Al-Kufa University Journal for Biology / VOL.11 / NO.1 / Year: 2019
Print ISSN: 2073-8854 Online ISSN: 2311-6544

Determination trace amount of Cu(II) in green cabbage leaves via new azo reagent compound

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Abstract

A new, simple, sensitive and rapid spectrophotometric method is proposed for the determination of trace amount of Copper (II). The method is based on the formation of a 1:2 complex with 3-(3-phenylpyrazylazo)2,7-naphthalendiol (3PPAN) as a new reagent is developed. The complex has a maximum absorption at 516 nm and (ε_{max}) of 9.5×10^{3} L. mol^{-1}. cm^{-1} .A linear correlation (1.25 –10. 0 μg. ml^{-1}) was found between absorbance at λ max and concentration. The accuracy and reproducibility of the determination method for various known amounts of Copper (II) were tested. The results obtained are both precise (RSD was 0.206%) and accurate (relative error was 1.14 %). The stability constant of the product was 5.00×10^{10} L. mol^{-1}. The method was applied successfully in green cabbage leaves .

Key words : Copper(II) determination, Cabbage; 3-(3-phenylpyrazylazo)2,7- naphthalendiol.

Introduction

The determination of metal ion at trace amount have been shown significant important in the fields of environment and biological studies. Literature date show that pyrozolone derivative have been widely used in the determination metal ions due to sensitive color, stability and very good chelatogenic characteristics [1-5].

Copper plays great role in the biological systems and used in many industries. Copper forms many complexes in the biological systems, ingredient of some bioactive molecules of enzymes and also in the storage and transport of active substances [6-8]. The biological complexes of Copper are important bioenzymes and have role in the biological systems. Important coenzymes studied are: methyl coenzyme, urease and acetyl coenzyme [9]. Several instruments method are present in the literature used to determine ion, and these methods inductively coupled plasma atomic emissions spectrometry [10, 11], flame and electro thermal absorption spectrometry [12-17], electrochemical methods [18, 19] and spectrophotometric techniques [20, 21] show good sensitivity but is limited because of expensive instrumentation and high cost for routine analysis.

According to the best of our knowledge, this reagent has not been reported in the literature as being used for any cation determination. In this study, we wish to report the 3-(3-phenylpyrazylazo)2,7-naphthalendiol (3PPAN) reagent as a selective reagent in spectrophotometric determination of micro a mounts of Copper (II).

2.Materials and Methods

The reagent was prepared by coupling 2,7-naphthlendiol with diazotate 3-amino-5-phenylpyrazole solution. A diazonium solution was prepared by taking 0.4922 g of 3-amino-5-phenylpyrazole in 50 ml of ethanol and concentrated hydrochloric acid with 5 ml of distilled water and adding sodium nitrite solution drop wise at (0 – 5°C). 2,7-naphthlendiol 0.5013 g was dissolved in 100 ml of ethanol and 30 ml of 0. 1 M were added at (0 - 5 °C). The mixture was left to stand overnight. The precipitate was filtered off and recrystallized from ethanol [22] Scheme1.

Scheme 1: Preparation of reagent (3PPAN)

3. Preparation of Copper(II) complex

The complex was prepared by stoichiometric amount from ligand in 50 ml of ethanol then added drop wise with stirring to a stoichiometric amount 1:2 for Copper salt in 25 ml hot distilled water. The solid brownish black product thus formed off, washed with ethanol and dried.

4. Apparatus

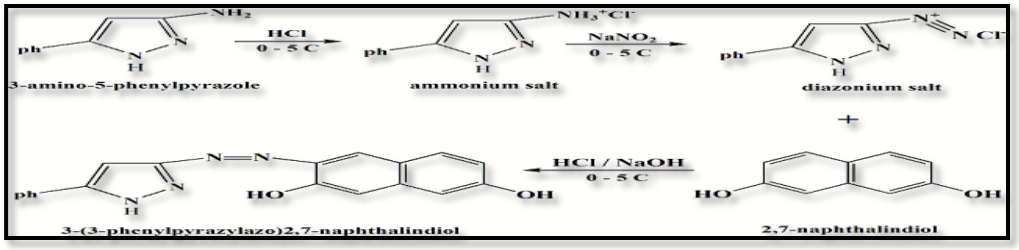
Spectrophotometric measurement were made with UV-Vis Spectrophotometer (LIBRA S60) double beam spectrophotometer using 1. 00 cm glass cells. Vibrational spectra were recorded on Test scan Testscan, (FTIR 8000 Series), Japan. Measurements of pH were made using an Hanna, HI9811-5 pH – meter equipped with a glass – saturated calomel combined electrode melting points of both ligand and complex were obtained with an electrothermalmelting point apparatus. Conductivity was measured in DMSO solution with an Alpha digital conductivity model-800.SHIMADZO atomic absorption spectrophotometer type AA-3600.

5. Reagents

All chemicals used were of analytical grades

Copper (II) stock solution (100 µg. ml^{-1})

Prepared by dissolving 0. 05 g of Copper Chloride in 500 ml of distilled water, working standard of Cu (II) solutions were prepared by simple dilution of the appropriate volume of the standard Cu(II) solution (100 µg. ml^{-1}) with distilled water. 3-(3-phenylpyrazylazo)2,7-naphthalendiol (3PPAN) (1 mM) 0. 0825 g of reagent was dissolved in 250 ml of ethanol.



6. General Procedure

In to a series of 10 ml calibrated flask, transfer increasing volumes of Cu(II) working solution 10 ppm to cover the range of calibration curve, add 2. 0 ml of 1Mm of (3PPAN)solution and pH was adjusted to 3.4. The complexes formed were solubilized in water and diluted up to 10 ml in a

standard flask. The absorbance of the resulting solution was measured at the respective absorption maxima against a reagent blank prepared under similar condition.

7. Results and Discussion

7.1 Spectra

The result of this work indicated that the reaction of Cu (II) with (3 PPAN) at pH 3.4 yield highly soluble product which can be utilized as a suitable assay procedure for Cu (II). This product has a maximum absorption at 458 nm at which the blank at this wavelength shows zero absorbance. The spectrum of (3 PPAN)solution gives strong absorption band belongs to n→π* of nitrogen atom of azo group at 308. 0 nm and with shoulder band due to transition π→π* of C=N group Fig. 1.

The bands of N=N and C=N were shifted to broad peak at high wavelength with high intensity at 516.0 nm upon complexation due to transition of π→π* charge transfer. Fig. 1,2

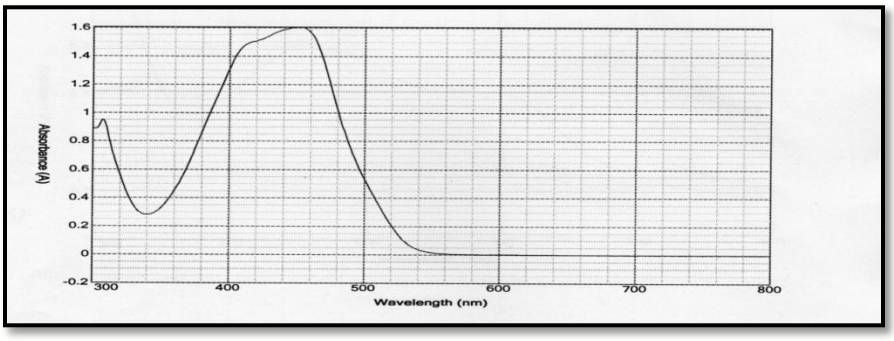


Figure 1: Absorption spectrum of the reagent *(3 PPAN)* against Ethanol as blank

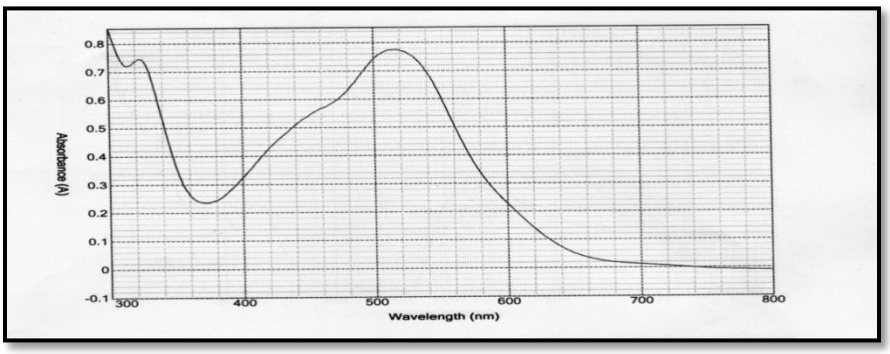


Figure 2: Absorption spectrum of [Cu (II) + *(3 PPAN)*] treated as described under procedure and against a reagent blank

7.2 pH effect

The pH of metal complex solutions was adjusted using dilute buffer solutions (0. 01M) CH_3COONH_4 ,NH_4OH and CH_3COOH and the effect on absorbance was studied Fig. 3. The absorbance of the complex was maximum and constant in the pH range given in table 2.

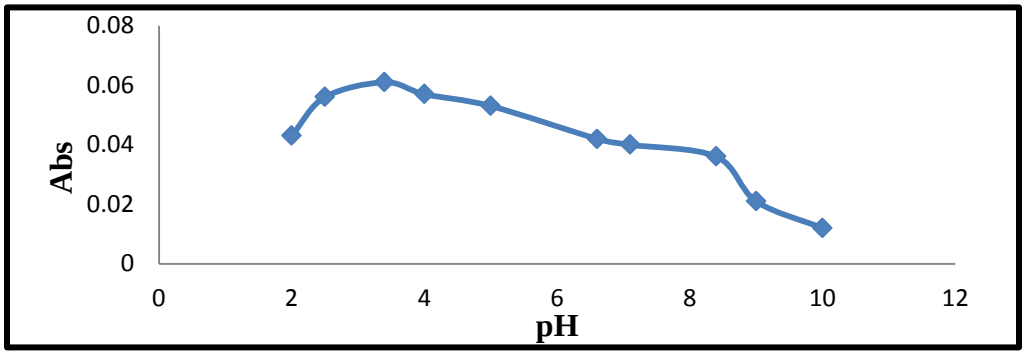


Figure 3 : pH effect

Table 1 : Characteristic Cu (II) – complex

Absorbtion maximum (nm)	516
Beer's law range (ppm)	1.25 – 10.0
pH	3.4
Sandell's sensitivity ($\mu g.cm^{-2}$)	6.6×10^{-3}
Molar absorptivity($L.mol^{-1}.cm^{-1}$)	9.5×10^{3}
Stability constand ($L.mol^{-1}$)	5.00×10^{10}
Melting point for reagent (C)	345 – 347
Melting point for Cu(II) complex(C)	256 – 258

7.3 Concentration Effect of (3PPAN)

Various concentration of (3 PPAN) was added to fixed concentration of Cu (II). 2. 0 ml of 1. 0 mM (3 PPAN) solution was sufficient and gave minimum blank value was increased causing a decrease in the absorbance of the sample. Therefore, 2ml of 1 mM of (3 PPAN) was used in all subsequent experiment Fig. 4.

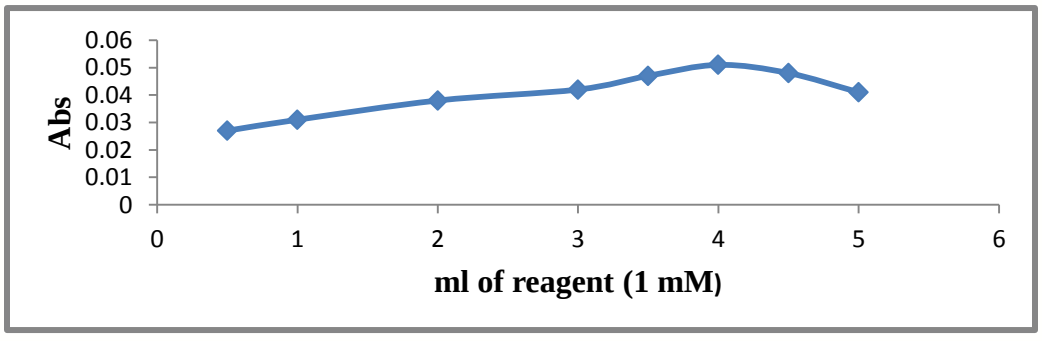


Figure 4: Effect of (3 PPAN) concentration

7.4 Effect of reaction time

The color intensity reached a maximum after the Cu (II) has been reacted immediately with (3 PPAN) and became stable after one minute, therefore one-minute development time was selected as optimum in the general procedure. The color obtained was stable for a least 24 hours. Figure 5.

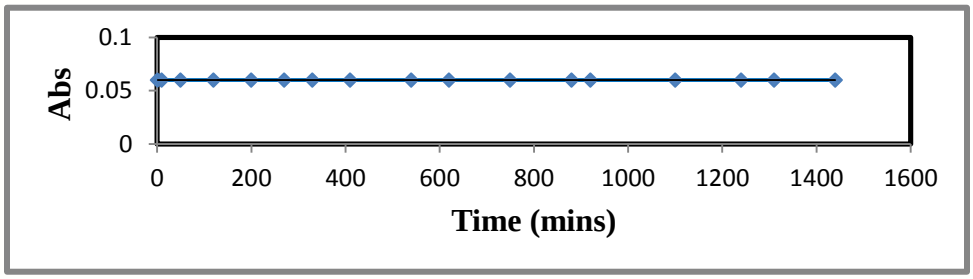


Figure 5 : Effect of reaction time

7.5 Effect of temperature

The effect of temperature on the colour intensity of the product was studied. In practice, the same absorbance was obtained when the colour was developed at room temperature (25 – 30 °C), but when the volumetric flask were placed in a water –bath at (40 – 70°C) a loss in colour intensity and stability were observed, therefore it is recommended that the colour reaction should be carried out at room temperature form (25 – 35 °C) for complex .Figure 6.

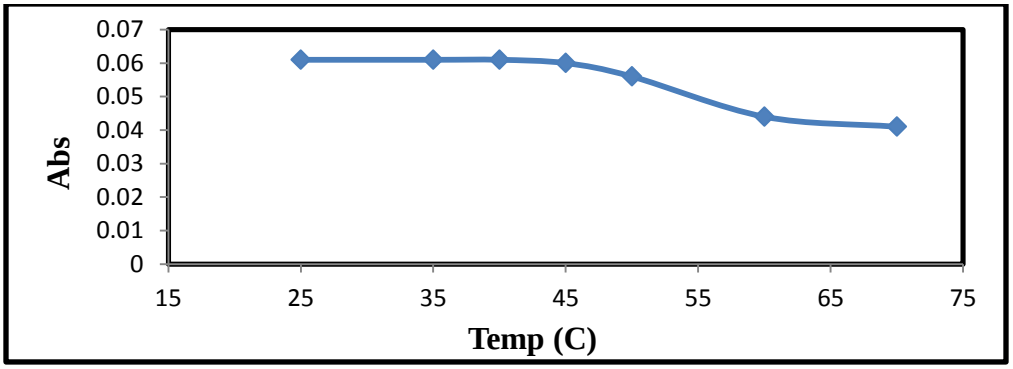


Figure 6: Effect of temperatures on Cu(II) complex

8. Calibration graph

The calibration equation for (1.25– 10.0 µg/ ml)Cu (II) isY = 0. 1501x + 0. 0321 (R2 = 0. 9948). Since the colored complex is stable for 24 hrs, the method can be applied to large series of samples. The molar absorptivity and sandal's sensitivity are given in Table 2.

9. Composition of the complex

The composition of complex was studied in the excess of reagent solution by the mole-ratio and Job's . Method Fig. 7, 8. A break at a 1:2 (M:L) mole ratio suggested the formation of complex where M= Cu (II) and L = (3 PPAN) under the given condition.

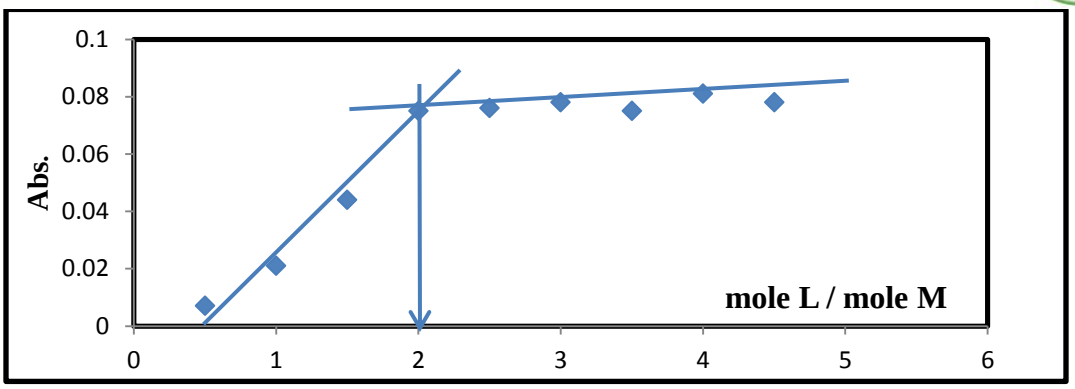


Figure 7: mole-ratio method for Cu [(3 PPAN)$_2$] complex

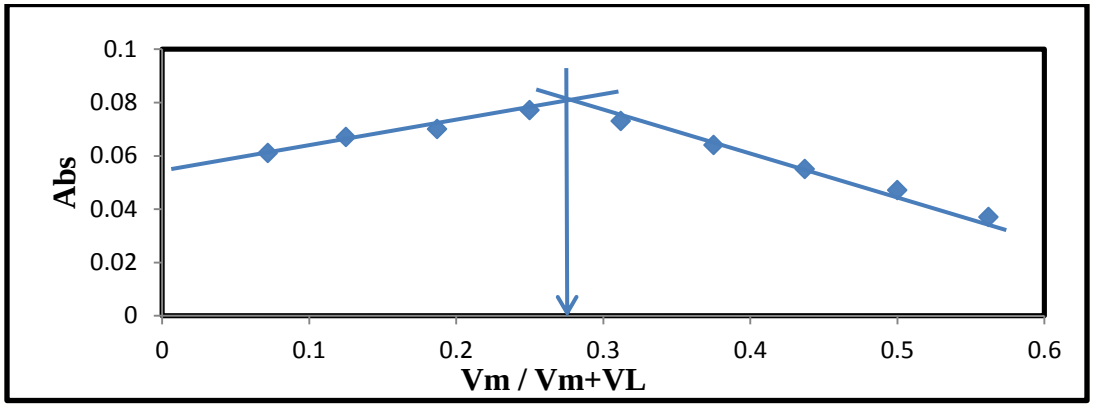


Figure 8: Job's method for Cu [(3 PPAN)$_2$] complex

10. Conductivity measurements

The solubility of the complex in dimethyl sulfoxide, methanol and Ethanol permitted of the molar conductivity of 10^{-3} M solution at 25°C and by comparison, the electrolytic nature for complex. The low values of the molar conductance data listed in Table. 3 indicate that the complex is non-electrolytes.

Table 2: Conductivity values of Cu[(3PPAN)$_2$] complex , s.mol^{-1}.cm^2

Molar conductivity in methanol	Molar conductivity in ethanol	Molar conductivity in DMSO
3.8	13.6	2.8

11. FT. IR of reagent and its complex

The FT. IR of the free ligand and its metal chelate were carried out in the (400 - 4000) cm^{-1}Range. The IR bands of the (3 PPAN) and its Cu (II)complex with their probable assignment are given in Table. 4 .The IR spectrum of ligand shows a broad band at 3387 cm^{-1} corresponding to O-H. This band is shifted to lower with low intensity at 3157 cm^{-1} frequency value upon

complexation suggesting chelation via the (M-O)However, the υ(N=N) stretching band in the free ligand is observed at 1575 cm^{-1} This band is shifted to lower with low intensity at 1539 cm^{-1} frequency value upon complexation suggesting chelation via the (M-N) [24].Fig 9,10.

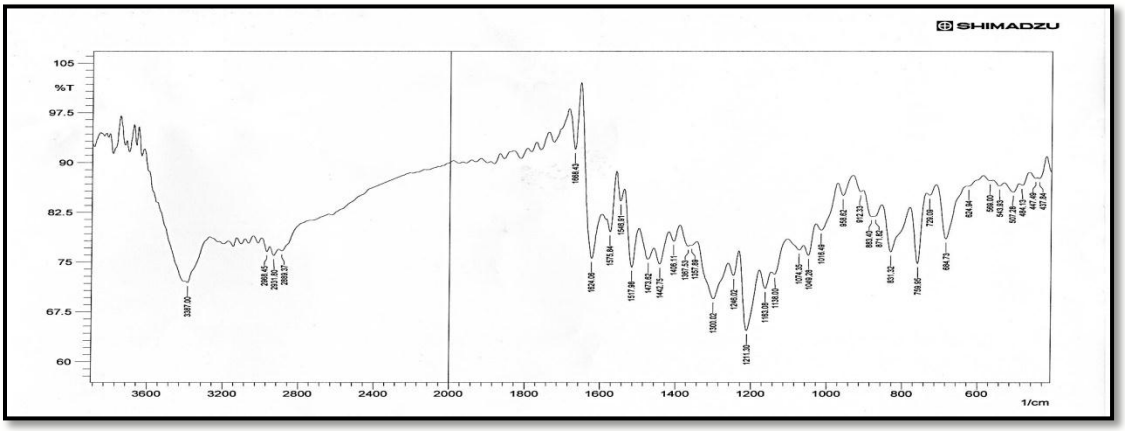


Figure 9. FT.IR for the reagent (3PPAN)

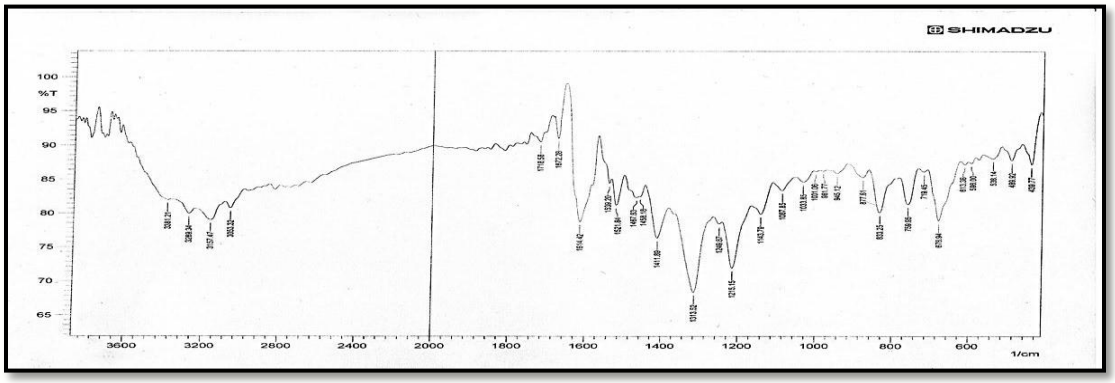
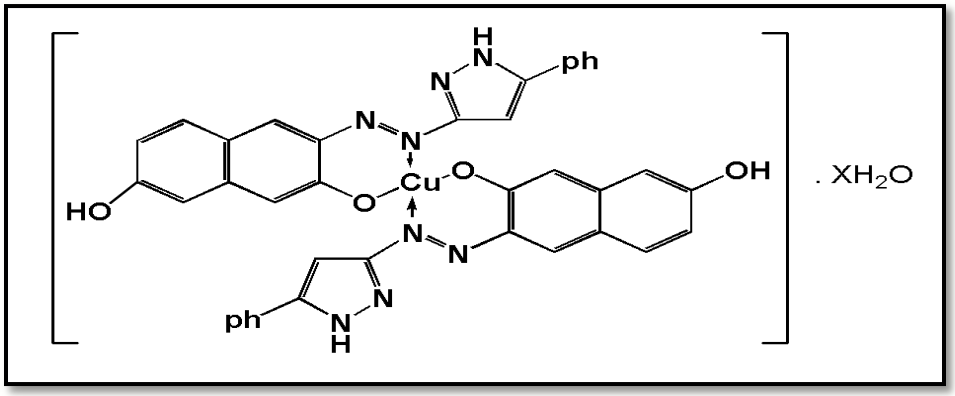


Figure 10. FT.IR for the Cu[(3 PPAN)$_2$] complex

On the basis of the FT.IR, stoichiometric and molar conductivity data the structure of complex can be suggested as the following:



Structure of Cu[(3PPAN)$_2$]

12. Applications

12.1 Estimation of Cu(II) in cabbage leaves

After washing some green cabbage leaves by distilled water, we put it in oven at (90 ^{o}C) for drying, the result is grinded and weight 1.0081 gm, add 10 ml from concentrated nitric acid and 2 ml from (H_2O_2) (30%). The mixture replaced in microwave for 20 sec, Added 10 ml deionized water following by filtration. Collecting the filtrate and transformed into 50 ml volumetric flask, the volume completed by distilled water and thesample is ready for UV-Visible and atomic absorption spectrometric analysis.[25].

Table 3: Determination of Cu(II) in green cabbage leaves

Amount found by our spectrophotometric method (ppm)	Amount found by atomic absorption (ppm)
0.3857	0.4233

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