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Determination of seasonal and developmental variation in oxalate content of *Anagallis arvensis* plant by titration and spectrophotometric method

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Abstract

Oxalates are one of the important toxicant from veterinary toxicology aspect as they are mostly related to nephropathy and kidney failure. The reasons behind these clinical signs are accumulation of oxalate crystals in the filtration units of body and damaging renal tubular epithelium. In the present study, oxalate content of mature and immature plants was estimated by 2 methods; permanganate titration and spectrophotometric method. The quantitative precipitation of oxalate as its calcium salt, followed by permanganate titration was done. This method presents little difficulty in aqueous solutions, and quantities of oxalic acid as low as 0.05 mg may be estimated with accuracy. With this standard titration method, the estimated oxalic acid content was 14.79% for mature plant parts (month of March) and 12.45% for immature (month of November) plant parts. Though it was most accurate process but time consuming, so spectrophotometric method was tried for evaluating the efficacy. In this method 0.5g of plant material was taken and the concentrations of oxalate were estimated in the plant extract and it was found to be 15.06% and 12.68% for mature and immature plant parts respectively. From this study it can be concluded that mature plant parts contains more oxalate than immature plant parts. Also regarding estimation methods, spectrophotometric method is better method than traditional titration method in the modern research field with little difference in accuracy but more effective.

Keywords: Oxalate, *Anagallis arvensis*, hypocalcaemia, spectrophotometric, seasonal variation

1. Introduction

Oxalic acid ($C_2H_2O_4 \cdot 2H_2O$; Mol. wt.: 126.07) is a dicarboxylic acid ($HOOC-COOH$), one of the strong organic acids. Oxalate is one of the important anti-nutritional factors present in different plants and their parts. Oxalic acid present in two forms: soluble oxalate usually forms with monovalent counter ions such as sodium (Na^+), potassium (K^+) and ammonium (NH_4^+), whereas insoluble oxalate forms with divalent calcium (Ca^{2+}), magnesium (Mg^{2+}) and iron (Fe^{2+}) ions [1]. Generally monovalent salts of oxalic acid are more water soluble or easily ionisable than divalent salts [2, 3, 4]. Ingested oxalate form insoluble complexes with dietary calcium (Ca). This leads to disturbances in calcium and phosphorus (P) metabolism involving excessive mobilization of bone mineral. The demineralised bones become fibrotic and misshapen, causing lameness and "bighead" in horses [5]. Ruminants are less affected, but prolonged grazing by cattle and sheep on some tropical grass species can result in severe hypocalcaemia [6]. The maximum permissible level of oxalate in human is 40-50 mg/day to avoid kidney stone and in ruminants it is < 2% [7]. Consumption of plants with high oxalate content has led to poisoning and death of livestock. High-oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people and may affect calcium absorption [8]. When a ruminant consumes an oxalate-containing plant, the oxalate is metabolized in four possible ways. First, soluble oxalate may be degraded by rumen microflora [9]. Second, soluble oxalate may combine chemically with Ca to become insoluble calcium oxalate, which is excreted in faeces, reducing absorption of Ca. Third, soluble oxalate may be absorbed from the rumen into the blood stream where it may combine with serum Ca to form insoluble oxalate crystals [10]. These crystals may then precipitate in the kidneys and can cause kidney failure [11]. Fourth, ingested insoluble oxalate from plants may pass through the digestive tract without a harmful effect on the body's metabolism [12].

In the present world, there are almost four lakhs (3,90,800) plant species available. Some of them were taken as food for human and forage or fodder for animals.

All the consumable plant's phytochemical and anti-nutritional properties have not been determined yet. Among all the plants, oxalic acid is synthesized by a wide range of plants. A few of them are forage plants that can cause oxalate poisoning in ruminants under certain conditions [13]. Very high oxalate-containing plants (more than 5% by dry weight) belong to the three families Amaranthaceae, Chenopodiaceae, and Polygonaceae [14]. In the present study, *Anagallis arvensis* [Family - Myrsinaceae] commonly called scarlet pimpernel was taken into consideration (Fig. 1). It is widely distributed all over the world but it is native to Europe. It generally grows as a weed in damp soil, gardens, waste places and in semi-exposed sites, in temperate regions particularly in the month of September-October and attains maturity by March-April in Indian condition. Experimental feeding of the plant material to various animals, such as horses and dogs, caused gastroenteritis [15]. Sufficiently high doses proved fatal [16]. In India it is commonly observed in turmeric field, particularly in marathwada region of Maharashtra (our study location). This plant contains different poisonous active principles like glycosides, volatile oil, saponin (anagallin), tannin, and oxalates in their edible parts. *Anagallis arvensis* had caused poisoning in sheep in Australia on various occasions [17] and its toxicity had confirmed by experiment [18]. Four cases of *Anagallis arvensis* poisoning were diagnosed in the Department of Sysandu, Uruguay during December, 1994 and January, 1995, in barely and stubble fields. Cattle of different ages were affected. Morbidity was 7-30% and case fatality was 50-86% [19]. So, the objective was decided to estimate the oxalate content of whole plant.

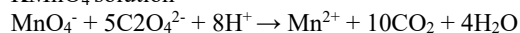
2. Materials and Methods

Whole plant parts of *Anagallis arvensis* were collected in the early morning hours from the turmeric fields of different region of Marathawada area and were kept at room temperature for 15 days to reduce the water content without exposing to direct sun light. During mature plant sample collection, flowering stage of the plants were chosen and same method of preservation were followed before estimation of oxalate content.

2.1 Determination of oxalate content by titration method:

Principle:

Oxalate ions are extracted from the plant parts by boiling them with dilute H₂SO₄ (0.5N). Then oxalate concentration estimated volumetrically by titrating the extract with standard KMnO₄ solution



Preparation of 0.5 N H₂SO₄: (500 ml) [S.D Fine Chemicals, Mumbai]

Concentrated H₂SO₄ was taken with purity of 98% (specific gravity= 1.84 g/lit). So 6.8 ml H₂SO₄ was added to distilled water to make 0.5 N H₂SO₄ by making the final volume 500 ml.

0.05 N KMnO₄ (100 ml) [L.R, Thomas Baker, Mumbai]

Molecular weight of KMnO₄ is 158 g. So 0.158 g KMnO₄ was added to 100 ml water for preparing 0.05 N KMnO₄.

Preparation of plant extract:

1 g of plant material was weighed in electric weighing balance and transferred to 30 ml of 0.5 N H₂SO₄ and was boiled in a water bath for 15 minutes. Then the extract was filtered with Whatman's No. 1 filter paper. Equal volume of deionised water was added. Then 10ml of filtered extract was taken and 40 ml 0.5 N H₂SO₄ was added. Final 50 ml of mixture was heated to 60°C and was titrated against 0.05 N KMnO₄. The end point was determined by permanent appearance of light pink colour.

2.2 Determination of oxalate content by spectrophotometric method

Collected plant samples were processed for estimation of oxalate content by spectrophotometric method described by Naik *et al.* [20] and the procedure was as follows:

Preparation of standard oxalic acid solution: (1mg/ml)

A standard solution of oxalic acid was prepared by dissolving 100 mg of oxalic acid (C₂H₂O₄.2H₂O; Mol. wt.: 126.07, Sara Fine Chemicals, Baroda-2) in distilled water and diluted to 100 ml with distilled water.

Working solution:

From the standard Oxalic acid solution, 0.1, 0.2, 0.3 and so on upto 1mg/ml concentration solution were prepared by proper dilution.

0.003 M KMnO₄ (100 ml) [L.R, Thomas Baker, Mumbai]

Molecular weight of KMnO₄ is 158. So 0.048 g KMnO₄ was added to 100 ml water for the preparation of 0.003 M KMnO₄.

2 N H₂SO₄ (500 ml) [S.D Fine Chemicals, Mumbai]

Concentrated H₂SO₄ was taken with purity of 98% (specific gravity= 1.84 g/lit). So 27.2 ml H₂SO₄ was added to 500 ml dist. Water to make 2 N H₂SO₄.

Preparation of graded standard solution for reading

A series of standard solutions of oxalic acid containing 0.1, 0.2, 0.3 and so on upto 1 mg/ml conc. were mixed with 5ml of 2 N H₂SO₄ and 2ml of 0.003 M KMnO₄ in separate test tubes and incubated at 37°C for 10 minutes.

Preparation of blank

Blank solution was prepared by adding 5ml of 2 N H₂SO₄ and 2ml of 0.003 M KMnO₄ simultaneously with solution mixture and incubated at 37°C for 10 minutes.

0.25 N HCl(100 ml) [Fisher Scientific, India]

Concentrated HCl was taken with purity of 35.5-38% (specific gravity= 1.19). So 2.1 ml conc. HCl was added to 100 ml distilled Water to make 0.25 N HCl.

Preparation of plant extract

0.5g of plant material was weighed in electric weighing balance and transferred to 30 ml of 0.25 N HCl and was boiled in a water bath for 15 minutes. Then the total extract volume was made 50ml by adding already prepared 0.25 N HCl.

Preparations of plant extract mixture for reading

1 ml of plant extract was added with 5ml of 2 N H₂SO₄ and 2ml of 0.003 M KMnO₄ at the same time of preparation of other solution mixture for reading and incubated at 37°C for 10 minutes (Fig. 2).

3. Results

3.1 For titration method

Table 1: Burette reading of mature *Anagallis arvensis* plant extract

Plant extract from <i>Anagallis arvensis</i>	Burette Readings		Con. Vol. of 0.05 N KMnO ₄ (Mean±SE)
	Initial	Final	
Sample 1	0	6.5	6.5 ± 0.28 ml.
	6.5	12.5	
	12.5	19.5	
Sample 2	0	7	7 ± 0.28 ml.
	7	13.5	
	13.5	21	
Sample 3	0	6.5	6.8 ± 0.15 ml.
	6.5	13.4	
	13.4	20.4	
Sample 4	0	6.5	6.6 ± 0.05 ml.
	6.5	13.1	
	13.1	19.8	

For sample 1, average Volume consumed = 6.5 ml

Calculation:

As we know $N_1V_1 = N_2V_2$

So $N_1 = 0.05 \times 6.5 / 10 = 0.0325$ N

From here, the strength of oxalate was calculated by multiplying Normality and Equivalent mass of oxalate ion. The final concentration was calculated to be 1.43 g/l. As we had taken total volume of 100 ml, so the oxalate content was

0.143 g. That means in 1 g of plant which was mixed with total of 100 ml dilute H₂SO₄ contained 0.143 g oxalate. Similarly for rest three samples, the values were 0.154, 0.1496 and 0.145 g respectively. The mean of all the values was found to be 0.1479±0.002 g. So the average percentage of oxalate content was 14.79%.

Table 2: Burette reading of immature *Anagallis arvensis* plant extract

Plant extract from <i>Anagallis arvensis</i>	Burette Readings		Con. Vol. of 0.05 N KMnO ₄ (Mean±SE)
	Initial	Final	
Sample 1	0	5.7	5.7 ± 0.05 ml.
	5.7	11.5	
	11.5	17.1	
Sample 2	0	5.5	5.6 ± 0.05 ml.
	5.5	11.2	
	11.2	16.8	
Sample 3	0	5.6	5.55 ± 0.02 ml.
	5.6	11.15	
	11.15	16.65	
Sample 4	0	5.7	5.8 ± 0.05 ml.
	5.7	11.5	
	11.5	17.4	

For sample 1, average Volume consumed = 5.7 ml

Calculation:

As we know $N_1V_1 = N_2V_2$

So $N_1 = 0.05 \times 5.7 / 10 = 0.0285$ N

From here, the strength of oxalate was calculated by multiplying Normality and Equivalent mass of oxalate ion. The final concentration was calculated to be 1.254 g/l. As we had taken total volume of 100 ml, so the oxalate content was

0.1254 g. That means in 1 g of plant which was mixed with total of 100 ml dilute H₂SO₄ contained 0.1254 g oxalate. Similarly for rest three samples, the values were 0.1232, 0.1221 and 0.1276 g respectively. The mean of all the values was found to be 0.1245±0.001 g. So the percentage of oxalate content was 12.45%.

3.2 For spectrophotometric method [Systronics Spectrophotometer 166]

Table 3: Spectrophotometer reading data for mature plant

Readings		I	II	I+II	Mean
Blank	(Ab)	2.125	2.115	4.24	2.12
0.1	(As)	1.856	1.848	3.704	1.852
0.2		1.888	1.892	3.78	1.89
0.3		1.672	1.674	3.346	1.673
0.4		1.52	1.532	3.052	1.526
0.5		1.398	1.392	2.79	1.395
0.6		1.271	1.275	2.546	1.273
0.7		1.17	1.174	2.344	1.172
0.8		0.997	0.989	1.986	0.993
0.9		0.869	0.863	1.732	0.866
1		0.823	0.825	1.648	0.824
Extract (Ae)	Sample 1	0.129	0.119	0.248	0.124
	Sample 2	0.135	0.143	0.278	0.139
	Sample 3	0.128	0.132	0.260	0.130
	Sample 4	0.133	0.121	0.254	0.127

Calculations

For preparation of a standard regression equation & graph, ΔA (Ab-As) need to be calculated. So the value of ΔA is given in the below table

Table 4: Calculation of ΔA (Ab-As)

Oxalic acid	Mean	ΔA=Ab -As	
Blank	2.12	(Ab) 0	
0.1	1.852	(As) 0.268	
0.2	1.89		0.23
0.3	1.673		0.447
0.4	1.526		0.594
0.5	1.395		0.725
0.6	1.273		0.847
0.7	1.172		0.948
0.8	0.993		1.127
0.9	0.866		1.254
1	0.824		1.296
Plant Sample 1	0.124	(Ae) 1.996	
Plant Sample 2	0.139		1.981
Plant Sample 3	0.130		1.99
Plant Sample 4	0.127		1.993

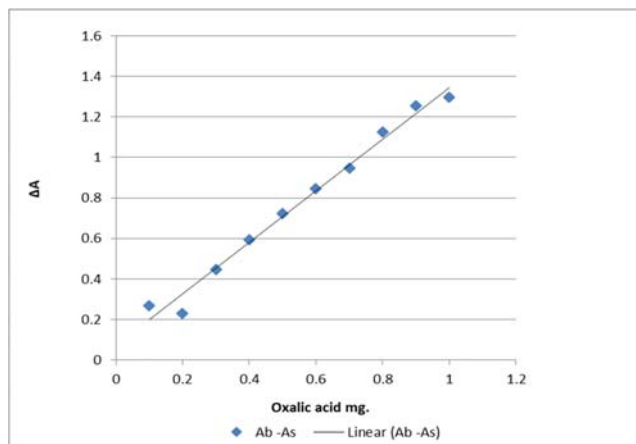


Fig 3: Regression line along with the regression equation & regression co-efficient (R²) for mature plant

The regression equation for the above line is ΔA= 1.273C + 0.073 with R² = 0.985

Where, ΔA is Ab -As

C is concentration

R² is regression co-efficient

After getting the equation, concentration of OA in the plant

extract was calculated.

OA conc. of the plant extract:-

ΔA= Ab-Ae, so ΔA for plant extract= 2.12-0.124= 1.996

1.996= 1.273 × C + 0.073

C= 1.996-0.073/1.273

C= 1.510 mg/ml of plant extract

Then calculate OA content in total plant extract. As 0.5 gm plant powder was taken in 50 ml of 0.25 N HCl, So 0.5 g plant powder contains 75.5 mg oxalate. From this, the amount of oxalic acid present in 100g plant material was calculated. 100 g of plant material contained 15.1 g Oxalic acid.

Similarly for rest three samples, the values were 14.98, 15.05 and 15.08 g respectively. The mean of all the values was found to be 15.05±0.02 g. So the average percentage of oxalate content was 15.05%.

Table 5: Spectrophotometer reading data for immature plant

Readings		I	II	I+II	Mean
Blank	(Ab)	1.73	1.716	3.446	1.723
0.1	(As)	1.153	1.125	2.278	1.139
0.2		1.091	1.113	2.204	1.102
0.3		0.959	0.983	1.942	0.971
0.4		0.888	0.832	1.72	0.86
0.5		0.802	0.816	1.618	0.809
0.6		0.679	0.689	1.368	0.684
0.7		0.591	0.581	1.172	0.586
0.8		0.52	0.53	1.05	0.525
0.9		0.356	0.362	0.718	0.359
1		0.308	0.320	0.628	0.314
Extract (Ae)	Sample 1	0.052	0.044	0.096	0.048
	Sample 2	0.039	0.045	0.084	0.042
	Sample 3	0.048	0.040	0.088	0.044
	Sample 4	0.063	0.053	0.116	0.058

Calculations

For preparation of a standard regression equation & graph, ΔA (Ab-As) need to be calculated. So the value of ΔA is given in the below table

Table 6: Calculation of ΔA (Ab-As)

Oxalic acid	Mean	ΔA=Ab -As
Blank	1.723	0
0.1	1.139	0.584
0.2	1.102	0.621
0.3	0.971	0.752
0.4	0.86	0.863
0.5	0.809	0.914
0.6	0.684	1.039
0.7	0.586	1.137
0.8	0.525	1.198
0.9	0.359	1.364
1	0.314	1.409
Plant Sample 1	0.048	1.675
Plant Sample 2	0.042	1.681
Plant Sample 3	0.044	1.679
Plant Sample 4	0.058	1.665

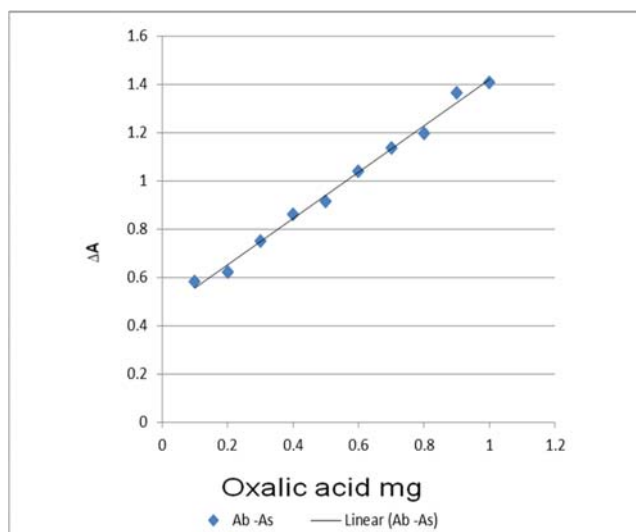


Fig 4: Regression line along with the regression equation & regression co-efficient (R²) for immature plant

The regression equation for the above line is $\Delta A = 0.957 C + 0.461$ with $R^2 = 0.992$

Where, ΔA is $A_b - A_s$

C is concentration

R^2 is regression co-efficient

After getting the equation, concentration of Oxalic acid in the plant extract was calculated.

OA conc. of the plant extract:-

$\Delta A = A_b - A_s$, so ΔA for plant extract = $1.723 - 0.048 = 1.675$

$1.675 = 0.957 \times C + 0.461$

$C = 1.675 - 0.461 / 0.957$

$C = 1.268 \text{ mg/ml}$ of plant extract

Then calculate OA content in total plant extract. As 0.5 gm plant powder was taken in 50 ml of 0.25 N HCl, So 0.5 g plant powder contains 63.4 mg oxalate. From this, the amount of oxalic acid present in 100g plant material was calculated. 100 g of plant material contained 12.68 g Oxalic acid. Similarly for rest three samples, the values were 12.74, 12.72 and 12.58 g respectively. The mean of all the values was found to be 12.68 ± 0.03 g. So the average percentage of oxalate content was 12.68%.

4. Discussion

In livestock, oxalate poisoning occurs less frequently but severity is a matter of concern [21]. Mostly high oxalate-containing plants (more than 5% oxalate by dry weight) belong to the three families Amaranthaceae, Chenopodiaceae, and Polygonaceae [14] and also Brassicaceae family [21]. Sometimes the oxalate content may go upto 30% on the basis of dry weight, depending on soil composition and season [8] which was an agreement to our findings. Generally, oxalate contents are highest in the leaves, followed by the seeds; levels tend to be lowest in the stem and fruits [22]. In present study, we had taken whole plant material for analysis of the oxalate content and that was the reason of high concentration oxalate. As *Anagallis arvensis* plant is herb, so all the parts of plant can be consumed by the ruminants and may lead to toxicity due to oxalate content. Similar finding regarding herbs contain more oxalate was observed by [23]. In addition, tropical plants contain higher amount of oxalate [24, 25] which was also noticed in our present study.

5. Conclusion

From this study, it was concluded that *Anagallis arvensis* plant is a high oxalate containing weed which on consumption for longer duration lead to oxalate poisoning in grazing animals. The mature plants contain more oxalate content (15.06% by spectroscopy method and 14.79% by titration method) than the immature plants (12.68% by spectroscopy method and 12.45% by titration method) but that difference not significant relation to toxicity. That means both mature and immature plants contain very high concentration of oxalate than the normal permissive limit for ruminants and human which may cause toxicity if consumed. Simultaneously spectrophotometric method is one of the convenient and accurate methods for estimation of oxalate content just like conventional permanganate titration method within less time.



Fig 1: *Anagallis arvensis* Plant sample



Fig 2: Colour change of standards and Samples in Spectrophotometric Method

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