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METHODOLOGY IN DETERMINATION OF OXALIC ACID IN PLANT TISSUE: A COMPARATIVE APPROACH

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ABSTRACT

Several methods have been described for determination of oxalic acid or oxalates in plants. These methods include Indole-oxalic acid reaction, titration with $KMnO_4$, rapid catalytic reduction by oxalic acid using Pyrocatechol violet (PCV) / Bromophenol blue / Victoria blue etc. In the present investigation, these methods are followed for determination of oxalic acid and compared for their sensitivity, simplicity and accuracy. It was observed that regression equation with R^2=0.898 was Y=13.93X + 0.315 for Indole reagent and with R^2=0.890, Y=-0.042X + 0.29 for Pyrocatechol-dichromate reaction. While, for Bromophenol blue-dichromate reaction with R^2=0.876, an equation, log ΔA =0.373C + 0.694, was derived. Calibration graph for method with Victoria blue was linear and regression equation was log (Ai/Aj)= 0.291B + 0.348 with correlation coefficient, 0.915. The gravimetric method for estimation of oxalic acid i.e. titration with $KMnO_4$, R^2= 0.997 gave an equation Y= 0.278X + 0.111. The new method for oxalic acid estimation using $KMnO_4$ is described. It showed consistency in results as compared to earlier accepted methods. The calibration graph for this method was linear and regression equation was ΔA = 0.966C - 0.027 with correlation coefficient 0.983. All these methods were followed for estimation of oxalic acid from the same extract from dried leaves of *Portulaca oleracea* L. From the observations it was noticed that the gravimetric method and the new method described appear to be sensitive and more accurate. On the basis of these observations it is concluded that the gravimetric method for the estimation of oxalic is consistent and accurate but time consuming and laborious as compared to spectrophotometric methods. The method with Indole-oxalic acid reaction appears to be sensitive at micro concentrations of oxalic acid. Bromophenol/PCV-dichromate reaction methods seem to be temperature sensitive. The new method described with oxidation of $KMnO_4$ was found simple, sensitive and more accurate as compared to other methods.

Key words: Methods, Oxalic acid, Plants.

INTRODUCTION:

Oxalic acid ($C_2H_2O_4.2H_2O$; Mol. wt.: 126.07) is one of the strong organic acids. It is a white crystalline solid, approximately 10% soluble in water at 20^0C [1]. It is constituent of cleaning solutions for removing paint, varnish and rust etc. Therefore, it is used extensively in laundries and bleaching of wood, straw and in leather manufacture [2].

Oxalic acid is a primary chelator of calcium. Hence, its effect in the human diet is very important. Because, oxalic acid can complex with calcium to form highly insoluble calcium oxalate crystals [3], which can cause hypooxularia in animals [4]. Oxalic acid has been detected in various organisms, plants and fungi. It has long been investigated from a medicinal viewpoint [5]. Besides this, it has been receiving much attention for its various ecological qualities such as, bioremediation of a wide variety of organic pollutants [6] with lignin biodegradation systems [7]; inactivation of copper-containing wood preservatives by wood rotting fungi [8]; detoxification of aluminium toxicity in aluminium resistant buckwheat [9]; crop damage caused by oxalic acid-producing phytopathogens [10] and the biofertilizer effect of ectomycorrhizal fungi [11].

In plants, oxalic acid and oxalates have been detected in varying quantities in all parts such as leaves, leaf stalks, flowers, tubers and roots [12]. The highest oxalic acid concentration commonly occurs in the leaves and that lowest in the roots [2]. In plants, oxalates may be present as the soluble sodium or potassium salts or as insoluble crystalline calcium oxalate. The formation of oxalate is typically intracellular, with the crystals forming specialized cells called crystal idioblasts. For many years, oxalic acid was considered as a metabolic end product and it was thought that calcium oxalate was formed to maintain low soluble level of this toxic acid [13], but recent studies have shown that oxalic acid is synthesized in response to increased calcium [14]. Glycolate, glyoxylate, oxaloacetate, and citrate have all been proposed as precursors of oxalic acid in plants, however, some studies were conducted, that indicated ascorbic acid could provide the substrate for oxalic acid synthesis which later on used for calcium oxalate formation [15, 16]. Calcium oxalate crystals are produced and accumulated in over 215 plant families [17]. Oxalate producing plants accumulate oxalate in the range of 3 to 80% (w/w) of their dry weight [18]. Most of the extreme oxalate accumulators (more than 5% oxalate by dry

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weight) belong to Caryophyllaceae, Chenopodiaceae and Polygonaceae, all within subclass Caryophyllidae.

Several methods are described for estimation of oxalic acid, including spectrophotometric ([19, 20], amperometric [21], polarographic [22], isotope dilution mass spectrometric [23], liquid chromatographic [24], gas chromatographic [25] and enzymatic [26] methods. All the methods listed above require special instrumentation and high cost of analysis as well.

In the present investigation, some of these methods are followed for estimation of oxalic acid and compared for their sensitivity, simplicity and accuracy. The main objective of this experimental work was to find out the most convenient and accurate method for estimation of oxalic acid from plants.

MATERIALS AND METHODS:

A standard solution of oxalic acid was prepared by dissolving 100 mg of oxalic acid ($C_2H_2O_4.2H_2O$; Mol. wt.: 126.07, S. D. Fine Chemicals, India) in distilled water and diluted to 100 ml with distilled water. Working solution was prepared freshly before use.

Oven dried leaves of *Portulaca oleracea* L. were used to determine oxalic acid content. Extraction was carried by following the traditional method described by [27]. 0.5g leaves were powdered and transferred to 50ml capacity volumetric flask. To which, 30ml 0.25 N HCl was added and kept in boiling water bath for about 15 min, cooled to room temperature and volume was made with 0.25 N HCl. This solution was used as extract for determination of oxalic acid.

Before starting the experiments with set protocols, an attempt was made to determine maximum absorbance of oxalic acid at different wavelengths on spectrophotometer. Assay mixture contained standard oxalic acid solution and different diluents such as distilled water, 0.25 N HCl, 0.0025N HCl and acidified distilled water.

Following are the methods which were followed for the estimation of oxalic acid:

1. Titration with KMnO$_4$

This method was described by [27]. Standard oxalic acid (1mg/ml) was added in 50ml capacity volumetric flask and approximately 30ml 0.25 N HCl were added. The flask was heated for an hour in boiling water bath and then cooled to room temperature and volume was made with 0.25 N HCl. 10 ml of the filtered solution were transferred to 15 ml marked centrifuge tube and it was kept in a refrigerator for 10 minutes at about 5-10 ^{0}C. 2 ml of the saturated solution of unhydrous sodium acetate and/or calcium acetate in dilute acetic acid were added to this tube. The tube was kept at 10 ^{0}C overnight to precipitate out oxalate. Next day the mixture was centrifuged for 5 min. at 5000 rpm. The supernatant was carefully decanted and precipitate was dissolved in 5 ml, 0.25 N HCl. Oxalate was re-precipitated from this and separated as before. The precipitate was then washed with 5 ml of washing reagent, an alcohol ammonia mixture, which was added down the sides of the tube. The precipitate was separated by centrifugation as before.

Finally, this precipitate was dried at 100^{0}C for 30 min. This dried precipitate was dissolved in 5 ml 2 N H$_2$SO$_4$ and was titrated hot against 0.02 N KMnO$_4$ which was standardized against 0.02 N oxalic acid solution with KMnO$_4$ as the self indicator.

2. Colour reaction with Indole reagent

This method was proposed by [28] which is based on the reaction described by [29]. According to them, a pink-coloured compound is formed in the reaction between indole and oxalic acid. Indole reagent was freshly prepared by dissolving 100 mg of indole in 100ml of concentrated sulfuric acid. Assay mixture contained 2 ml standard oxalic acid solution at various concentrations, ranging from 0.100 to 1.00 mg per ml, prepared in 1 N H$_2$SO$_4$. Blank was prepared with 2 ml of 1 N sulfuric acid instead of oxalic acid solution. Then 2 ml of indole reagent was added in each test tube including blank, allowing the reagent to run down the side of the tube to minimize heat development. All test tubes were placed in water bath at 80 to 90^{0}C for 45 minutes. Cooled to room temperature and absorbance was measured at 525 nm on spectrophotometer (Shimadzu, UV-1900).

3. Oxidation of Bromophenol blue (BPB) by Dichromate

The method given by [30] was followed. The basic principle of this method is oxidation of Bromophenol blue by potassium dichromate in presence of oxalic acid. The colour of Bromophenol blue is decreased due to its oxidation by dichromate with the help of oxalic acid (oxalic acid catalyses the oxidation of Bromophenol blue. The reaction mixture contained 100 – 600µl of standard oxalic acid, 250 µl of 1.00×10^{-3} M BPB (0.670g of BPB dissolved in 2 ml, 0.01 M NaOH solution and diluted to 100 ml with distilled water), 400 µl of 0.1 M K$_2$Cr$_2$O$_7$ and 450 µl of 1 M H$_2$SO$_4$. The final volume was made to 10 ml with distilled water. It was kept in boiling water bath for 10min. and the reaction was quenched by adding 1ml of 2 M NaOH. The absorbance was read at 600 nm on spectrophotometer (Shimadzu, UV-1900). The absorbance for non-catalytic reaction (blank) i.e. without oxalic acid (Ab), and for catalytic reaction (As) was recorded. The difference (ΔA), between Ab and As was calculated to select the condition for determination and log(Ab-As) was calculated to determine oxalic acid concentration.

4. Oxidation of Pyrocatechol Violet (PCV) by Dichromate

This method was described by [31]. The basic principle for this method is the same as for Bromophenol blue-Dichromate reaction described by [30], only difference being, PCV is used instead of Bromophenol blue. Solutions of standard oxalic acid (1 mg/ml), 5 ml of 0.80 M H$_2$SO$_4$, 1 ml of PCV (4.0×10^{-4} M) and 0.35 ml Potassium dichromate (0.01 M) were mixed in test tubes. After addition of K$_2$Cr$_2$O$_7$ solution, the decrease in absorbance was recorded at 450 nm on spectrophotometer (Shimadzu, UV-1900) at different time intervals from 10-180 sec. The absorbance was labeled as A$_b$ for blank and

A$_s$ for sample. The calibration curve was constructed by plotting the difference in the absorbance (ΔA_s - ΔA_b) vs. oxalic acid concentration.

5. Oxidation of Victoria blue by Dichromate

In this method, Victoria Blue B (VBB) was used instead of Pyrocatechol violet / Bromophenol blue. This method was proposed by [32]. However, the principle behind experimental work is the same as that described for Pyrocatechol violet method. Standard oxalic acid solution (1mg/ml) was prepared in distilled water. Assay mixture contained different concentrations of std. oxalic acid solution (0.1 to 1 mg/ml) with 1 ml of 1 M H$_2$SO$_4$, 1 ml of 0.080 M K$_2$Cr$_2$O$_7$ and diluted to 20 ml with distilled water. To this, 1 ml 0.0001 M Victoria Blue B solution (prepared by dissolving 0.00506 g of VBB in 20 ml of hot water and diluting to 100 ml) and 4 ml distilled water was added. Blank was prepared with distilled water instead of oxalic acid solution. The mixture was immediately placed into water bath for reaction at 60^{0}C. After 9 min. the mixture was cooled to quench the reaction with tap water for 2 min. The absorbance value A$_i$ of the blank solution (non-catalytic) and A$_j$ of the sample solution (catalytic) was recorded on Shimadzu UV-1900 spectrophotometer at 610nm. The calibration curve was constructed by plotting values of log (A$_i$/A$_j$) for different concentrations of oxalic acid against corresponding oxalic acid concentrations.

RESULTS AND DISCUSSION:

In the present investigation, the experimental work was concentrated on the comparison of methods to determine the oxalic acid content of plant material. Therefore, one gravimetric and five spectrophotometric methods were followed and compared for their sensitivity, simplicity as well as accuracy. Linear regression analysis is a useful tool for comparing methods of measurement [33]. Hence, Pearson correlation coefficient R^2 and linear regression equation were used to determine which method would be sensitive / accurate as compared to other methods for oxalic acid determination. Use of correlation coefficient and linear regression equation is common to determine the sensitivity of biochemical analytical method. The correlation coefficient and regression equation indicate inter-dependence of two values. In spectrophotometric determinations the absorbance of compound is helpful to determine its concentration. The basic principle of any spectrophotometric method is Beer-Lambert's law, which states the relationship between light and the absorbance. Beer-Lambert's law is expressed as, A=E×C×L, where, A is absorbance, E is molar extinction coefficient; C is concentration of solution and L is length of light path.

1. Oxalic acid + Distilled water

Before preparation of assay, absorbance peak of standard oxalic acid solution was determined on Shimadzu UV-190 Spectrophotometer and it was 190 nm (Fig. 1). Blank was prepared with distilled water.

Correlation coefficient (R^2 = 0.523) for the assay mixture of oxalic acid and distilled water has been determined and recorded (Fig. 2). From the results it can be concluded that this combination i.e. oxalic acid + distilled water does not follow Beer-Lambert's law and hence recording the absorbance of the solution at 190 nm appears not to be useful in quantitative determination of oxalic acid.

2. Oxalic acid + 0.25 N HCL

In this experimental design an assay mixtures containing oxalic acid at various concentrations and 0.25 N HCl were used. Systronics UV-VIS double beam spectrophotometer-2201 was used for measurement of absorbance at 190 nm. Blank was 0.25 N HCl. The observations (absorbance readings) indicated no correlation between oxalic acid concentration and absorbance (Fig. 3).

Same experiment was carried by using chemicals manufactured by other manufacturer and absorbance was recorded at 190 nm on Systronics UV-VIS double beam spectrophotometer-2201. As shown in Fig. 4, there was some change in absorbance readings but here the oxalic acid concentration did not follow Beer-Lambert's law with absorbance.

3. Oxalic acid + 0.25 N HCl at 292nm

In earlier experiments absorbance peak of assay mixture containing oxalic acid and 0.25 N HCl was not determined. The highest absorbance for different concentrations of oxalic acid with 0.25 N HCl was recorded at 292 nm on Systronics UV-VIS double beam spectrophotometer-2201. Blank was prepared only with 0.25 N HCl. Fig. 5 shows the absorbance of light at 292 nm by the different concentrations of oxalic acid with 0.25 N HCl.

From the results, it is clear that there is some correlation between oxalic acid concentration and the absorbance at 292nm.

4. Oxalic acid + acidified distilled water

Standard solution of oxalic acid (0.1mg/ml in distilled water) has a specific pH. Therefore, in this experiment pH of different solvent systems used for preparation of std. oxalic acid solutions was measured (Table 1).

From the above observations, it is clear that pH of standard oxalic acid solution and acidified distilled water is nearly the same. Acidified distilled water was prepared by adding a drop of conc. HCl in 500 ml of distilled water. Therefore, mixture of standard oxalic acid solution and acidified distilled water was taken for assay. This mixture showed an absorbance peak at 190 nm on Systronics UV-VIS double beam spectrophotometer-2201(Fig. 6).

Assay of standard oxalic acid solution and acidified distilled water showed significant increase in absorbance with increasing concentrations of oxalic acid. However, acidified distilled water could not be used to determine oxalic acid content from plant tissue because in plant's tissue oxalic acid is mostly in the form of salt (calcium oxalate) [3]. Calcium oxalate is highly insoluble in water and very little soluble in organic solvents.

Hence, acidified distilled water as a solvent for calcium oxalate is not suitable. However, pure oxalic acid could be quantified by this exercise.

5. Oxalic acid + 0.0025 N HCL

Earlier observations (Table 1) have shown that pH of 0.0025 N HCl is 3.10. Therefore, absorbance of mixture of standard oxalic acid and 0.0025 N HCl was recorded on Systronics UV-VIS double beam spectrophotometer-2201 at 208 nm (Fig. 7). Blank was prepared only with 0.0025 N HCl.

Fig. 7 shows no correlation between oxalic acid concentrations and its absorbance at 208nm. Hence, assay mixture of oxalic acid in 0.0025 N HCl appears not be suitable for estimation of oxalic acid in plants.
Several workers have described a number of methods for estimation of oxalic acid. Few of them are discussed here for their sensitivity and accuracy.

6. Titration with $KMnO_4$

The method described by [27] is world-wide accepted and followed. However, this method is laborious and time consuming (requires 2 days for experimental work) as compared to other methods. But the results obtained by this method are consistent and accurate. As shown in Fig. 8, the highest R^2 value i.e. 0.997 was recorded for burette readings ($KMnO_4$ ml) vs. oxalic acid concentration with regression equation y = 0.278x + 0.111.

7. By Indole reagent

The method of Indole-oxalic acid reaction is more convenient and simple but at higher concentrations of oxalic acid, this method is insensitive [28]. However, in current investigation absorbance of oxalic acid at different concentrations varying from 0.1 to 1.0 mg/ml at 525nm shows correlation coefficient R^2=0.932 and equation y = 0.912x-0.070 (Fig. 9). The intensity of pink coloured compound in assay mixture is directly proportional to the concentration of oxalic acid. This method seems to be sensitive at low concentrations of oxalic acid and less time consuming than traditional titration method described by [27].

8. Oxidation of Bromophenol blue (BPB) by Dichromate

Basic principle of this method is catalytic oxidation of bromophenol blue by potassium dichromate in presence of oxalic acid. Oxalic acid is used as a catalyst during oxidation of bromophenol blue. Decrease in absorbance due to oxidation of BPB is recorded and presented in the form of log ΔA for different concentrations of oxalic acid varying from 0.2 to 1.4 mg. The calibration graph was linear in the concentration range of oxalic acid. The regression equation is logΔA = 0.373C + 0.694 with correlation coefficient of 0.876, where C is the concentration of oxalic acid in mg/ml and ΔA is Ab-As (Blank absorbance - sample absorbance) (Fig. 10).

From the above observations, this method seems to be simple, requiring less time but not sensitive as compared to traditional gravimetric method.

9. Oxidation of Pyrocathecol Violet (PCV) by Dichromate

The basic principle of this method is the same as discussed earlier in oxidation of bromophenol blue (BPB) by dichromate. Pyrocathecol violet is used instead of BPB. As compared to the method in which BPB is used, this method is rapid and requires specific instrumentation i.e spectrophotometer with magnetic stirrer. This method is temperature sensitive and requires constant 30^0C temperature for completion of reaction. The calibration curve is linear in the concentration range of 0.1 to 1 mg/ml oxalic acid. The regression equation obtained is A = -0.017B + 0.283 with correlation coefficient of 0.676, where A is absorbance of PCV and B is the concentration of oxalic acid (Fig. 11).

The reduction in absorbance is very rapid and hence some errors are possible in recording the absorbance. The constant temperature of 30^0C is also a key factor in this measurement.

10. Oxidation of Victoria blue by Dichromate

Victoria Blue B (VBB) is an analytical reagent used in this method. The catalytic oxidation of VBB by Potassium dichromate occurs in presence of oxalic acid. The colour intensity of VBB decreases with increase in oxalic acid concentration. Therefore, a decrease in colour intensity is directly proportional to the concentration of oxalic acid. The calibration curve obtained is linear with equation log (Ai/Aj) = 0.291B + 0.348 with correlation coefficient R^2= 0.915, where log (Ai/Aj) is absorbance and B is concentration of oxalic acid (Fig. 12).

From Fig. 12 it is clear that catalytic oxidation of VBB by dichromate in presence of oxalic acid is sensitive and this appears to be an accurate method as compared to earlier methods with PCV and/or BPB but not that accurate to the gravimetric method. However, this method requires less time and labour than those in titration with $KMnO_4$ method.

From all the above work it can be found that there is a need of new spectrophotometric method which is simple, accurate, less time consuming and which involves only few chemicals for the assay. Therefore, a new method with oxidation of $KMnO_4$ by oxalic acid in presence of H_2SO_4 is described here.

11. Oxidation of $KMnO_4$ by oxalic acid

Standard oxalic acid solution (1 mg/ml) was prepared with distilled water. 100 ml of 0.003 M $KMnO_4$ was prepared from appropriate dilution of 0.01 M $KMnO_4$ in distilled water. 500 ml of 2 N H_2SO_4 was prepared in distilled water. Assay mixtures contained different concentrations of oxalic acid ranging from 0.1 to 1 mg, 5 ml of 2 N H_2SO_4 and 2 ml of 0.003 M $KMnO_4$. This mixture was incubated for 10 min. at room temperature (27 $\pm$ 2^{0}C). After 10 min. absorbance was recorded at 528 nm on Shimadzu, UV 1900 spectrophotometer. Reagent blank was prepared with

distilled water. Absorbance for blank solution was recorded as Ab and for sample it was As. The calibration curve obtained (Fig. 13) is linear in concentration range of 0.1 to 1 mg/ml of oxalic acid. The regression equation is $\Delta A = 0.966C - 0.027$ with correlation coefficient 0.983, where C is the concentration of oxalic acid in mg/ml and ΔA is Ab-As.

Table 2 summarizes the regression equations and correlation coefficients obtained for all the above methods. The method described here is based on the principle of titration method proposed by [27]. The steps such as precipitation with saturated solution of calcium acetate in dilute acetic acid and washing with ammonia solution was omitted. The heating on boiling water bath was not required. This method is simple, sensitive, accurate, requires few chemicals and less time consuming as compared to all the earlier methods. The correlation coefficient is about +1, which means, it exhibits high accuracy in determination of oxalic acid.

The above methods were employed for estimation of oxalic acid from a plant sample. All these methods were followed to estimate oxalic acid from *Portulaca oleracea* L. leaves. *Portulaca oleracea* L. is commonly known as 'Purslane' belonging to family Portulacaceae. It is one of the high oxalate accumulating plants [35]. It is a leafy vegetable, commonly found in cultivated fields, along roadside and gardens. Hence, in the present work it was used as a plant sample for oxalic

acid estimation. The oxalic acid content of the leaves of *Portulaca oleracea* is depicted in Table 3.

The results obtained for oxalic acid content of leaves of *P. oleracea* are different in different methods employed (Table 3). The method described by [28] based on indole-oxalic acid reaction exhibited 2.95g/100g oxalic acid in dry tissue of *P. oleracea*. While, in Fowler and Bright's gravimetric method (1935) it is 6.34g/100g. The methods with catalytic oxidation using different dyes have recorded 4.19, 2.87 and 5.02g/100g dry tissue of oxalic acid content for Bromophenol blue, Pyrocatechol violet and Victoria blue respectively. However, according to the new method proposed in this work it is 6.32 g/100g oxalic acid in dry tissue of *P. oleracea*.

Portulaca oleracea is a high oxalate accumulating plant. The leaves of *P. oleracea* contain 6.9% oxalic acid in dry tissue [35]. While, 6.22 g/100g of oxalic acid in the fresh leaves of *P. oleracea* was reported by [34]. Following the new method described in this investigation the oxalic acid content of the leaves of *P. oleracea* determined is 6.32 g/100g dry tissue.

CONCLUSION:

It is concluded that the traditional gravimetric method of [27] is sensitive and accurate but time consuming and laborious. The new method described in the investigation (oxidation of $KMnO_4$) is simple, sensitive and more accurate than other spectrophotometric methods.

Table 1: pH of standard oxalic acid solution and different solvent systems used

Sr. No.	Solution/Solvent	pH
1.	pH of standard oxalic acid solution (0.1mg/ml of distilled water)	**3.73**
2.	pH of distilled water	7.15
3.	pH of 0.25 N HCl	1.47
4.	pH of 0.025 N HCl	1.79
5.	pH of 0.0025 N HCl	**3.10**
6.	pH of acidified distilled water	**3.59**

Table 2: Regression equations and correlation coefficients (R^2) for various methods

Sr. No.	Methods	Regression equations	R^2
1.	Oxalic acid + Distilled water	y = 0.055x + 0.081	0.523
2.	Oxalic acid + 0.25N HCl	y = 0.043x - 0.008	0.625
3.	Oxalic acid + 0.25N HCl	y = -0.007x + 0.099	0.085
4.	Oxalic acid + 0.25N HCl at 292nm	y = 0.007x + 0.002	0.809
5.	Oxalic acid + acidified distilled water	y = 0.074x - 0.002	**0.956**
6.	Oxalic acid + 0.0025N HCl	y = 0.026x + 0.022	0.227
7.	By Indole reagent	y = 0.912x - 0.070	0.932
8.	Titration with $KMnO_4$	y = 0.278x + 0.111	**0.997**
9.	Oxidation BPB by Dichromate	log ΔA = 0.373C + 0.694	0.876
10.	Oxidation of PCVby Dichromate	A=-0.017B+0.283	0.676
11.	Oxidation of VBB by Dichromate	log (Ai/Aj) = 0.291B + 0.348	0.915
12.	Oxidation of $KMnO_4$ by oxalic acid*	ΔA= 0.966C - 0.027	**0.983***

* Proposed new method.

Sr. No.	Methods followed for oxalic acid estimation	Oxalic acid content (g/100g dry weight).
1.	Indole reagent (Bergerman and Elliot, (1955)	2.95
2.	Titration with KMnO$_4$ (Fowler and Bright, (1935)	**6.34**
3.	Oxidation of Bromophenol blue (BPB) by Dichromate (Xu and Zhang, (2000)	4.19
4.	Oxidation of Pyrocathechol Violet (PCV) by Dichromate (Ensafi and Emadi, (2004)	2.87
5.	Oxidation of Victoria blue by Dichromate Yan *et al.*, (2004)	5.02
6.	Oxidation of KMnO$_4$ by oxalic acid*	**6.32**

*Proposed method
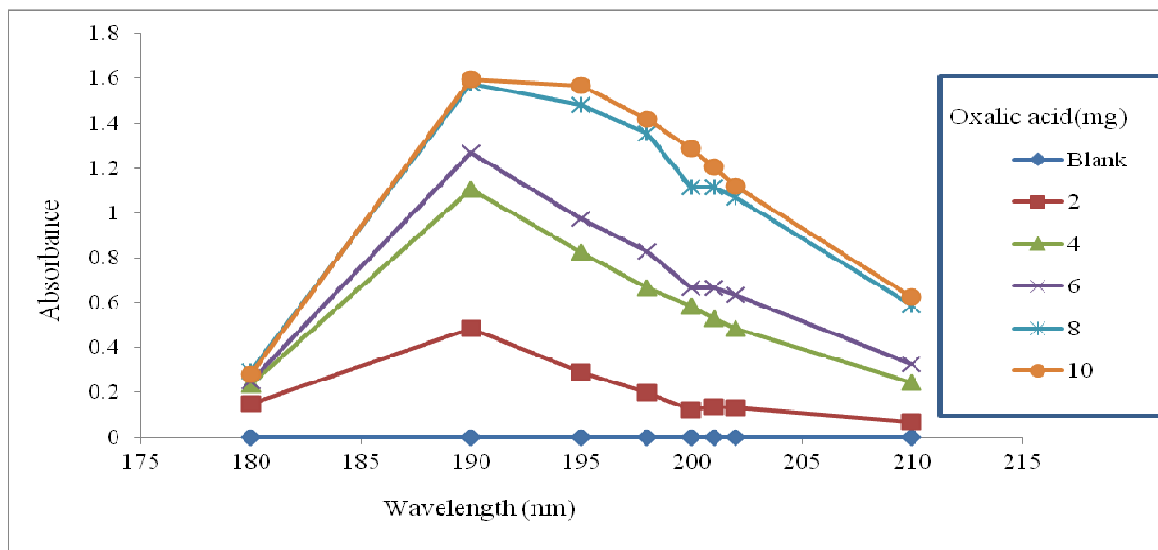
Fig. 1: Absorption spectra of oxalic acid at different concentrations showing maximum absorbance peak at 190 nm.
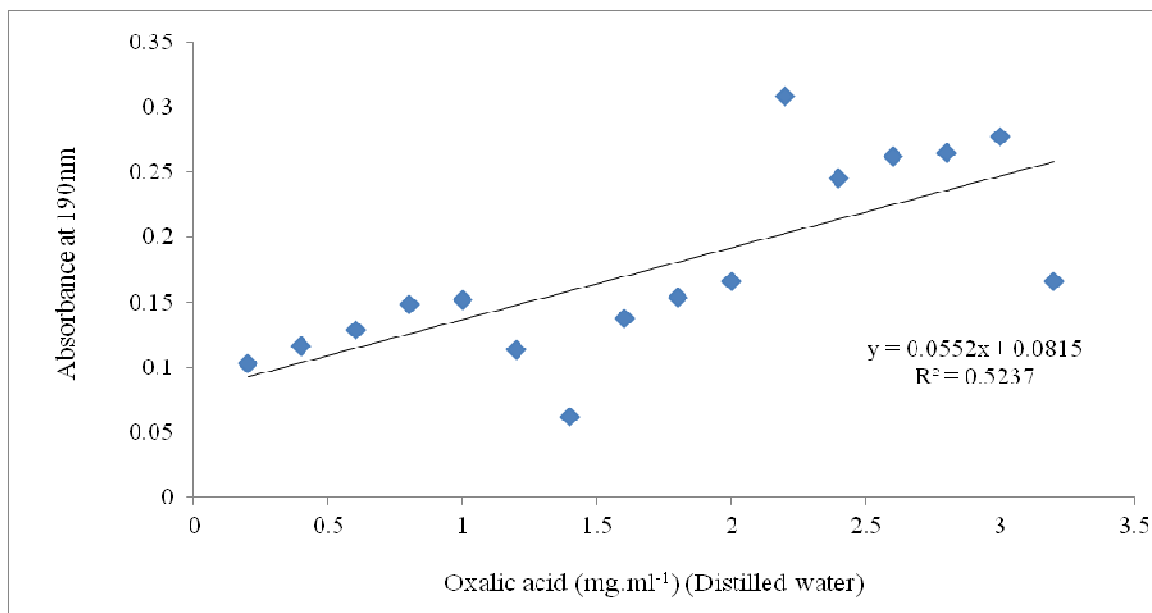
Fig. 2: Absorbance of light of 190 nm by oxalic acid (in distilled water) at various concentrations.
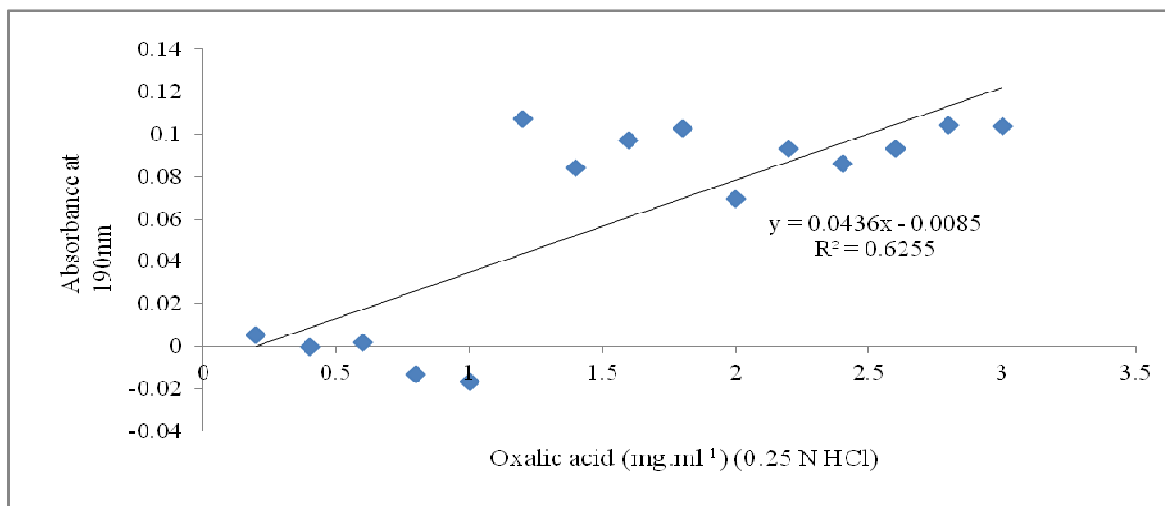
Fig. 3: Absorbance of light of 190 nm by oxalic acid (in 0.25 N HCl) at various concentrations.
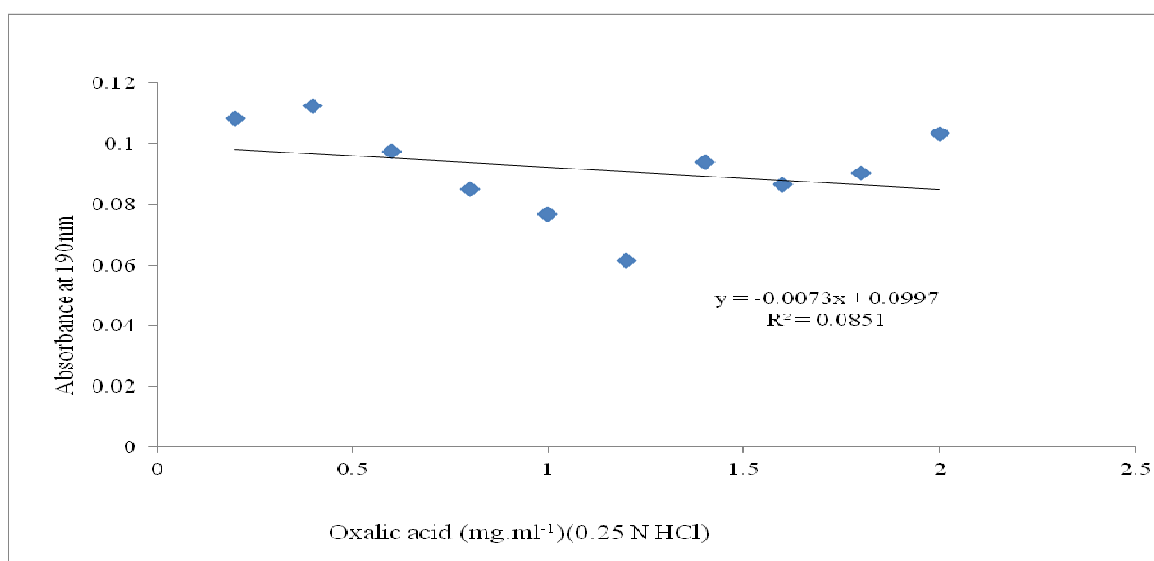
Fig. 4: Absorbance of light of 190 nm by oxalic acid (SD Fine Chemicals)(in 0.25 N HCl) at various concentrations.
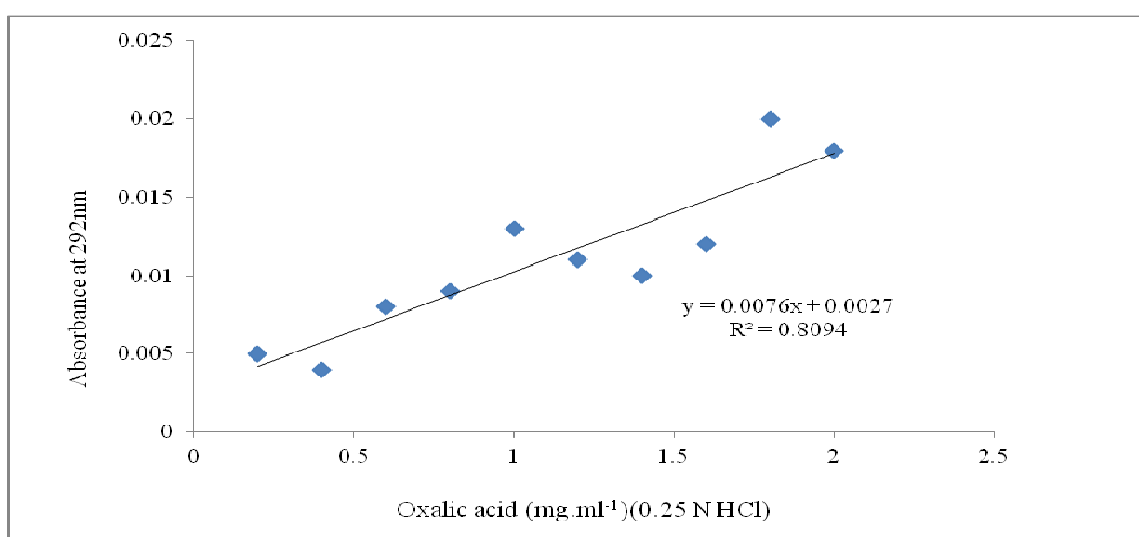
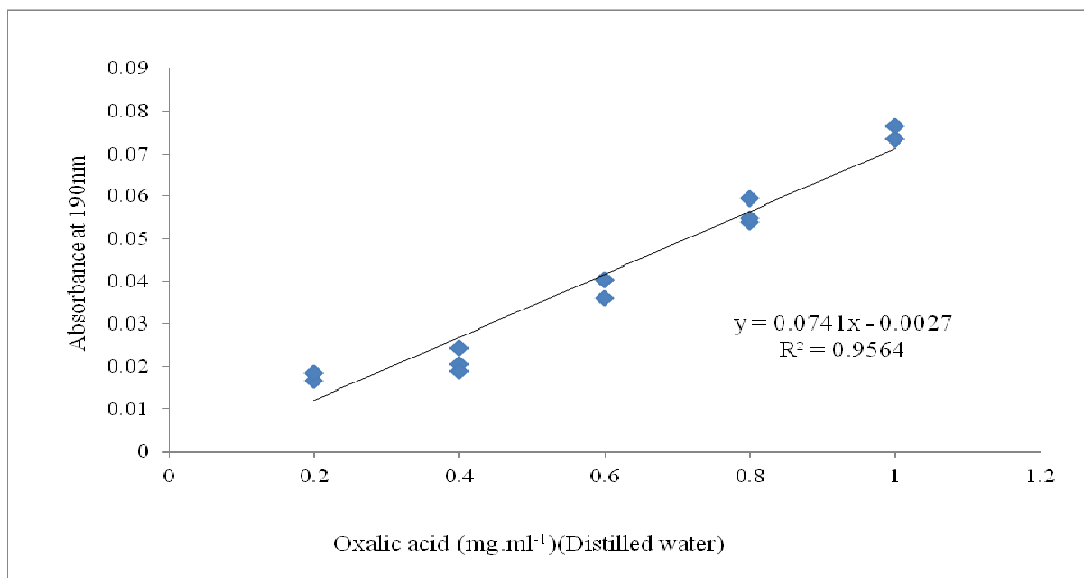**Fig. 5:** Absorbance of light at 292 nm by oxalic acid.

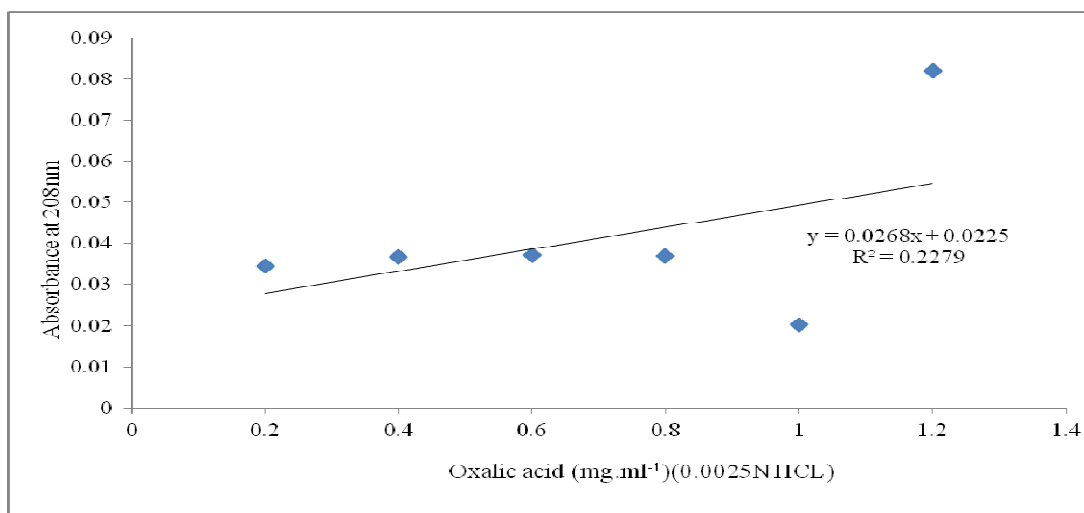**Fig. 6:** Absorbance of light at 190 nm by oxalic acid in acidified distilled water.

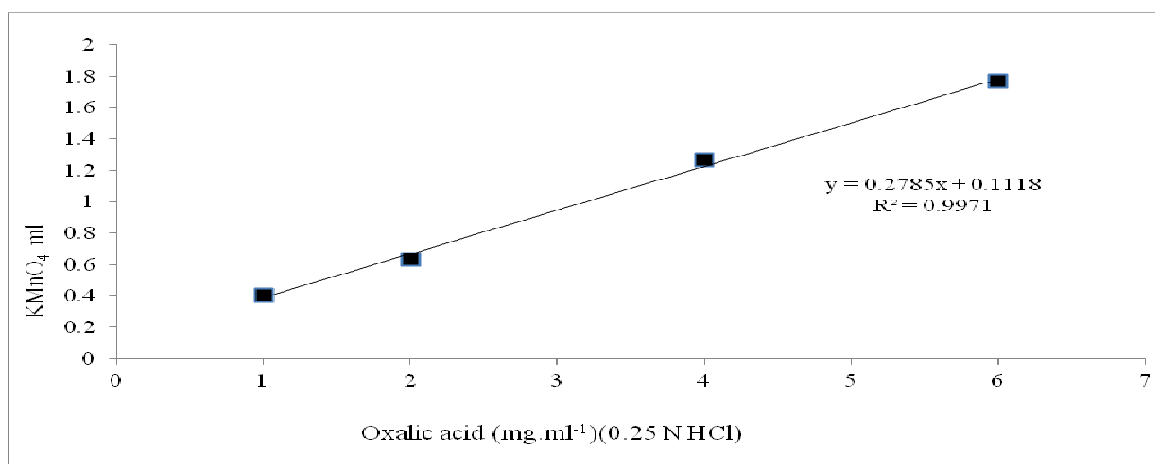**Fig. 7.** Absorbance of standard oxalic acid in 0.0025 N HCl at 208 nm.

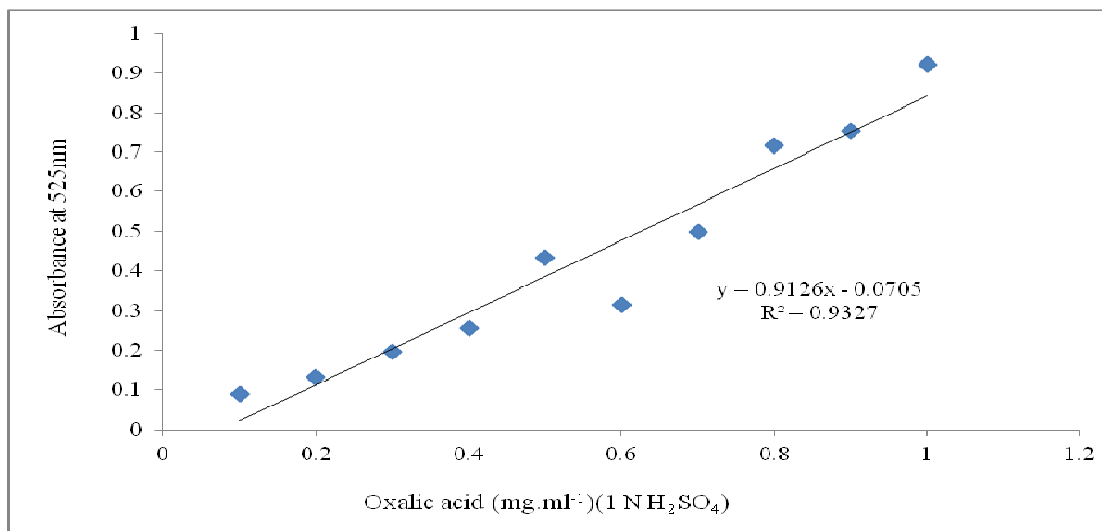**Fig. 8**: Standard oxalic acid concentration vs. burette readings i.e. ml of KMnO₄ used for titration.

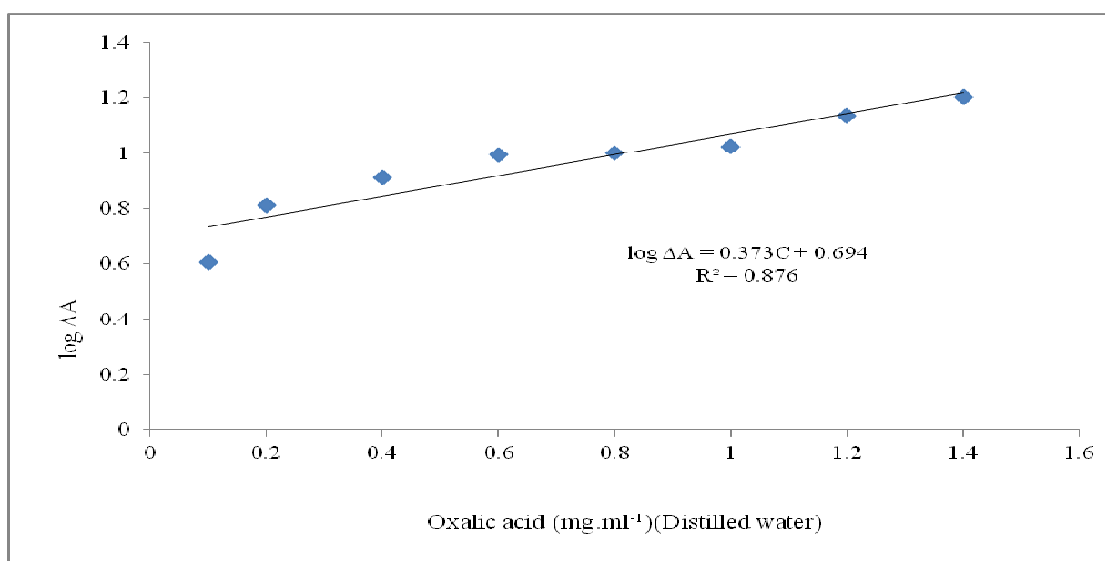**Fig. 9**: Absorbance by oxalic acid in Indole reagent method.

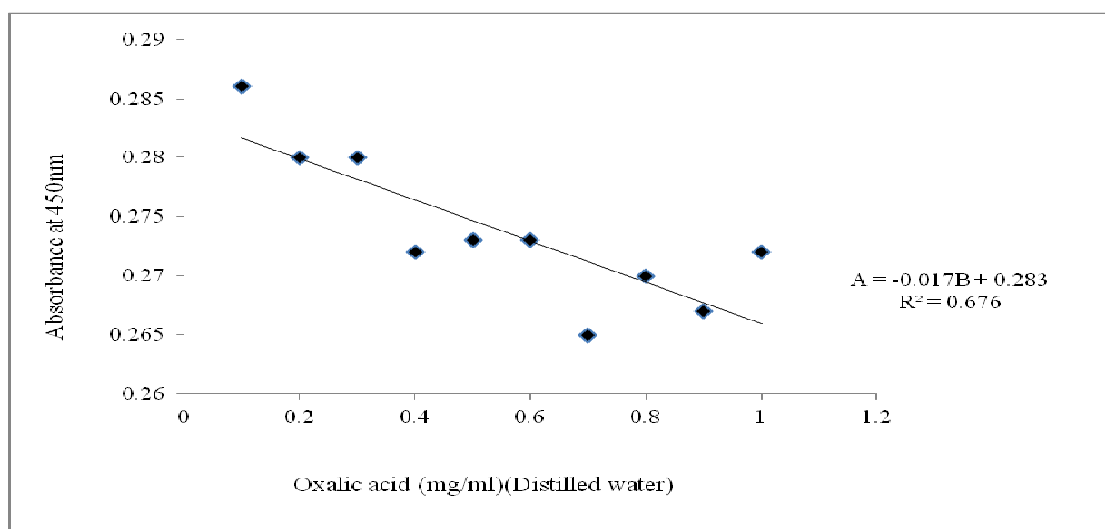**Fig. 10**: Calibration of concentration of oxalic acid vs. log ΔA.

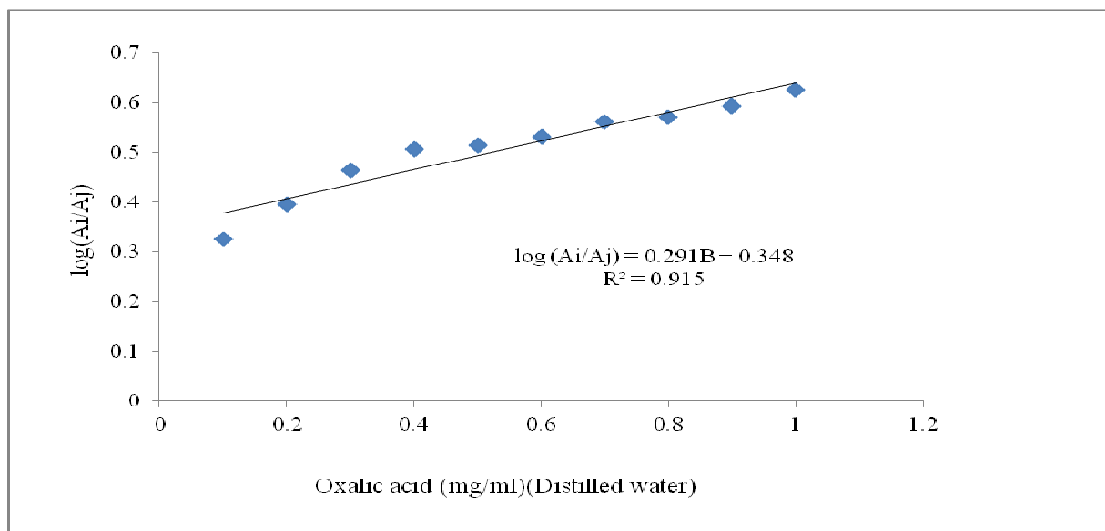**Fig. 11**: Calibration curve of oxalic acid concentration vs. absorbance of PCV.

Fig. 12: Calibration curve of oxalic acid concentration (0.1-1mg) vs. log (Ai/Aj)
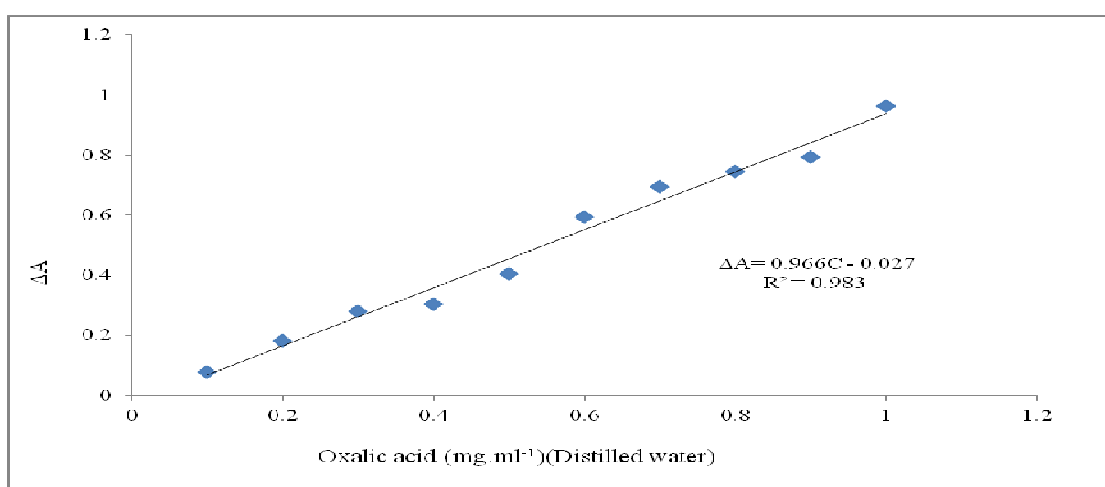

Fig. 13: Calibration curve of oxalic acid (mg/ml) vs. ΔA.

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