may lead to significant loss of minerals when it is consumed in large content of oxalate rich foods [(1)]. Free oxalic acid / oxalate bind with calcium ions in the body precipitate as insoluble calcium oxalate crystals and may lead to hypocalcaemia and urolithiasis [(2)]. Generally kidney stones are comprised of high concentration of calcium oxalate with following small amount of calcium carbonate[(3)], calcium phosphate[(1)]. Oxalic acid is somewhat present in few amounts in the plants. In case of *Oxalis corniculata L.* is rich in oxalic acid content in leaves. This herb is well known as procumbent yellow sorrel or creeping wood sorrel belonging to family Oxalidaceae. Its distribution is high throughout worldwide. Its leaves are edible having a tangy taste [(4)].

In the present study, efforts have been carried out to investigate total oxalic acid content in this plant extract spectrophotometrically with reference to standard oxalic acid. Analysis of oxalic acid determination was performed on basis of the reaction mechanism between permanganate and oxalate.

Permanganate Reducing Antioxidant Capacity (PRAC) assay was used to evaluate total antioxidant capacity of sample. It involves in the reaction mechanism between permanganate and oxalate.

Reaction mechanism:

In the PRAC assay, a systematic and chemically acceptable decomposition of the overall autocatalytic reaction is shown below [(5)]:

$$2MnO^{-}_{4} + 6H^{+} + 5H_2C_2O_4 \rightarrow 2Mn^{+}_{2} + 8H_2O + 10CO_2 \quad (1)$$

Potassium permanganate, $KMnO_4$ is a strong oxidizing agent. Permanganate, MnO_4^- is an intense dark purple color. Reduction of purple permanganate ion to the colorless Mn^{+2} ions, the solution will turn from dark purple to a faint pink colour. No additional indicator is therefore needed. The reduction of permanganate requires strong acidic conditions. In the assay, permanganate will be reduced by oxalate, C_2O_4 in acidic conditions. Oxalate reacts very slowly at room temperature.

Material and Methods:

Chemicals:

Folin & Ciocateu's phenol reagent, sodium carbonate, ammonium acetate, aluminum chloride, potassium permanganate ($KMnO_4$), oxalic acid, gallic acid, rutin and ascorbic acid were purchased from Loba chemicals Ltd.

Methanolic Extraction:

Whole plant of *Oxalis corniculata* was uprooted and washed with tap water several times and put into a conical flask. Sufficient volume of methanol was added to the conical flask and covered with a cotton plug on the mouth of conical flask. It was kept in maceration for 7 days at 30°C in order to maximize the extraction. After 7 days it was filtered through Whatmann filter paper and transferred to a suitable container and kept for analysis.

Determination of A_{50} of methanolic extract of *Oxalis corniculata* in PRAC assay:

A calibration curve was determined by preparing a serial dilution of six solutions of potassium permanganate and registering the absorbance for each of them.

In order to quantitatively compare the antioxidant activities, we proposed the following formula:

A_{50} = t (std)/t (plant) × c (std)/m (plant) × V (std)/V (plant sample) × V (extract) / 100

where, *A*50 is antioxidant activity expressed, reflected in the time until the sample induces a decrease of the potassium permanganate concentration up to one half, compared against a ascorbic acid (mMol equivalent standard/g plant), t(sample) is the time until the sample induces a decrease of the permanganate concentration up to one half (min), t(std) is the time until ascorbic acid induces a decrease of the permanganate concentration up to one half (min), c(standard) is ascorbic acid concentration (mMol/ml) {0.01mmol/ml}, m(plant) is weight (g) of the plant sample submitted to extraction {4g}, V(plant sample) is volume of the plant extract submitted to the analysis {0.2 ml}, V(standard) is volume of the standard submitted to the analysis {1ml}, and V(extract) is volume (ml) of the obtained extract {40 ml}.

PRAC assay of Methanolic extract of *Oxalis corniculata*

Standard calibration curve of oxalic acid

0.1mg Oxalic acid was dissolved in 100ml of distilled water. A series of five solutions of oxalic acid in range of 100-900µl was transferred to test tubes containing 900-100µl of distilled water. 350µl of 0.2M H_2SO_4 was added to test tube.150µl of $KMnO_4$ was added individually and allowed to take reaction for few minutes. A calibration curve was determined by registering the absorbance at 530nm for each of them.

The redox reaction is involved between antioxidant sample and potassium permanganate in acidic media, leading to sample discoloration until no colour was observed.

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Methanolic extract of *Oxalis corniculata* in different concentration ranging from 100µl to 900µl was introduced in test tubes containing an oxidative mixture of 0.15ml 0.1mM potassium permanganate; 0.5ml 2M sulfuric acid, and 2ml distilled water. Samples were screened immediately against 530 nm after addition. Subsequent decrease of potassium permanganate concentration was observed. Oxalic acid was used as positive standard.

The contents of oxalic acid in *Oxalis corniculata* extract were expressed as oxalic acid equivalents (OAEs). All assays were conducted in triplicate and its mean was calculated. Percent Inhibition of PRAC = A_o - A/ A_o x100 where, A_o is the absorbance taken immediately after addition of permanganate solution to extract and A is the concentration of extract after 10 min.

Statistical analysis:

All experiments were performed in triplicates and the results were expressed as mean ± standard deviation. Data was analyzed using student't' test for two sets while one-way analysis of variance (ANOVA) for more than two sets. Significant differences were considered when means of compared sets differed at $P < 0.05$. Data was carried out using SPSS v.16.0 (Statistical Program for Social Sciences) software.

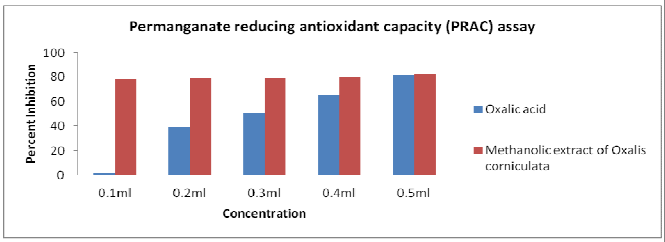
Results:

A_{50} of methanolic extract of *Oxalis corniculata*:

The result from calculation of the given formula was obtained as A_{50} of MEOC=0.006.

Permanganate reducing antioxidant capacity (PRAC) assay:

Methanolic extract of *Oxalis corniculata* increases antioxidant capacity with increasing concentration in terms of percent inhibition. Standard oxalic acid increases gradually with increasing concentration in a dose dependent manner.



Discussion:

Cacig *et al* [6] have firstly introduced PRAC method as a simple spectrophotometric method for evaluation of antioxidant capacity. It was observed colour change from dark pink to orange red in the initial reaction. Increasing concentration of plant extract has shown decrease in absorbance due to presence of oxalic acid. However after first phase initiation of reaction, it was monitored increase in absorbance with increasing concentration of plant extract. This indicates that formation of MnO_2 particles induces increase false absorption in the short term due to the light diffusion [6].

Permanganate reducing antioxidant capacity is a novel antioxidant assay:

It provides the assessment of antioxidant capacity based on the photocatalytic degradation of potassium permanganate in acidic medium. It measures in the reduction of Mn (VII) to Mn (II) or Mn (IV) depending on the potency of antioxidant sample [6]. It involves in the redox reaction between antioxidant sample and potassium permanganate in acidic media which leads to change in colour until disappearance. Discolouration in permanganate solution indicates the increased reducing power present in antioxidant. In contrast of antioxidant assays like

FRAP, CUPRAC and PMA show the increased in absorbance in analogous with increasing concentration of antioxidant sample in a dose dependent manner [7]. PRAC shows diminished absorbance with corresponding to increasing concentration of antioxidant sample resembling to free scavenging activities like DPPH, ABTS. In most of free radical scavenging activities, it involves in quenching free radicals released by oxidant therefore capacity in free radical scavenging activities is expressed in terms of percent inhibition. PRAC has unique trait by its results are expressed in terms of percent inhibition unlike to these antioxidant assays. Acidic potassium permanganate by chemiluminescence reaction has been reported as a quick chemical test to estimate total antioxidant status of wines, fruit juices and teas with help of chemiluminescence detector [8].

Conclusion:

This study may be useful to screen the total oxalic content in plants and foods in terms of oxalic acid equivalent (OAE). It would initiate to determine higher levels of oxalate in foods. The approach based on acidic potassium permanganate may be a great potential for rapid screening, evaluation and characterization of the total antioxidant capacity of constituents in whole foods, phytochemicals in plants, bioactive compounds. On the basis of results obtained from PRAC assays, the methanolic extract of *Oxalis corniculata* has shown a significant total antioxidant capacity. Comparative studies between PRAC and other antioxidant activity of methanolic extract of *Oxalis corniculata* should be further investigated.

References:

1) Noonan SC, Savage GP. Oxalate content of foods and its effect on humans. *Asia Pacific J Clin Nutr* 1998; 8(1): 64-74.
2) Huges J, Norman RW. Diet and calcium stones. *Can Med Asso J* 1992; 146: 137-143.
3) Durgawale P, Shariff A, Hendre A, Patil S, Sontakke A. Chemical analysis of stones and its significance in urolithiasis. *Biomedical Research* 2010; 21(3):305-310.
4) Ram Avatar Sharma, Aruna Kumari. Phytochemistry, Pharmacology and therapeutic application of *Oxalis corniculata* linn. - A Review. *Int J Pharm Pharm Sci* 2014; 6(3):6-12.
5) Krisztia´n Kova´cs, Be´la Vizva´ri, Miklo´s Riede, Ja´nos To´th. Decomposition of the permanganate/oxalic acid overall reaction to elementary steps based on integer programming theory. *Phys Chem Chem Phys* 2004, 6: 1236-1242.
6) Cacig SI and Szabo MI. Spectrophotometric method forthe study of the antioxidant activity applied on Ziziphus jujoba and Hydrangea paniculata aqueous extracts. (Zbornik Maticesrpske za prirodne nauke) Proceedings for Natural Sciences, Matica Srpska, Novi Sad, Serbia, 2006: 87–93.
7) Phatak RS, Hendre AS. Total antioxidant capacity (TAC) of fresh leaves of *Kalanchoe pinnata*. *Journal of Pharmacognosy and Phytochemistry* 2014; 2 (5): 32-35.
8) Conlan XA, Stupka N, McDermott GP, Barnettb NW, Francis PS. Correlation between acidic potassium permanganate chemiluminescence and in vitro cell culture assay: Physiologically meaningful antioxidant activity. *Anal Methods* 2010; 2: 171-173.

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