# Study on Extraction and Characterization of Chlorophyll Dyes from Spinach, Ivy Gourd and Bottle Gourd Leaves

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**Abstract**

In this study, spinach leaves ***(Spinacia oleracea)***, ivy gourd leaves ***(Coccinia grandis)*** and bottle gourd leaves ***(Lagenaria siceraria (Mol.))*** were extracted by methanol solvent. The extracting temperature treated was 100 °C for 2 hr. Chlorophyll from three pigments has been confirmed by UV-Vis analysis. From absorbance and transmittance spectra, optical band gap was determined for spinach dye, ivy gourd dye and bottle gourd dye. The residual parts of spinach powders, ivy gourd powders and bottle gourd powders were examined by FTIR analysis and EDXRF analysis. FTIR was used to determine the various functional groups present in the adsorbent. EDXRF analysis was used to determine the concentration of elements of the residual parts of the three leaves.

**Keywords**: Chlorophyll, Dye, Pigments, UV-Vis, FTIR, EDXRF,

## Introduction

The chlorophylls are one of a number of pigments usually contained in green organelles of higher plants (E.D Dikio *et al.*, 2008). Initially, it was assumed that chlorophyll was a single compound but in 1864 Stokes showed by spectroscopy that chlorophyll was a mixture. If dried leaves are powdered and digested with ethanol after concentration of the solvent, 'crystalline' chlorophyll is obtained, but if ether or aqueous acetone is used instead of ethanol, the product is 'amorphous' chlorophyll (Haworth P *et al.,* 1983, Omolora *et al.*, 2014).

Chlorophyll assists this transfer as when chlorophyll absorbs light energy, an electron in chlorophyll is excited from a lower energy state to a higher energy state. In this higher energy state, this electron is more readily transferred to another molecule. This starts a chain of electron-transfer steps, which ends with an electron being transferred to carbon dioxide. Meanwhile, the chlorophyll which gave up an electron can accept an electron from another molecule. This is the end of a process which starts with the removal of an electron from water. Thus, chlorophyll is at the centre of the photosynthetic oxidation-reduction reaction between carbon dioxide and water (Ogawa T. *et al.*, 1996, Thornber J.P. *et al.*, 1975, and Wang X. *et al.*, 2012).

In this work, natural dyes (chlorophyll based) were extracted from spinach leaves, ivy gourd leaves and bottle gourd leaves. The absorbance and transmittance spectra of chlorophyll based three samples were determined by using SHIMAZHU UV-1800 spectrophotometer. The residual parts of spinach powders, ivy gourd powders and bottle gourd powders were examined by FTIR and EDXRF analysis.

## Experimental procedure

The natural dyes were extracted with methanol by the following procedure. Fresh spinach leaves, fresh ivy gourd leaves and fresh bottle gourd leaves were washed with water and dried at room temperature. After drying, they were crushed into powder with agate motor. Each powder (1 g) was dissolved with 25 ml of methanol in the beaker.

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These solutions were kept at room temperature for about one week. After one week, these solutions were stirred with magnetic stirrer at 700 rpm for 1hr and were heated at 100 °C with water bath for 1 hr. And then, the residual parts were filtered out and the resulting filtrates were used as dye solution. Glass substrates were kept in each solution for 15 hrs. These films were kept to be dried with room temperature.

Optical properties of dye in UV and visible regions were analyzed by using SHIMADZU UV-1800 photospectroscopy. The various functional groups present in the adsorbent and concentration of elements of the residual parts of spinach powders, ivy gourd powders and bottle gourd powders were examined by FTIR and EDXRF analysis.

## Results and Discussions

### UV-Vis analysis

UV-Vis absorption values of dye films were measured in (SHIMADZU UV-1800) spectrophotometer. The UV-Vis photospectra of spinach leaves, ivy gourd leaves and bottle gourd leaves were recorded with respect to bare the substrate placed in the reference beam using beam spectrophotometer.

The absorption peaks of chlorophyll dye extracted from spinach leaves at room temperature are 536.00 nm, 616.00 nm and 663.00 nm in figure 1. The cut off wavelength of spinach dye film absorption spectrum was 520.00 nm. The absorption peaks of chlorophyll dye extracted from ivy gourd leaves at room temperature are 534.00 nm, 616.00 nm and 664.00 nm in figure 2. The cut off wavelength of ivy gourd dye film absorption spectrum was 515.00 nm. The absorption peaks of chlorophyll dye extracted from bottle gourd leaves at room temperature are 504.00 nm, 538.00 nm, 608.00 nm and 665.00 nm in figure 3. The cut off wavelength of bottle gourd dye film absorption spectrum was 495.00 nm.

All absorption peaks lie between the ranges of 504.00 to 665.00 nm. The absorption abilities of dye solutions are closely that of chlorophyll at room temperature.

The energy band gap of each wavelength was calculated by using Plank's photoelectric equation.

$$\mathrm{E} = h\nu = \frac{\mathrm{hc}}{\lambda}$$

Where E (eV) is the energy band gap, h is Plank's constant, c is the velocity of light and $\lambda$(nm) is the wavelength.

The optical band gap of spinach dye film from absorption spectrum was calculated to be 2.42 eV. The optical band gap of ivy gourd dye film from absorption spectrum was calculated to be 2.37 eV. The optical band gap of bottle gourd dye film from absorption spectrum was calculated to be 2.51 eV. Table (1) showed the variation of energy band gap of spinach leaves, ivy gourd leaves and bottle gourd leaves at room temperature.

The transmittance spectra were analyzed by plotting hν vs $(\alpha h\nu)^2$, based on following equation.

$$\alpha h\nu = A\,(h\nu - E_g)^{n/2}$$

Where $\alpha$ is the absorption coefficient, A is a constant and n is the exponent that depends upon the quantum selection rules for the particular material. A straight line is obtained when $(\alpha h\nu)^2$ is plotted against photon energy (hν), which indicates that the absorption edge is due to a direct allowed transition (n =1 for direct allowed transition). The intercept of a straight line on hν axis corresponds to the optical band gap ($E_g$) and its values determined.

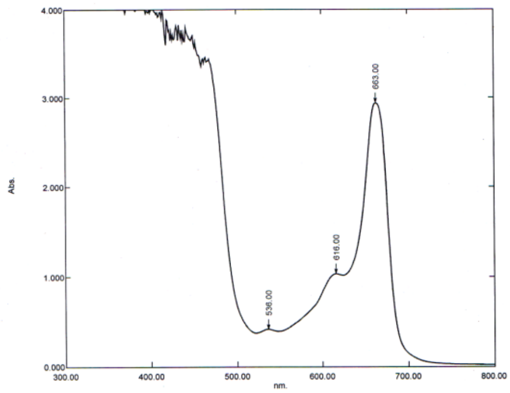


Figure (1). Absorbance spectrum of spinach dye thin film

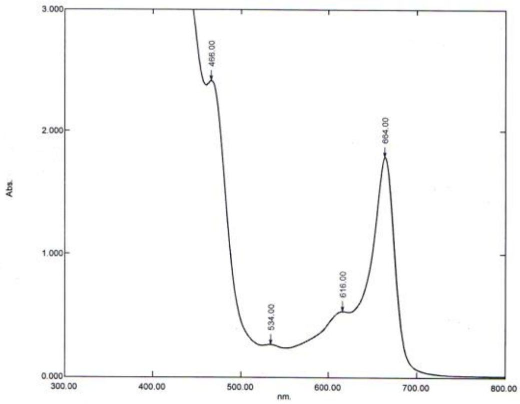


Figure (2). Absorbance spectrum of ivy gourd dye thin film

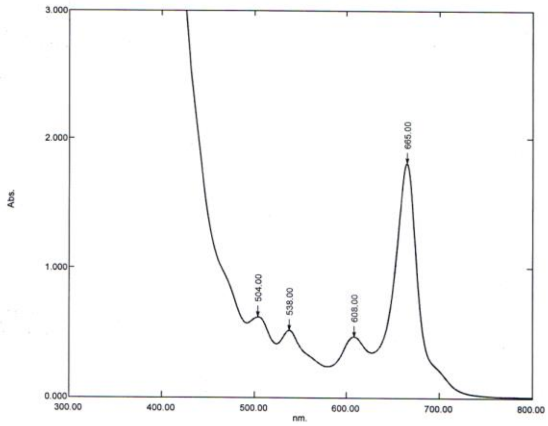


Figure (3). Absorbance spectrum of bottle gourd dye thin film

Table (1). Variation of energy band gap of spinach leaves, ivy gourd leaves and bottle gourd leaves at room temperature from absorbance spectra

| No | Type of dyes | Energy band gap |
|---|---|---|
| 1. | Spinach leaves | 2.42 eV |
| 2. | ivy gourd leaves | 2.40 eV |
| 3. | Bottle gourd leaves | 2.51 eV |

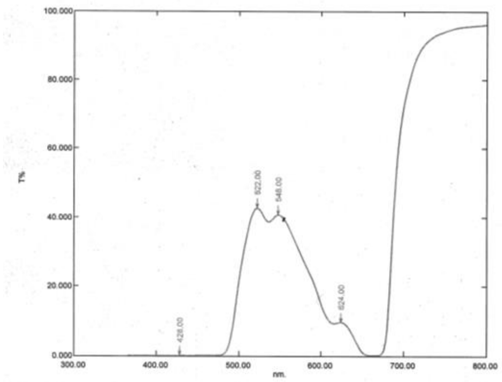


Figure (4). Transmittance spectrum of spinach dye thin film

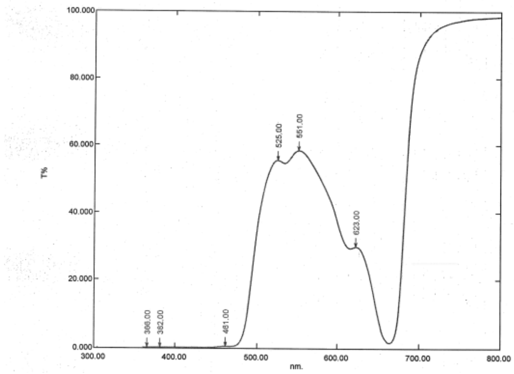


Figure (5). Transmittance spectrum of ivy gourd dye thin film

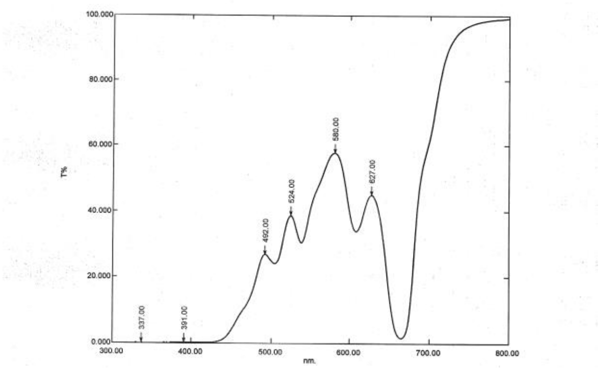


Figure (6). Transmittance spectrum of bottle gourd dye

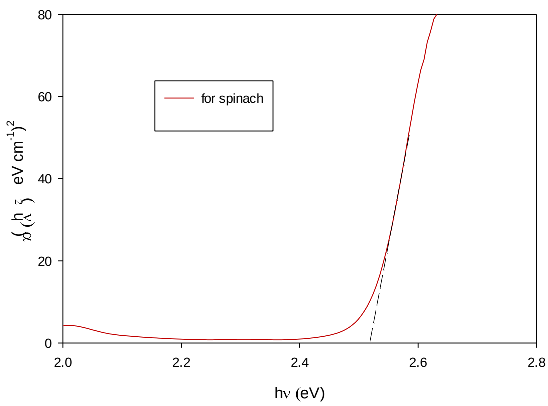


Figure (7). Optical energy band gap of spinach dye at room temperature from transmittance spectra

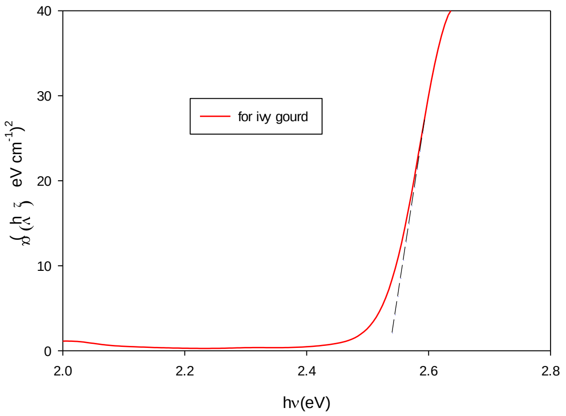


Figure (8). Optical energy band gap of ivy gourd dye at room temperature from transmittance spectra

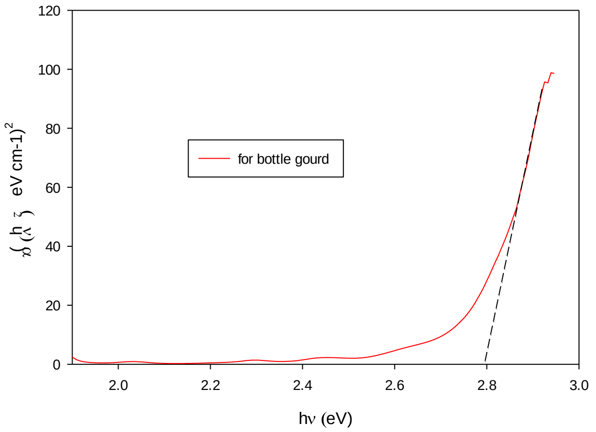


Figure (9). Optical energy band gap of bottle gourd dye at room temperature from transmittance spectra

Table (2). Variation of energy band gap of spinach leaves, ivy gourd leaves and bottle gourd leaves at room temperature from transmittance spectra

| No | Type of dyes | Energy band gap |
|---|---|---|
| 1. | Spinach leaves | 2.51 eV |
| 2. | ivy gourd leaves | 2.53eV |
| 3. | Bottle gourd leaves | 2.73 eV |

**EDXRF (Energy Dispersive X-ray Fluoresce) analysis**

The results of relative intensity of the mineral elements in spinach leaves, ivy gourd leaves and gourd bottle leaves (counts/s) are shown in table (3). The results showed the relative intensity of every element in the spinach leaves. Figure 10 showed EDXRF spectrum of spinach residual powder. It was determined that the relative contents of calcium (48.699%), potassium (39.689%), iron (4.991%), sulfur (2.062%), manganese (1.043%) and zinc (1.001%) were higher than the upper range of mineral elements that can be measured. The content of Ca was higher in spinach leaves.

Figure (11) showed EDXRF spectrum of ivy gourd residual powder. EDXRF spectrum of ivy gourd leaves was found to contain a certain amount of calcium (56.220%), potassium (31.949%), and iron (3.972%) and sulfur (3.901%). The content of Ca was also higher in ivy gourd leaves.

Figure (12) showed EDXRF spectrum of bottle gourd residual powder. EDXRF spectrum of bottle gourd leaves was found to contain a certain amount of potassium (49.938%), calcium (35.204%), phosphorus (4.778%), iron (3.753%), sulfur (2.927%) and titanium (1.236%). The content of K was also higher in bottle gourd leaves. Elements potassium (K) and calcium (Ca) were mainly found in these three samples.

Table (3). Relative intensity of the mineral elements in spinach leaves, ivy gourd leaves and bottle gourd leaves (counts/s)

| Sample | Spinach leaves | Ivy gourd leaves | Bottle gourd leaves |
|---|---|---|---|
| Ca | 48.689% | 56.220% | 35.204% |
| K | 39.689% | 31.949% | 49.938% |
| Fe | 4.991% | 3.972% | 3.753% |
| S | 2.062% | 3.901% | 2.927% |
| Mn | 1.043% | 0.458% | 0.605% |
| Zn | 1.001% | 0.665% | 0.779% |
| P | 0.955% | 0.642% | 4.778% |
| Ti | 0.547% | 0.960% | 1.236% |
| Sr | 0.511% | 0.507% | 0.148% |
| Rb | 0.317% | 0.425% | 0.124% |
| Cu | 0.145% | 0.191% | 0.299% |
| Y | - | 0.076% | - |
| Zr | 0.051% | 0.033% | - |
| Kr | - | - | 0.148% |
| Br | - | - | 0.052% |

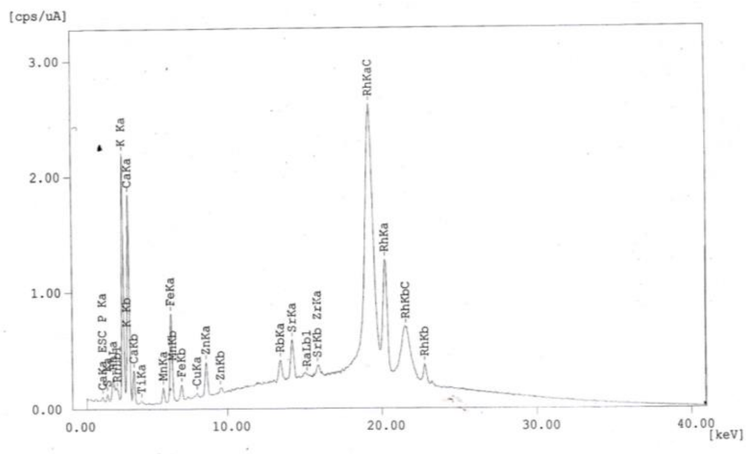


Figure (10). EDXRF spectrum of spinach residual powder

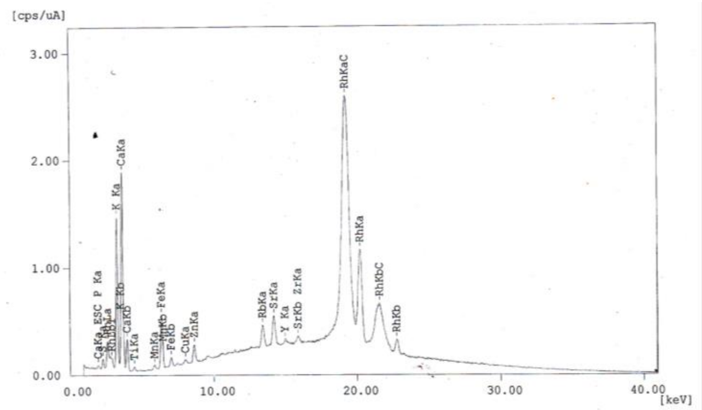


Figure (11). EDXRF spectrum of ivy gourd residual powder

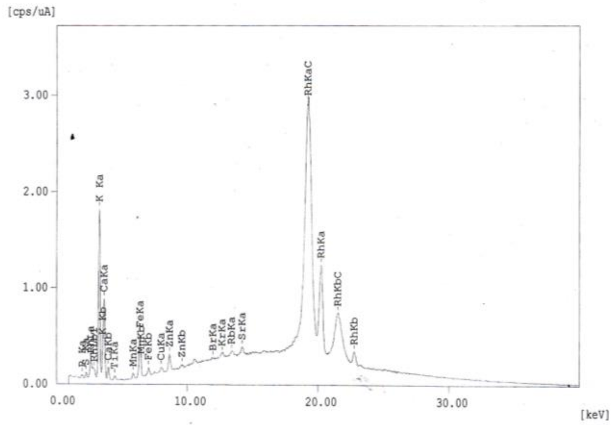


Figure (12). EDXRF spectrum of bottle gourd residual powder

**FTIR (Fourier Transform Infrared Spectrometry) analysis**

Figure (13) showed FTIR spectrum of spinach residual powder. FTIR spectra analysis indicated 3471 $cm^{-1}$ representing R-C (O)-$NH_3$ bonded and N-H symmetric & asymmetric stretching. Alkenes C=C stretching was observed at 1643.41 $cm^{-1}$. $CH_3$C-H bend and –(CH $(CH_3)_2$ bonds were observed around 1384.94 $cm^{-1}$. The absorption band at 1323.21 $cm^{-1}$ corresponded to C-F stretching. C-O stretching was observed at 1149.6$cm^{-1}$ and 1105.25$cm^{-1}$. Alkyl halides C-Cl stretching and atomic compound C-H bond were observed at 781.20 $cm^{-1}$.

Figure (14) showed FTIR spectrum of ivy gourd residual powder. The absorption band of FTIR spectrum of ivy gourd residual powder at 3414.12 $cm^{-1}$ corresponded to R-C (O)-$NH_3$ bonded and N-H symmetric & asymmetric stretching. The band at 2918.40 $cm^{-1}$ and 2848.96 $cm^{-1}$ corresponded to strong C-H stretch and strong and broad O-H stretch. Alkenes C=C stretching was observed at 1641.48 $cm^{-1}$. The function group of aromatics and C-C stretch was observed at 1419.66 $cm^{-1}$. The IR band due to C-F stretch is observed at 1325.14 $cm^{-1}$. The peaks 1244.13$cm^{-1}$ and 1030.02 $cm^{-1}$ were be observed =C-O-C sym & asym stretching. The peak of C-O-C stretch of ethers and C-O stretch of Alcohols were observed at 1149.66 $cm^{-1}$. C-O stretch of Alcohols was observed at 1103.32 $cm^{-1}$ and 1076.32 $cm^{-1}$. C-Br stretch of alkyl halides was observed at 601.81 $cm^{-1}$ and 570.95 $cm^{-1}$.

Figure (15) showed FTIR spectrum of bottle gourd residual powder. The absorption band of FTIR spectrum of bottle gourd residual powder at 3443.05 $cm^{-1}$ corresponded to R-C(O)-$NH_3$ bonded and N-H symmetric & asymmetric stretching. Alkenes C=C stretching was observed at 1651.12 $cm^{-1.}$ The function group of aromatics and C-C stretch was observed at 1415.80 $cm^{-1}$. C-N stretch of aliphatic amines was observed at 624.32 $cm^{-1}$. C-O-C stretch of ethers and C-O stretch of Alcohols were observed at 1149.66 $cm^{-1}$. C-O stretch of Alcohols was observed at 1103.32$cm^{-1}$ and 1072.46$cm^{-1}$. C-Br stretch of alkyl halides was observed at 624.32 $cm^{-1}$.

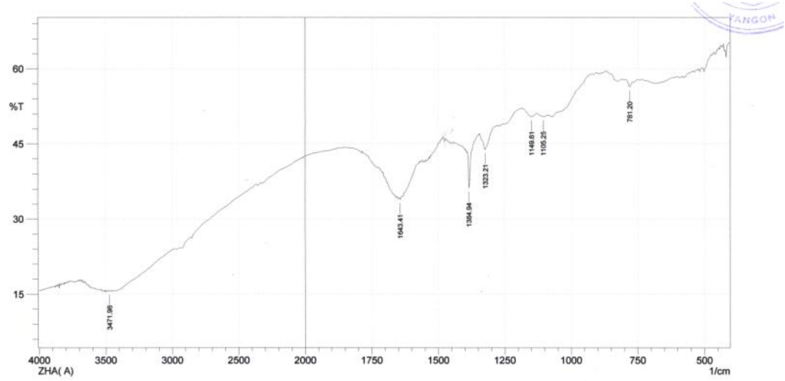


Figure (13). FTIR spectrum of spinach residual powder

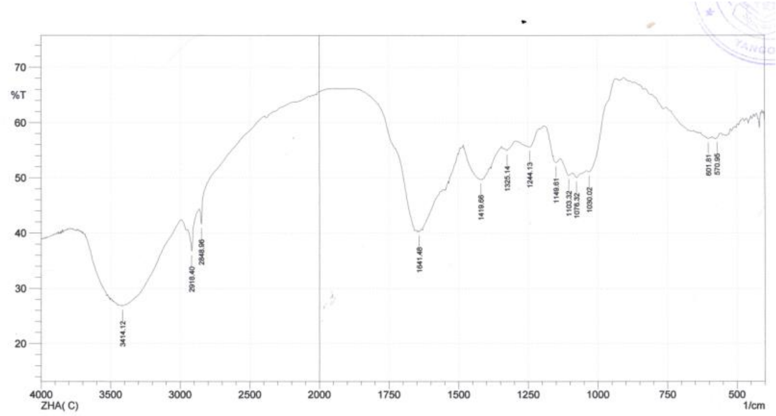


Figure (14). FTIR spectrum of ivy gourd residual powder

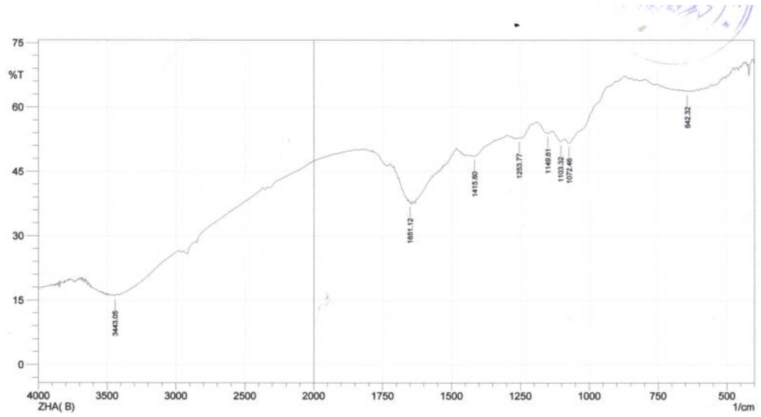


Figure (15). FTIR spectrum of bottle gourd residual powder

## Conclusion

Extraction of spinach, ivy gourd and bottle gourd dyes and its optical properties have been studied. According to UV-Vis results, absorption peaks of the three samples were found between 665.00 nm and 466.00 nm. In fact that, all absorption peaks in the visible region indicated that the three samples exhibited the chlorophyll members. The band gap calculated from absorption spectra is about 2 eV. As the obtained results, it is concluded that these dyes are quite credible and application for dye sensitizer in DSSC. EDXRF analysis examined that potassium (K) and calcium (Ca) were mainly found in these three samples. The functional groups of alcohols, alkenes, alkanes, aromatics amines, aromatics and alkyl halides were observed in these three dye samples from FTIR analysis.

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