

Evaluation of Oxygen Molecule in *Ficus religiosa* by Spectrophotometer

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Abstract:

Ficus religiosa is common tree found in all over Indian subcontinent belongs to family *Moraceae*. It is used as traditional medicine for about 15 disease including Asthma, Diarrhea and sexual diseases. Ficus leaves extract decreases stress hormone level (cortisol). Ficus plant shows positive result in pollution control. Research work focuses on rapid sensitive method to determine amount of oxygen molecule in natural leaf extract of fresh leaves of ficus plant although no scientific publication yet published to determine oxygen molecule in leaf sample of ficus plant by using mentioned method in paper and method published until do not approach in sensitivity. In this study spectrophotometric principle used for determining oxygen molecule in leaf sample. The result showed that oxygen amount in leaf extract was found about 0.023 gram/lit and 0.035×10^{23} molecule in 5 ml of leaf sample. This paper suggests that oxygen in leaf sample is catalyst for all functioning cell and do work well when sufficient amount of oxygen and nutrient available. Cell sustains basic level to hyper oxygenated cell, it stimulates immune response, cell division hence this study beneficial in areas of plant physiology, environmental biology and medicinal pharmacology.

Key word : *Ficus religiosa*, *Moraceae*, Hormone, spectrometry, hyper, oxygenated, oxygen

INTRODUCTION

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. [1] *Ficus religiosa* (*Moraceae*) commonly known as 'Peepal tree' is a large widely branched tree with leathery, heart-shaped, long-tipped leaves on long slender petioles and purple fruits growing in pairs. [2-3] Plants are especially versatile in handling of carbohydrates first plants are autotrophs and able to convert CO_2 and H_2O into carbohydrates and oxygen [11]. The general equation of photosynthesis was first given by van Niel i.e. given below [4]

overall reaction: $6\text{CO}_2 + 12\text{H}_2\text{O} = 6\text{O}_2 + \text{carbohydrates}$

accurate data on determination of amount of oxygen from leaf sample essential for documenting changes in respiration rate in the ratio of volume of CO_2 evolved to the volume of O_2 consumed in respiration when carbohydrates used as substrates and completely oxidised their ratio of CO_2

consumed and O₂ evolved is equal to 1.[8]

Van Niel[4] first demonstrated that in photosynthesis- CO₂ reduced to carbohydrates and suggest that O₂ comes from water not from carbon dioxide[4].

The concentration of oxygen evolved in photosynthesis and expressed in term of mg/ml in leaf sample in leaf sample much greater difficulty and method publish until do not approach in sensitivity, The method described in this paper was designed to measure the concentration of oxygen in leaf sample of *ficus religiosa*. Paper described new investigation a rapid sensitive and selective method reported for determination of selective method evolved oxygen with a new reagent the proposed method based on reaction occur and formation of complex with oxygen molecule and new reagent [12]

Complex absorbance measured at particular wavelength and estimate complex formed in mg/ml and quantify concentration by Beer Lambert law[5] and estimate oxygen

Material & Method

REAGENTS USED:

1M MnSO₄

4M KOH (pH 12)

Liquid Nitrogen

Extraction buffer (Isolation buffer), pH 7.

PRINCIPAL :

To determine amount of oxygen in leaf sample first fresh leaves of plant weight 5gm taken as sample crush homogeneous and suspend in Isolation buffer and add 500 u of 1M MnSO₄ added in sample, where Mn⁺ ions oxidised by oxygen present in sample [9]

concentration by molar concentration[ref.S.M.Mahadik 13] using avogadro rule [6]

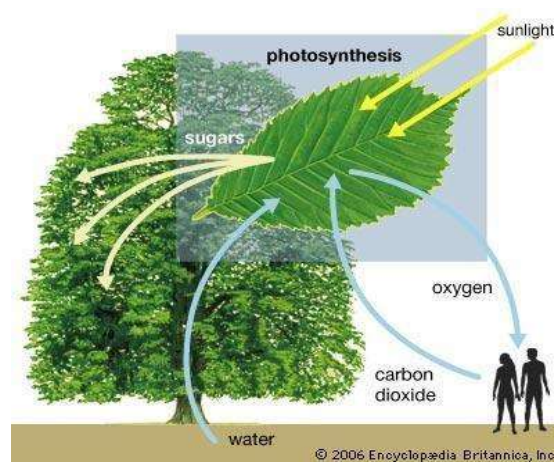


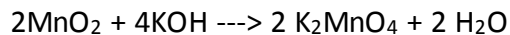
Fig.1.1 : Photosynthetic pathway of oxygen biosynthesis in plant.



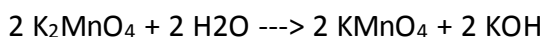
Fig 1.2 : *ficus religiosa* plant



2MnO₂ fused with alkali in aqueous medium in presence of air gives K₂MnO₄[14]



Potassium manganate is converted into permanganate by electrolytic oxidation in alkaline media[9]



After this reaction solution turn into dark violet colour take O. D. @540 nm Quantify the K₂MnO₄ by using standard curve (fig.1.5)

Calculate oxygen moles in potassium permanganate and estimate oxygen concentration in leaf sample by Avogadro number rule [13][6] (see calculation).

PROCEDURE

Ficus religiosa leaves obtained from young plant of *ficus religiosa* and was identified by comparing morphological characteristics[2-3], The young and fresh leaves were removed and weighed 5 grams by using electronic weighing balance now 5 grams leaf sample were chilled and homogenised by using liquid nitrogen and extracted in standard isolation buffer [8] in concentration of (5gm in 50 ml) filter leaf sample by using cellulose filter paper and then 8 sterile test tube were taken and mark them B,1,2,3,4,5,buk,UK ,standard KMNO₄ was prepared and pipetted out in different test tube ranging from 0.02 to 0.08 respectively (see table 1) made up to 5 ml with isolation buffer and O.D. was measured @ 540 nm and

standard graph plotted against concentration to absorbance (fig.1.4) the same procedure was followed for sample extract (skip dilution of KMNO₄) and add 0.5 ml 1 M MnSO₄ and incubate for 10 min and then add 0.5 ml KOH respectively in both test tube(buk and uk) after 20 min incubation dark purple-radish colour sample measure @540 nm[10]

Fig.1.2: standard KMnO₄ solution diluted in test tube B,1,2,3,4,5, (left to right)

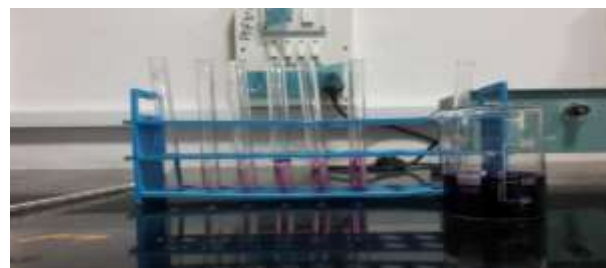


Fig 1.3: Test tube UK and BUK (treated with KMnO₄ and KOH) after incubation

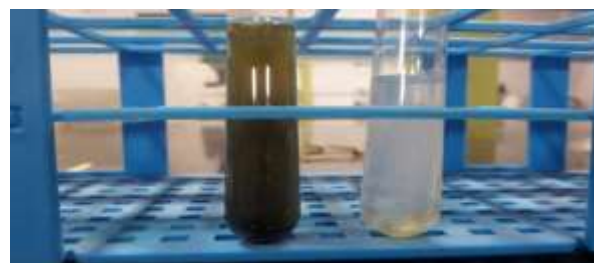


Fig.1.4 : Quantitative estimation of KMnO₄ Standard Curve against Absorbance to concentration in mol / liter.

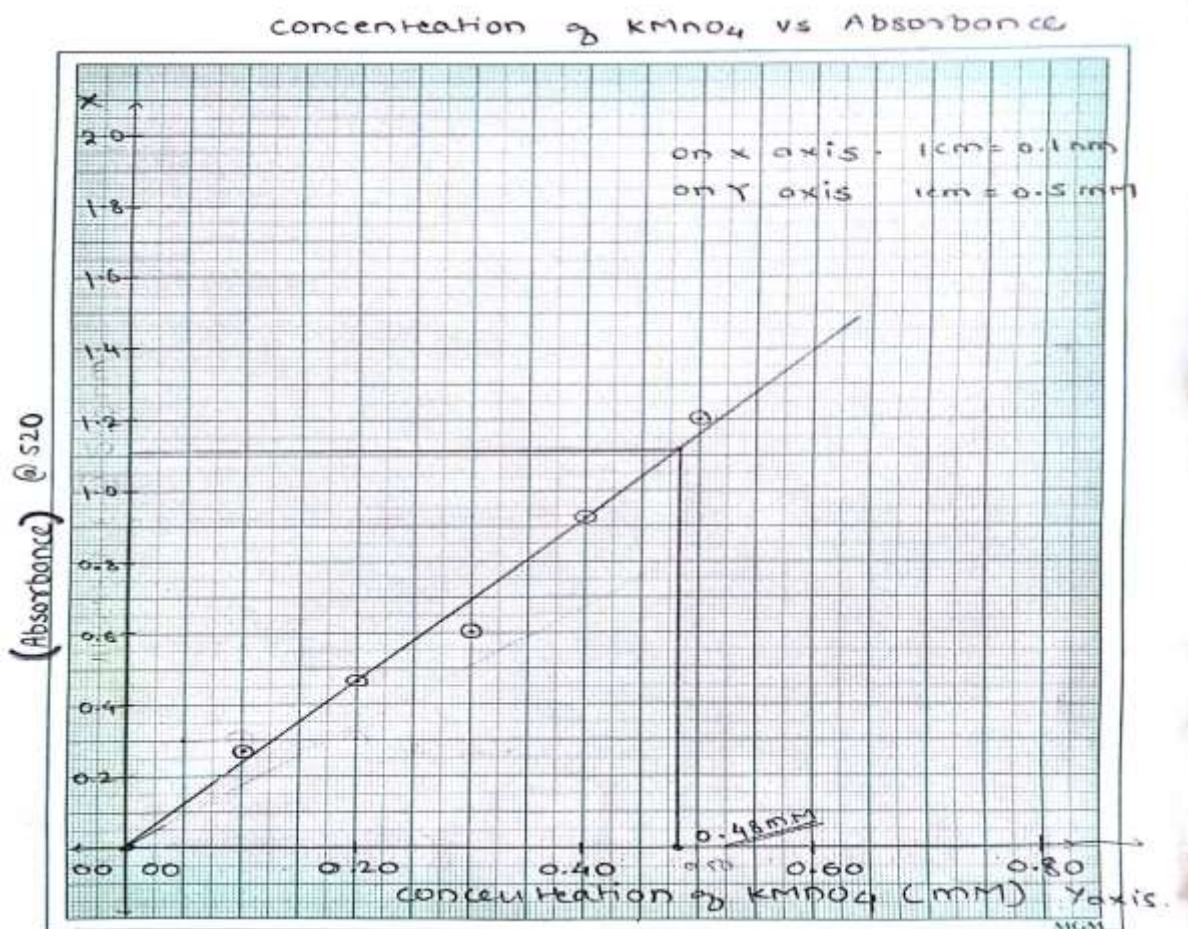


Table 1 : Quantitative Estimation of Oxygen.

sr.no.	Test tube no.	Concentration of $KMnO_4$:	Amount of Extraction buffer	1 M $MnSO_4$ {ml}	4 M KOH {ml}	Optical density@ 520 nm	Concentration Mol/ltr
1	B	0.00	5	-	-	0.00	0.00
2	1	0.02	4.98	-	-	0.30	0.0002
3	2	0.04	4.96	-	-	0.46	0.0004
4	3	0.06	4.94	-	-	0.6	0.0006
5	4	0.08	4.92	-	-	0.92	0.0008
6	5	0.10	4.9	-	-	1.20	0.0010
7	BUK	-	5	0.5	0.5	-	-
8	UK	-	5	0.5	0.5	1.12	0.00048

Calculation

O.D @ 520nm = 1.12

mole of KMnO_4 = 0.00048 mol/ltr (by Std graph)

mass of KMnO_4 = 0.07584 gms

$(0.0006) \times \text{M.W. KMnO}_4 \text{ 158.04/ 1 mole} = 0.0948 \text{ gm /ml}$

mole of O = $0.0948 / 16 = 0.00474$

Avogadro's rule [ref.6,13,]

1 mole = 6.022×10^{23}

$0.00474 \times 6.022 \times 10^{23} = 0.028 \times 10^{23}$

mass of oxygen = $0.00474 \times 16 / 4 = 0.0236 \text{ gm/liter}$

Result and Discussion:

The result of oxygen molecule determination and quantification in leaf extract of *ficus religiosa* summarized in Table 1 and calculation oxygen present in leaf sample react with reagent summarized in procedure and leaf extract undergo oxidation from MnO_2 this reaction shows high amount of oxygen present in leaf sample and sample turn greenish to brownish colour and clear zone @ upper level now after incubation 4 M KOH was and maintain alkaline condition the sample turn dark violet colour this indicate that manganese oxide react with KOH and forms K_2MnO_4 , concentration of KMnO_4 estimate by using standard graph and confirm by spectrophotometric analysis, concentration of oxygen is directly proportional to amount of amount of dioxygen total oxygen amount estimated by using avargardo rule i.e. total no. of moles of oxygen present in total no of KMnO_4 (see calculation) and result obtain after calculation i.e. 0.023 gm / ltr, 0.0059 moles / ltr, and 3.5×10^{23} molécules in 5 ml leaf sample of *ficus religiosa*.

Conclusion:

Oxygen plays important role in cellular

metabolism .It act as catalyst for all cell when sufficient oxygen and nutrient available cell perform optimum level with less energy . It stimulates cell division, Immune response, decrease level of stress hormone. This study shows, ficus plant contain high amount of oxygen, it is 203 mg/dl. When leaf extract consume this oxygen entered in cell and helps to smooth functioning and perform optimum level of cell metabolism with less energy .Oxygen molecule plays important role in photosynthesis this process also consume carbon-dioxide from atmosphere and end up with carbohydrate and oxygen by this method amount of oxygen release in photosynthesis can be calculated and also carbon-dioxide consumption by RQ (Respiratory Quotient) thus respiration and photosynthesis are interlinked ^{5*}and feed reactant to each other .Oxygen also destroys the harmful bacteria in our bodies without affecting the beneficial bacteria that we need.Hence determining oxygen value beneficial in areas of plant physiology and environmental biology, medical pharmacology.

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