

# Combining Natural Reaction Analogues and First Derivative Spectrophotometric Method to Enhance the Visible Spectra of a Non-Polar Crude Leaf Extract

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Research Article

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## ABSTRACT

**Aim:** This study was aimed at using natural reaction analogues and first derivative spectrophotometry to enhance the visible range of the non polar leaf extract.

**Study Design:** The leaf of *Alchornea cordiflora* was extracted so as to enrich the extract with chlorophyll holochromes and carotenoids. The ethanolic extract was designated as MX and used as the non polar leaf extract in the experiment.

**Place and Duration of Study:** Department of Biochemistry, University of Cape Coast, Ghana, between March 2011 and August 2011.

**Methodology:** A non-polar crude leaf extract was treated with naturally occurring reaction analogues of acidification, alkalinisation, oxidation and reduction. The spectra of the resulting solutions were analyzed by difference and first derivative spectrophotometric methods.

**Results:** The treatment led to the enhancement of the chromophoric content and the general quality of the individual spectrum. The wavelength regions: 420-500, 440-500, 460-500, 500-600 nm of spectral improvement were selective to their respective analogue test substance and conditions used in a reaction

**Conclusion:** The 500-600 nm range was better than the Soret and the red regions to monitor the copper complexation with acidic non-polar leaf extracts of *Alchornea cordiflora*. Alkalinization, oxidation and reduction conditions also resolved the multiple peaks of the Soret region into distinct peaks that can be useful for monitoring and quantification of bioinorganic preparations from the leaf extract of *Alchornea cordiflora*.

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## 1. INTRODUCTION

The current drive towards the use of plant crude extracts as against pure synthetic ligands for the synthesis of inorganic nanostructures is purely borne out of health and environmental concerns (Leela and Vivekanandan, 2008). In addition, coordination compounds with mixed ligands are more versatile than mono-liganded complexes in organic reactions (Thahira et al., 2007). The vast diversity of metabolites in plants makes plant crude extracts attractive reagents for the preparation of bioinorganic compounds with mixed activities and for environmentally benign and biocompatible nanostructures (Leela and Vivekanandan, 2008; Govindarazu et al., 2010). The use of plant extracts for the synthesis of bio-inspired nanostructures will require simple, cost effective but robust means of monitoring in order to understand the mechanism and kinetics of the reaction to ensure less defective but more homogenous chemical composition of the product. UV-visible spectrophotometry has been the method of choice of routine comparative monitoring the synthesis of chemical compounds (Silverstein et al., 1991). It is fast, simple, easy to assemble and applicable to wide range of compounds and conditions. The conventional methods of UV-visible monitoring in synthesis rely on spectral changes of either the product as it is formed or the reactants as they are consumed. The two approaches are daunting in inorganic synthesis involving non-polar leaf extracts in view of their complex spectra. The visible spectrum of a non-polar leaf extract is characterized by the superposition of chlorophylls and carotenoids peaks in the Soret region and the overwhelming broad chlorophyll peaks in the red region (Croce et al., 2001). This study was therefore aimed at evaluating the combined effects of natural reaction analogues and first derivative spectrophotometric method (Dere et al., 1998) on the enhancement of the visible spectrum of a crude non-polar leaf extract of *Alchornea cordifolia* that could be useful for monitoring bioinorganic synthesis. *Alchornea cordifolia* grows in the wild in the sub-Saharan Africa countries. The leaf extract of this plant is used in traditional medicine as anti-inflammatory, anti-bacteria, anti-fungus and anti-parasitic (Mavar et. al., 2004). In Ghana the leaf extract is also used for food coloration and dyeing of cloths. In addition to pigments such as chlorophyll and carotenoids, the leaf also contains chemicals such as tannins, phenolic acids, flavonoids, and alkaloids (Mavar et. al., 2004). Improved method of monitoring the synthesis of bioinorganic compounds using the leaf extract of *Alchornea cordifolia* will expand the use of the plant and orientate research on its compositional standardization, and formulation for a successful commercial exploitation. For the above reasons the plant was used in this study as a model for medicinal leaf extracts and also to access its potential use in bioinorganic preparations.

## 2. MATERIALS AND METHODS

### 2.1 Collection, Preparation of Non-Polar Extract, MX

The leaf of *Alchornea cordifolia* was selected for this study since it is widely used in Ghana for food coloration and general dyeing purposes and also for its easiness of organic extraction. The leaves were collected and identified by the plant taxonomist at the University of Cape Coast, Ghana. The leaves were kept at -20 °C prior to extraction after a brief washing in cold running water. 100 gm of thawed leaves were extracted overnight by

steeping the leaves in 1000 ml of chilled 100% acetone. Acetone was removed by rotary evaporation and the remaining green turbid residue was diluted up to 100 ml with distilled water and kept at 4 °C as the bulk acetone fraction prior to use. The 100 ml bulk acetone fraction was extracted three times with 100 ml of 100% hexane for each run. After evaporation of hexane the residues were combined and dissolved in 20 ml of absolute ethanol and used as the non-polar crude leaf extract MX for visible spectrophotometric analysis.

## **2.2 Analogue Reactions**

The analogues of the naturally occurring reactions, 1–5 were performed by treating the reagent MX with the appropriate analogue test substance. Different compositions of ethanol-aqueous solvent system were tested for each analogue reaction to ensure proper mixing of the non-polar components of MX and the inorganic test samples. Inappropriate combination of the two solvents could result in precipitation of a turbid MX solution. The following media containing the various analogue test samples (underlined) were set for the analogue reactions (1-5):

1. 1800  $\mu$ L ethanol, 0.01 M HCl, pH 2.2
2. 1800  $\mu$ L ethanol, 0.02 M NaOH, pH 9.0
3. 2100  $\mu$ L ethanol, 850  $\mu$ L water, 0.3 % H<sub>2</sub>O<sub>2</sub>
4. 1500  $\mu$ L ethanol, 700  $\mu$ L water, 0.04 M NaBH<sub>4</sub>
5. 1700  $\mu$ L ethanol, 1200  $\mu$ L of 0.1M HCl, 20  $\mu$ M CuSO<sub>4</sub>

The analogue test substances were prepared with double distilled water. Ethanol, acetone and hexane were analytical grade.

## **2.3 Spectrophotometric Analysis**

All spectrophotometric readings were recorded with visible spectrophotometer (Jenway 6100) equipped with a 1-cm light path length. For each test, absorption data were recorded for sample within the wavelength range of 400 to 700 nm at 10 nm wavelength intervals. 200  $\mu$ L of reagent MX was equilibrated in each of the above reaction media at 30 °C for 10 minutes for completion of reaction before absorbance was recorded with borosilicate cuvette for a final reaction volume of 3.0 ml. Spectra for the control samples of reagent MX were recorded in reaction media without the analog test substances. The media 1-5 were used as blanks and subtracted from their respective spectra. First derivative spectra were obtained by mathematically manipulating the output data of the zero order absorption spectra on spreadsheets using Microsoft Excel 2007 program.

## **3. RESULTS AND DISCUSSION**

Many of the reported use of plant-extracted solutions for the preparation of bioinorganic substances have been performed in aqueous systems where metal salts and the extracted solutions are soluble. For plant aqueous extracts, polyols and water-soluble heterocyclics are the major components capable of reducing metal centers (Chobot and Hadacek , 2010). This implies that a large proportion of plant metabolites made up of non- polar molecules have not been explored for the synthesis of bioinorganic substances. The problem associated with the use of non-polar leaf extracts in bioinorganic synthesis is their limited solubility in aqueous systems where most metal salts are soluble. The complex spectrum of

a non-polar leaf extract also makes it difficult to identify reactant (leaf extract) or product signal that can be used for monitoring the reaction. The solutions to these problems are highlighted in the sections 3.1 to 3.4. The use of hexane leaf extract obtained from the bulk acetone leaf extract, as against the direct use of hexane or acetone leaf extract for the analyses was aimed at enriching the hexane leaf extract with chlorophyll holochromes and carotenoids and also to prevent turbidity of MX treatments. MX treatments produced clear solutions in 50 to 70 % ethanol in the stated combinations as shown in Section 2.2 with the analogue test substances and copper sulfate. Acid-base and redox reactions were explored in this study as the naturally occurring reaction analogues since they are the commonest physiological reactions of the cell. Copper complexation with the non-polar leaf extract was used to test the two main goals of this study. First, the mixing of the inorganic substances and the non-polar extracts at microscale level to give a clear solution with stable color suitable for spectrophotometric analysis and second, the generation of an enhanced reagent's (leaf extract) peak distinctive enough to be used for monitoring the synthesis of bioinorganics. The spectral analysis of the products of the reactions 1 to 5 is presented as Figures 1 to 5 respectively. A and B parts of each figure are the zero and first derivative spectral scans of each product. The aim is to reveal the extent of change and chromophoric content of each wavelength window of analysis and the selectivity of the change to the analogue reaction.

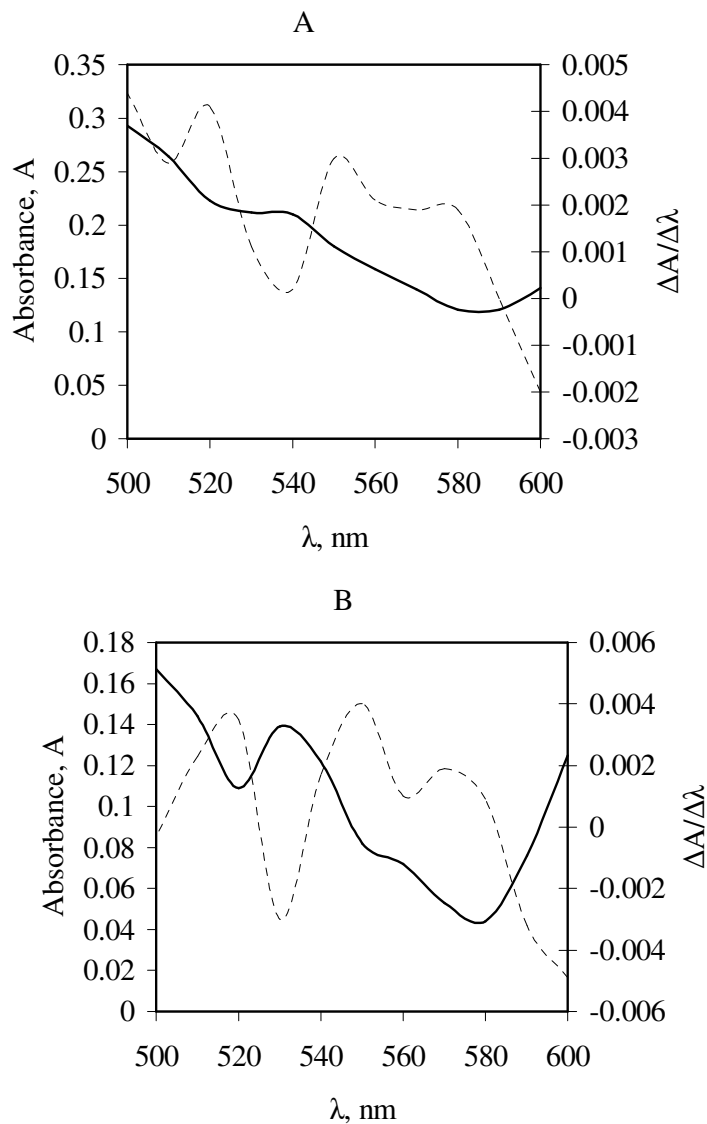
### **3.1 Spectral Analysis of MX in Acidifying Condition**

In Figure 1, there was a clear enhancement of absorption bands in the wavelength range of 500-600 nm after acid treatment. The three overlapping derivative peaks of the control (A) sample were resolved after acid treatment (B) providing more spectral details within this window. The absorbance of a major peak around 535 nm was lower under acid treatment but the general spectral quality was improved with the treatment. The wavelength range 500 to 600 nm is transparent to pure chlorophyll and carotenoid mixtures and in the case of crude green leaf extracts can only be seen with chlorophyll band tailings that may constitute about 10-20 % of the maximum chlorophyll peak (Croce et al., 2001). The region can serve as the most reliable monitor for metal complexes of plant-extracted ligands compared to the complex Soret region which was not affected by the acid treatment. Thus in addition to the general effect of pH changes on absorption the treatment of extracts with hydrochloric acid must have led to the accumulation of ligands including the pheophytins (Nelson and Feruzzi, 2008) which resulted in the general increase in the chromophoric content of the zero and first derivative absorption spectra in the 500 to 600 nm range as shown in Figure 1.

### **3.2 Spectral Analysis of MX in Alkaline Medium**

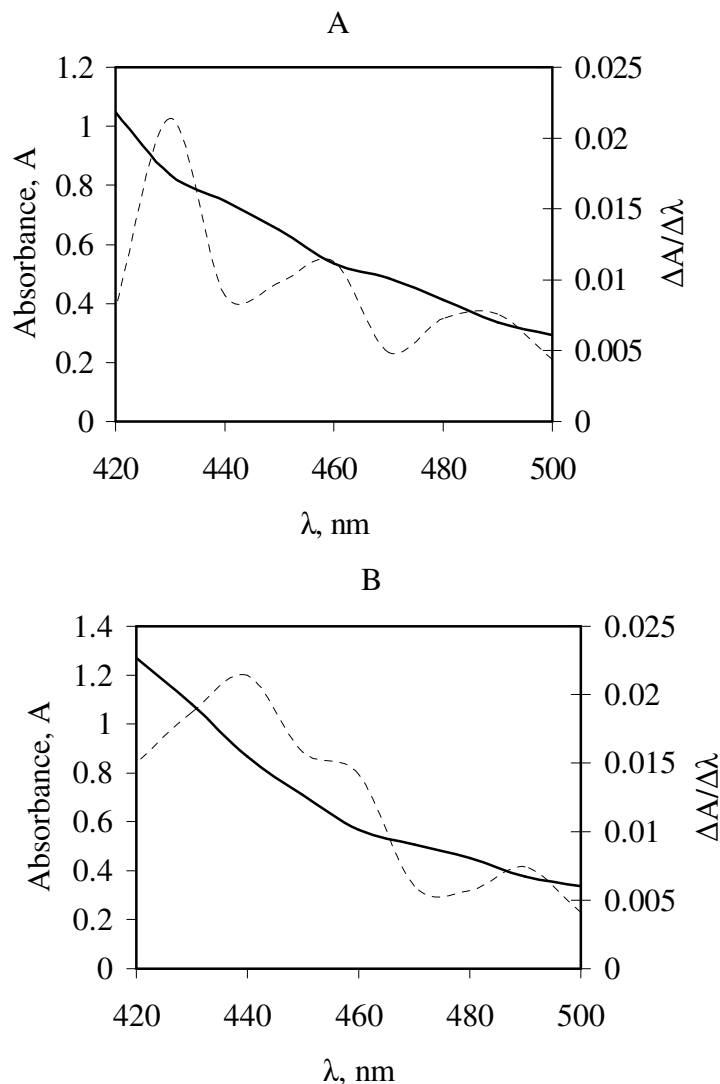
In Figure 2, the first order spectra of the untreated (A) and treated (B) samples of reagent MX were more informative than the zero order spectra. A shift of the derivative signal in the range of 420-440 nm without a corresponding shift in the adjacent signal after alkali treatment may be due to the different origins of the two adjacent signals in the control spectra (A). The alkali treatment did not affect the yellow-green (500-600 nm) and the red (600-700) regions. The Soret region (350-450 nm) is composed of chlorophyll and carotenoid peaks and therefore the affected peak could be as a result of the saponification of the carotenoid components.

This implies that the unaffected adjacent derivative signals in the 450 nm to 500 nm range could be due to the chlorophyll molecules since these could not have been deesterified by the mild sodium ethoxidic condition of the analogue reaction (2) (Fernandez et al., 2000).



**Figure 1. Visible scanning of leaf extract MX, in neutral (A) and acidic (B) media.**

The acidic medium contained 0.01 M HCl at pH 2.2 Zero order (—) and first order (.....) derivatives were determined for absorbance values recorded within the wavelength range 500 to 600 nm



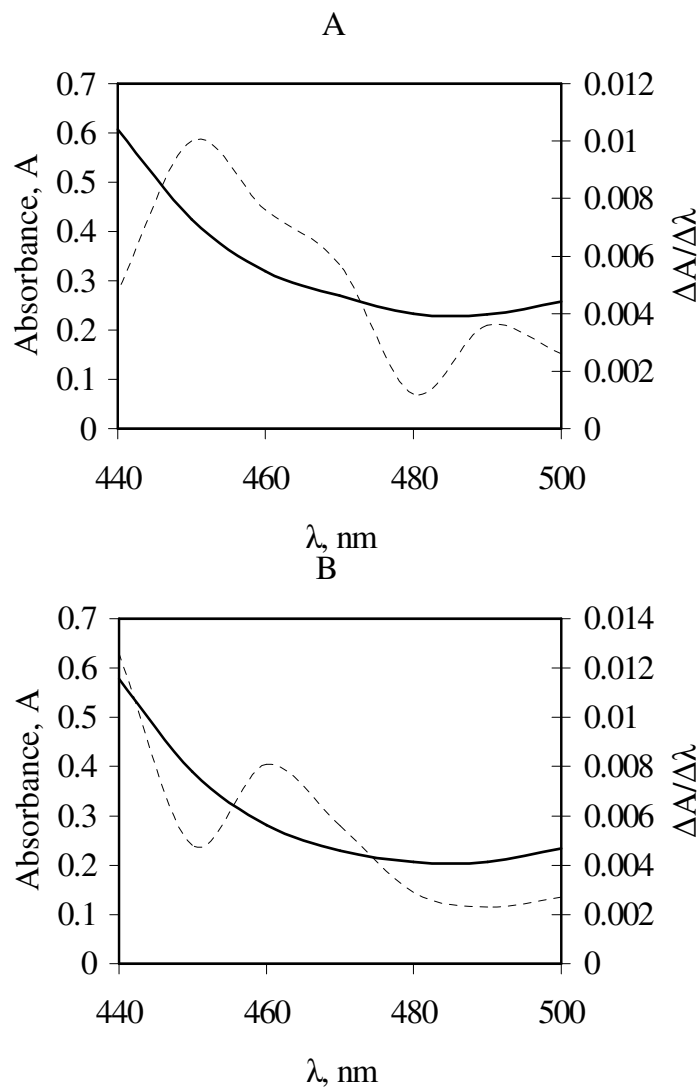
**Figure 2. Visible scanning of leaf extract MX, in neutral (A) and alkaline (B) conditions.**

The alkaline medium contained 0.02 M NaOH at pH 9.0. Zero order (—) and first order (.....) derivatives were determined for absorbance values recorded within the wavelength range 420 to 500 nm

### 3.3 Spectral Analysis of MX in Oxidizing Medium

In the 440-500 nm spectral window of Figure 3, the effect of oxidation on MX was not visible in the normal spectra of the treated and untreated MX. However, the first derivatives of the two spectra displayed shifting and quenching of signals resulting in a single derivative signal in this window after treatment. Hydrogen peroxide has been shown to oxidize both chlorophyll and carotenoid molecules with or without catalysis (Fenton or peroxidase)

(Takahama et al., 2007). The first derivative peak around 455 nm (Figure 3B) is a sharp contrast to the corresponding zero and first order spectra of Figure 3A.



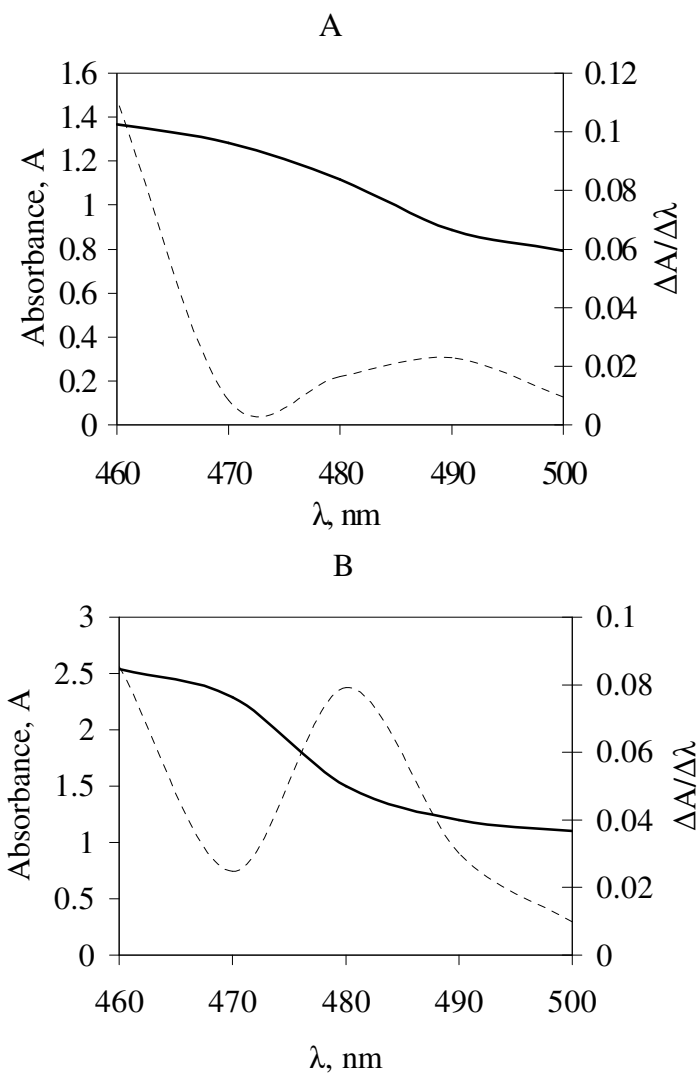
**Figure 3. Visible scanning of leaf extract MX, in control (A) and oxidizing (B) conditions.**

The oxidative agent was 0.3 %  $H_2O_2$ . Zero order absorption spectrum (—) was converted to first order spectrum (.....) for the wavelength range 440 to 500 nm

### 3.4 Spectral Analysis of MX in Reducing Medium

In Figure 4, the first derivative spectrum of the  $NaBH_4$ -treated sample gave around 25 % increase in signal height. The symmetrical nature of this signal with an increase in depth of the adjacent valleys might indicate a unique chromogenic reaction product responsible for the observed transformation. The first derivative pattern observed in Figure 4 was similar to Figure 3. However, the reduction gave a maximum derivative peak around 475 nm as

shown in Figure 4B. Sodium borohydride has opposite spectral effect on chlorophylls and carotenoids causing red shift in chlorophylls and blue shift in carotenoids. However, it has been found that compared to chlorophylls, carotenoids are in general quite resistant to reduction. A dilute solution of sodium borohydride can readily reduce the formyl group in chlorophyll b to its hydroxymethyl derivative while esters, vinyl and carboxyl groups of carotenoids are only reduced with difficulty (Chadwick et al., 1987). Thus, the unique first derivative peak maximal of the treated MX shown in Figures 3 and 4 could be due to changes in the chlorophyll pigments and not in the carotenoid molecules.



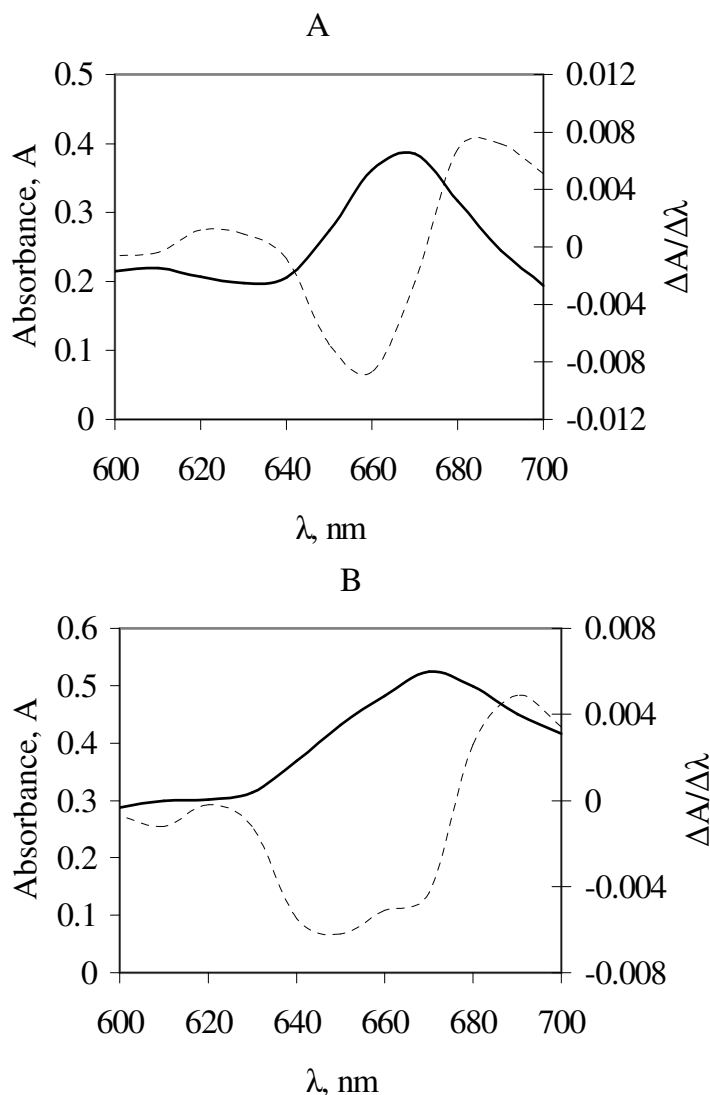
**Figure 4: Visible scanning of leaf extract MX, in control (A) and reducing (B) conditions**

The reductive agent was 0.04 M NaBH<sub>4</sub>. Zero order absorption spectrum (—) was converted to first order spectrum (.....) for the wavelength range 460 to 500 nm



### 3.5 MX-Copper Complexation Observed by Enhanced Spectrum

In Figure 5, the significance of the unique extraction protocol, the selectivity and sensitivity of the naturally occurring reaction analogs and first derivative spectroscopy to spectral enhancement were tested by experiment reported by Nelson and Feruzzi, (2008) showing the replacement of magnesium in chlorophyll by iron in acidic condition with modifications.

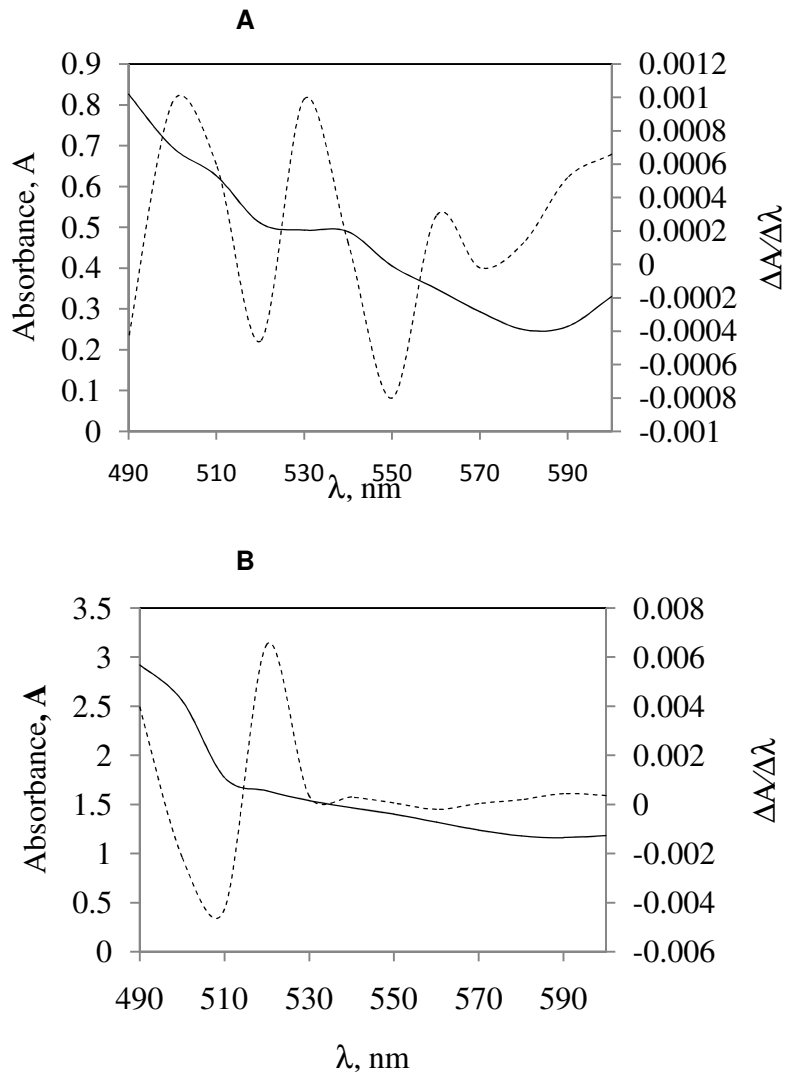


**Figure 5: Visible scanning of leaf extract MX, in control (A) and copper complexing (B) conditions.**

*Copper-complexing medium contained 20  $\mu$ M CuSO<sub>4</sub> in HCl solution. Zero order absorption spectrum (—) was converted to first order spectrum (.....) for the wavelength range 600 to 700 nm*

The treatment of acidic ethanolic solution of leaf extract MX, with copper sulfate resulted in the replacement of Mg (II) ions by Cu (II) ions in chlorophyll molecules as shown by the

splitting of a single minimum of Figure 5A into two minima in Figure 5 B. The occurrence of the acid modified MgChl (pheophytin, H<sub>2</sub>Chl) peak at  $\lambda_{\max}$  around 675 nm and the more stable CuChl peak at  $\lambda_{\max}$  around 655 nm was better observed with first derivative spectrum than by the zero order spectrum as shown in Figure 5B. In Figure 6A the enhancement of the chromophoric content in the 500-600 nm range of Figure 1 was tested as the reagent's (leaf extract) own signal that can possibly monitor the accumulation of Cu-pheophytin complex product of Figure 5. As shown in Figure 6B, treatment of the acidic MX with copper sulfate resulted in a single symmetrical derivative peak in this wavelength window making this window a plausible reagent's (leaf extract) own signal that could be used to monitor Mg (II) titration with Cu(II) in chlorophyll molecules in MX as shown in Figure 5B.



**Figure 6: Visible scanning of leaf extract MX, in control (A) and copper complexing (B) conditions.**

*Copper-complexing medium contained 20  $\mu$ M CuSO<sub>4</sub> in HCl solution. Zero order absorption spectrum (—) was converted to first order spectrum (.....) for the wavelength range 500 to 600 nm*

#### 4. CONCLUSION

Many traditional preparations obtained from food and medicinal plants owe their efficiency on the multi-component nature of their crude rather than on any purified compound (Chatterjee et al., 2010). Consequently a number of broad-based chemical analysis and applications of their crude extracts have been reported (Chatterjee et al., 2010). Bio-inspired inorganic structures are usually prepared with the crude extract of the bio-material and analyzed without resort to purifications. Spectrophotometric analysis of crude non-polar leaf extracts or their inorganic derivatives are quite daunting in view of their complex components. In this study chemical and first derivative treatments achieved enhanced and stable spectral signals in the Soret, the uninformative yellow-green (500-600 nm) and the red spectral regions of a non-polar leaf extract. The improved chromophoric content has been shown as possible monitor for copper pheophytin complexation. Alkalinity, oxidative and reductive reactions resulted in specific chemical changes that gave unique absorption peaks which were treated with first derivative to give enhanced spectra in the windows 420-500 nm; 440-500 nm and 460-500 nm respectively. The approach could complement the use of color visualization, increasing absorbance and shift in wavelengths to monitor the synthesis of bioinorganic substances.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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