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Original Research Article

Isolation and Structural Investigations of Green Pigment (Gp) from Pineapple Peels

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Abstract

This study describe the philosophy of Green Chemistry Principles when applied to the treatment of pineapple peels waste. Green pigments were isolated and identified from pineapple fruit peels extracts by using renewable methods like two phase system extraction (TPE) by ether: acetone at 40°C. The extract was analyzed and purified via flash column chromatography under certain condition (FCC) .The fractional components monitored with thin layer chromatography (TLC) at room temperature and identified utilizing spectroscopic analysis tools such as UV-visible, FT-IR, ^{1}H-NMR ,^{13}C-NMR and GC-Mass analysis .Two components of green pigments (GP) were identified .They are chlorophyll types(a) and (b) respectively.The advantages of this method include friendly environment, low cost of preparation, isolation, identification, and ease of scale detection.

Key words: Pineapple,Green pigments, Column chromatography, Phytal side chain.

الخلاصة

في هذا العمل طبقت مبادي الكيمياء الخضراء لمعالجة قشور فاكهة الاناناس.تم فصل وتشخيص الصبغة الخضراء من مستخلص قشور الاناناس باستخدام طرق الاستخلاص المستزد بالاسيتونوالايثر عند درجة حرارة ٤٠ م . تم معاملة مزيج المستخلص وتنقيته من خلال كروموتغرافيا العمود المعجلة ضمن ظروف محددة. تم فحص المكونات المعزولة بواسطة كروموتغرافيا الطبقة الرقيقة عند درجة حرارة المختبر وتشخيصها بالطرق الطيفية كطيف الاشعة فوق البنفسجية والمرئية , الاشعة تحت الحمراء, الهيدروجين والكربون النووي المغناطيسي ,كروموتغرافيا الغاز وطيف الكتلة. تم تشخيص نوعين من الصبغة الخضراء (أ , ب) على التوالي. تميزت هذه الطريقة بكونها صديقة البيئة بالاضافة الى قلت كلفته التحضير والفصل والتشخيص وسهولة قياسها وتحديدها .

الكلمات المفتاحية :الاناناس , الصبغة الخضراء, كروموتغرافيا العمود, سلسلة الفاتيل الجانبية.

Introduction

The solid waste production in our country is growing day by day in volume and in toxicity. More and more of everyday products contain toxic chemicals put our environment in grave danger. According to the most important concept of green chemistry principles, which is referred to as, 'the fact itis better to prevent waste material than to treat or clean up waste after it is formed"[1]. A lot of published researching were introduced in this field. Diapic N., et al extracted chlorophyll pigments from Parrotiapersica,Hamamelisvirginiana, Vitisvinireravarpinot noir , Prunusserrulataand Fothergillagardenii autumnal leaves[2-7]. Ewa Studied the molecular design and environmental determinants of plant color concentration[8].Donghua l., et al studied the effect of some biomarker characters like Cr(VI) against Chlorophyla,b ratio with their structure, composition and function [9] .On the other hand Isolation of chlorophylls *a* and *b* from spinach leaves performed employing both as counter-current chromatography techniques[10]

and extraction processing [11].Gautam et al and Iara Studied the amount and activity of bromelain present in the stem and fruit peel of the pineapple plant[12,13]. More recently Suman and Kaushik Worked on identifying the proper dose of inorganic Nitrogen fertilizer in relation to the growth stage and leaf color chart[14].

Due to the importance of GP in human health the choice of Green pigment extracted from the waste of peel pineapple fruit was the main goal of this publication. Green pigment is a mixture of chemical components fond in the plant that absorb light and utilize it in order to make energy in plant. Moreover, the measurement of photosynthetic pigments can provide basic information on the physiological status of a plant[15]. The spectroscopic analysis was applied to elucidate the structural characterization of those compounds.

Materials and Methods[16]

1. Sample preparation:

Pineapple peels were collected from the market regionin,Hillaprovenance.The leaves were washed and cuts into small pieces, dried under vacuum, then crushed in a mortar and pestle for homogenization. The samples were kept in a dry place at room temperature for further evaluation. (**Figure 1a)**.

2. Extraction of pigment :

6 gm of sample were prepared from pineapple peels **(Figure 1a)** in a soxhlet tube thimble tube and extracted using three different solvents (methanol, acetone, anddiethyl ether) respectively usingsoxhlet apparatus**(Figure1b)**.The extraction time for each solvent was 45 minutes until the solvent became colorless. The crude extract was directly transferred to a cooling centrifuge for about 10 minutes at 2000 rpm at 8°C. The extracted sample was then dried using anhydrous $MgSO_4$and the supernatant transferred to a lypholyzer apparatus to remove all the excess of the solvent (after drying process all the solvents were recovered with a yield of 85%). The best percentage yield observed in case of acetone then ether and methanol were (255 , 220, and 112 mg/5 gm.) extract respectively.

3. Analytical methods:

a. the crude extract of 90% acetone was tested by thin layerchromorography (TLC) using aluminum backed silica 60 (20×20) TLC plates with F254 florescence indicator and developed using acetone/ ether in ratios of 10:1, 10:3 and 10:5 as mobile phase. The best separation was observed the (10:1). Three spots clearly identified when the TLC plat was examined by E-Graph Image Server 5 version 2.0.4 (ATTO Corporation). The retention factors (R_f) were 0.21, 0.28, and 0.52 respectively **(Figure 2a)**.

Figure 1a,b,c: Section of pineapple peels used for extraction the green pigments.

Figure 2a: TLC of the pigment extraction after (ATPE).

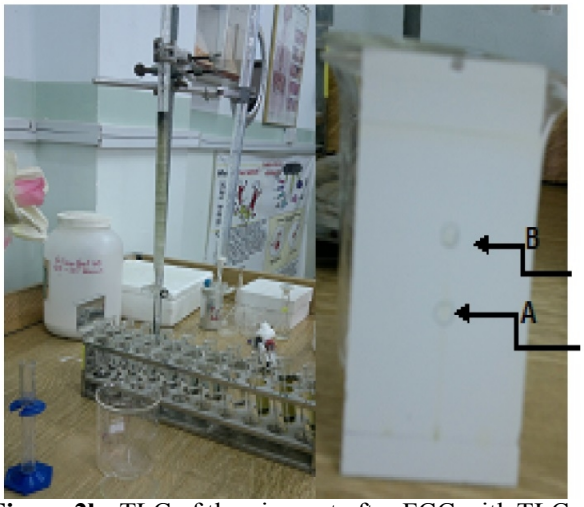

Figure 2b: TLC of the pigment after FCC with TLC

b. The sample was then transferred to a 30× 500 mm glass chromatography column packed with Silica gel (250-400) mesh size (stationary phase). The mobile phase employed was acetone:etherin (10:2) ratio **(Figure 1c)**. The flow rate for elution was 3ml /min. and 10 fractions were collected over 40 minutes and tested within TLC.Fractions 6 and 7 exhibited two of different spots withR_f= 0.43 for **A** and 0.68 for **B,** (**Figure 2b**).Removing the solvent under vacuum with cooling (lypholyzer). An oily green pigments obtained for **A** and **B** components respectively.

4. Equipment:

Variousspectroscopic tools were used for identification in this application. The UV-Visble spectra were recorded on a Pye-Unicam 8700 spectrophotometer (200-900 nm), IR spectra were recorded on a Bruker ,Tensor 27 FT-IR spectrophotometer (4000-400 cm^{-1}),using an ATR unit, Nujol mulls and liquid films between potassium bromide cells.E-Graph Image Sever 5 version with UV source 2.0.4 ATTO Coperation[17].Proton and Carbon Nuclear Magnetic Resonance (^{1}HNMR &^{13}C-NMR), Bruker, Ultra Shield Spectrometer 300 MHz, with tetramethylsilane (TMS) as standard, and $CDCl_3$ + D_2O as a solvent [18]. The Gas Chromotography with Mass Analysis (GC-MS), QP-2010 Ultra (Shimadzu Instrument, Japan)[19].Column chromatography Glass Column (30 ×500 mm) packed with silica gel 250- 400 mesh, 1 gm/25 gm)[17].Freeze Dryer (Christ, Alpha 1-4 LD, with Vacuum Pump RZ 6,up to 4-10^{-4}mbar.

Results and Discussion

The fundamental importance of this application was recycling impurities in the food waste from human nutrition resources to keep our environment safe and secure. The use of green chemistry principles were applied for the isolation, and identification of one the most important biochemical pigments in living organisms. Historically, numerous methods were used for elucidation of the molecular structures of chlorophyll and its derivatives **(Figure3)**[20]. More than four spectroscopic techniques were used in this research to prove and support the presence of GP in the waste pineapple peels . In UV-Vis spectra of the extract exhibit several absorptions bands at (λ_{max}) between 320-455 nm. This indicates a mixture of several pigments ,which were separated by column chromatography. Fourier transformer spectra (liquid disk and ATR mode) of the isolated compounds exhibit good evidences of the presence of oily **GP type a** and its derivatives**(Figure 4)**. Two different types of carbonyl groups (ester & ketone) appear at two stretching frequency absorptions at

v= 1737 and 1711 cm^{-1} respectively in rings (I and IV) . The conjugated system of the double bond -C=C- in the porphyrinisocyclic rings observed at stretching frequency v= 1463 cm^{-1}. Larg intensity of stretching absorption bands atv =2919 and 2862 cm^{-1} refer to the Ar-CH, CH_2 and CH_3 in the side chain groups and porphyrin ring. In addition C-O, C-N and CH bending which is exhibited at v = 1266 -1110 cm^{-1}.**Figure (4b)**shows strong broad band stretching at v = 3367 cm^{-1} , for OH carboxylic group, strong stretching absorption atv = 2020 -2850 cm^{-1} , CH, aromatic and alphatic groups, two types of stretching frequency absorption atv = 1736 and 1632 cm^{-1}, carbonyl ester &keto respectively, and the rest of spectra for -C=C- conjugated ,CH bending,C-N ,and C-O appeared at 1465 -1381 and 1244-1070 cm^{-1}respectively .

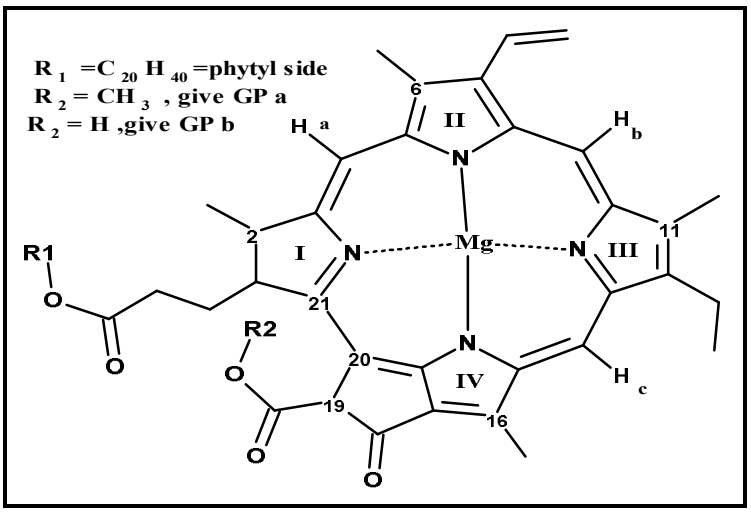

Figure 3: Scheme of the chemical structure GP A and B

On the other hand ^{1}H-NMR spectrum in $CDCl_3$(**Figure 5a)**showed the presence of Green Pigment type A as follows : three protons (Ha,Hb,and Hc) of in the isocyclicporphrin ring exhibited singlet at δ = 8.74, 8.49 ,and 8.25 ppm ; three allylic protons bonded in C7 exhibited at δ = 8.07-8.09 ppm d,1H,and 2H,dd atδ= 6.55-6.42 ppm respectively. Also one proton s,1H of C19 at 6.16 ppm, two protons in C1&C2 were exhibit atδ= 4.13 ppm d, 2H . Three types of four methyl groups bonding to C6 ,C11,C16,and C19 exhibited as follows; at δ= 3.69 ppm s,2CH_3, 6H refer to C6 and C16, s,CH_3,in C11 atδ= 3.34 ppm, s,3H.Atδ= 3.29 ppm refer to methoxy group, where as the methyl group,3H at C2 exhibit atδ= 2.16 -2.11 ppm. The rest of signals belong to the ethyl in C12 pyrol ring II and phytyl side chain in C1 ring I . They were exhibited betweenδ= 2.56 to 1.50 ppm[21].The other supporting evidences of isolation of GP type A was done by ^{13}C-NMR in **(Figure 5b).** Two types of carbonyl C=O groups exhibited in down field asC=O, keto,IV ring atδ= 186.45 ppm and 2C=O, ester groups exhibited atδ= 174.53 ppm respectively. The other type of carbons such as C3,C18,C21,C5,C10,C8 ,C15 ,and C13 exhibited atδ=160.33,155.13, 154.02,150.37,148.11,147.09,146.23, and 140.04 ppm respectively. Also there are many signals with different intensity observed at δ= 139.58 , 136.49, 130.85, 126.44 and 119.44 ppm which they belong to C12, C7,C6,C11 and C17 with carbon allylic group bonded at position C7. The rest of the signals clearly observed at δ= 65.10 to 22.91 refer to phytal side chain **(R1, Figure:3)**. From another side the elucidation of GP type B were done by GC-Mass analysis (**Figure 6a &b)**with the following condition, temperature injector

programming 40 to 100 °C, column temperature 250-340 °C and the detector temperature was 350°C .the chromatogram in (**Figure 6a**) ,exhibit a broad band width at 4.5-6.0 with peak height at 163.This provides good evidence and supports the presence of GP type b in its isolated form. Moreover the mass spectrum of the GP b exhibits molecular ion peak EI-MS^+ at m/z = 636 ($[M+Na]^+$), also additional signal exhibited at m/z= 617 ($[M+H]^+$)fragment ion peak, and fragment ion at m/z= 592($[M^+$+H]-Mg) plus the rest of fragments at m/z= 504,453, 335,266 with base peak 200 respectively. Through these clear evidence the identification of isolated compounds matching 90% with literature publications[22].

The isolated pigment has many advantages in medicine and industry such as:

1. GP helps control Hunger and promote losing weight [23].
2. It's controls body odor by reduction odor ,constipation and gas[24].
3. Encourages healing and helping in wounded therapy [25].
4. Removing toxic metals by binding with them to prevent absorption [26].
5. Protects DNA against fried foods [27], and potent antioxidant properties by protect cells from the oxidative damage by eliminating free radicals generation [28].
6. Effective against *Candidiaalbicans* growth [29]. Also able to reducing systematic redness and swelling [30].
7. Promotes healthy iron levels by new GP derivatives [31].

In addition to all these benefits, the waste of leafs (by-products) have a low commercial value, therefore can directly returned to the fields as soil improvements . Also the wastes are rich in the enzyme bromelain that could be isolated and purified in order to generate a product with a higher quality value. Thus further studies are needed to elucidate such benefits.

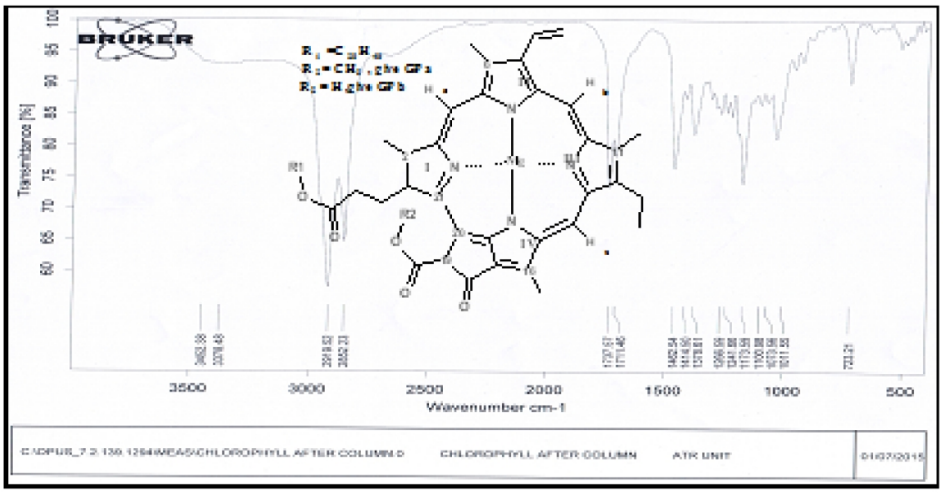

Figure 4a: FT-IR spectrum of GP type A after column chromatographic analysis.

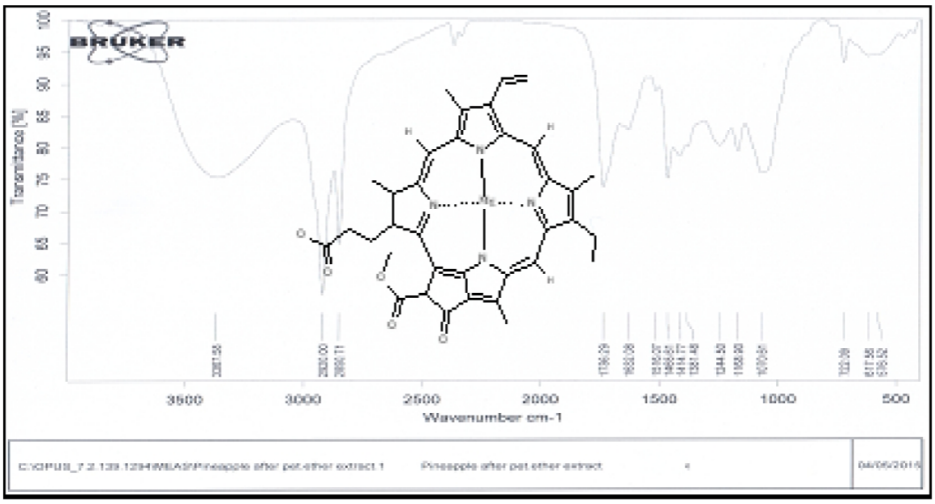

Figure 4b : FT-IR spectrum of Gp type B derivative by liquid cell disk analysis

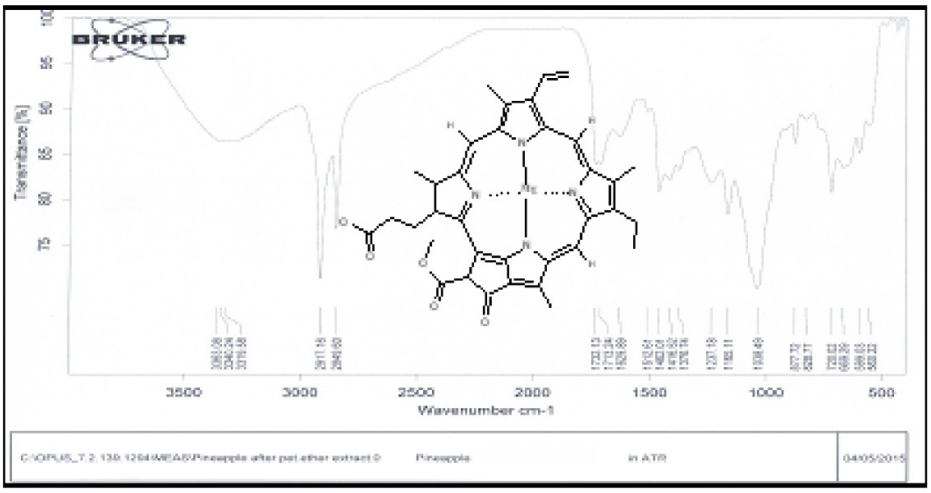

Figure 4c : FT-IR spectrum of Gp type B derivative using ATR unite instead of liquid cell.

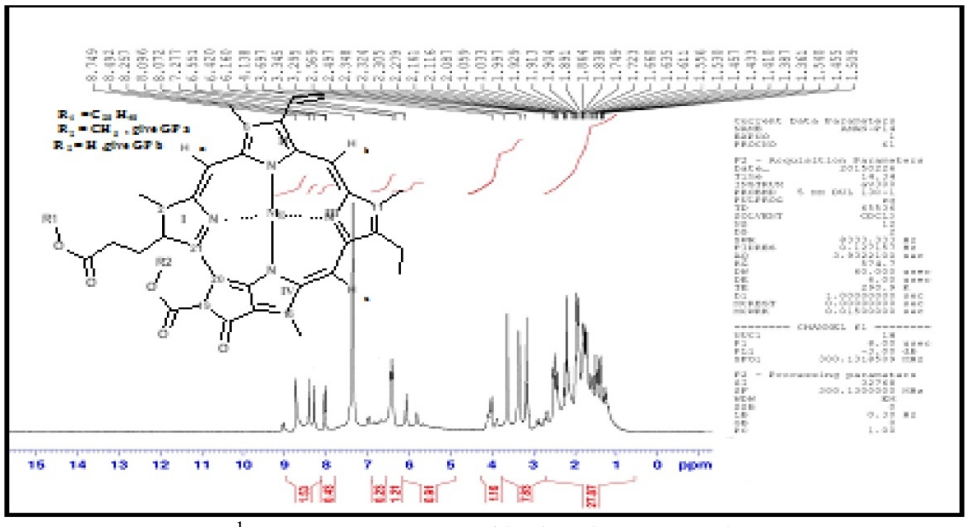

Figure 5a: ^{1}H-NMR spectrum of isolated compound GP type A

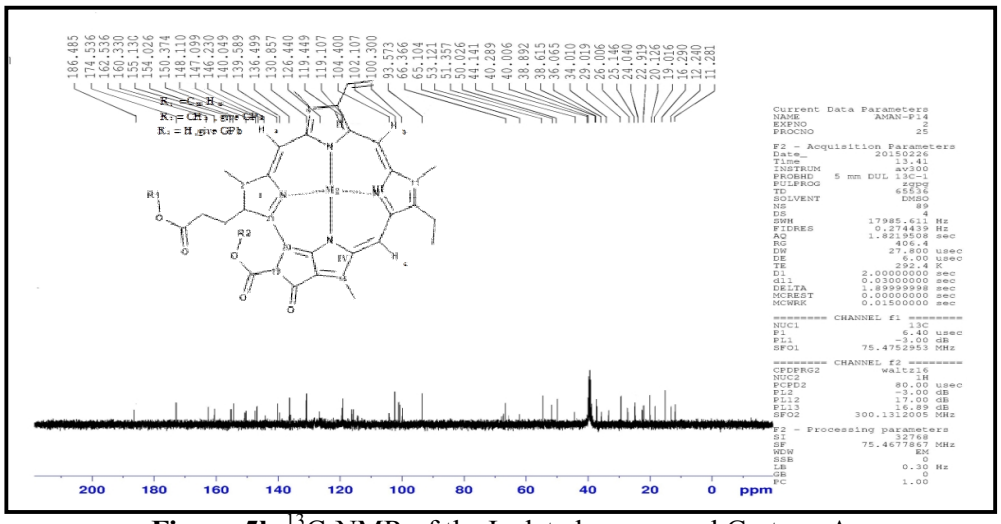

Figure 5b: ^{13}C-NMR of the Isolated compound Gp type A

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[Comment]

====== Analytical Line 1 ======

[GCMS-QP2010 Ultra]

IonSourceTemp	:150.00 °C
Interface Temp.	:40.00 °C
Solvent Cut Time	:0.00 min
Ionization Mode	:NCI
Detector Gain Mode	:Relative
Detector Gain	:0.94 kV +0.00 kV
Threshold	:0

[Comment]

====== Analytical Line 1 ======

[GCMS-QP2010 Ultra]

IonSourceTemp	:150.00 °C
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Solvent Cut Time	:0.00 min
Ionization Mode	:NCI
Detector Gain Mode	:Relative
Detector Gain	:0.94 kV −0.00 kV
Threshold	:0

Sample Inlet Unit: :DI

[DI Temperature Program]

Rate (°C/min)	Final Temp (°C)	Hold Time (min)
80	100	0.00
40	350	1.00
0	0	0.00
0	0	0.00
0	0	0.00

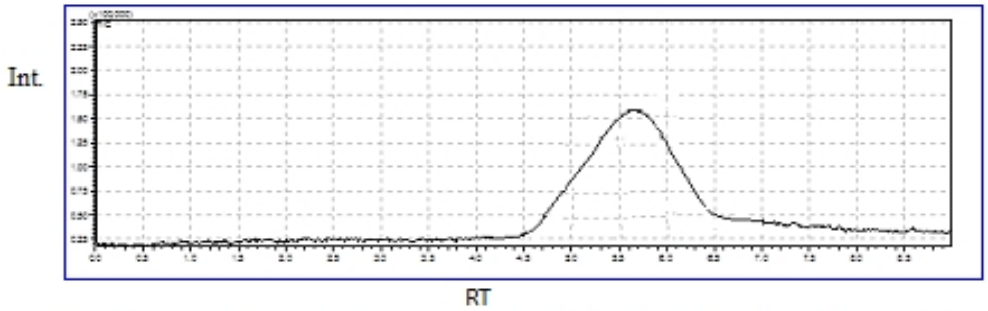

Figure (6a) Gas Chromatography Analysis(GCA) by TIC for the Isolated GP type B.

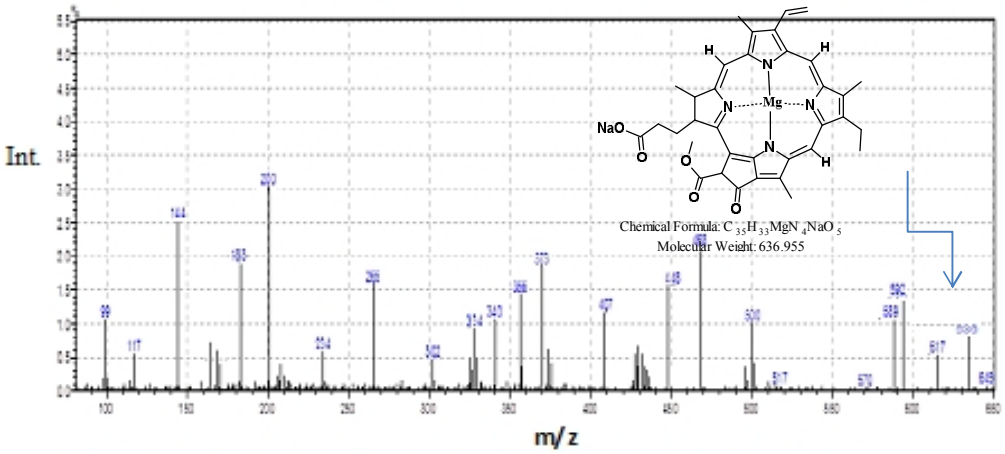

Figure (6b): Mass Analysis Spectrum (m/z) for Green pigment (GP) type B

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