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Research Article

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Isolation and characterisation of coumarin from the stem bark of *Polyalthia longifolia*

Adlis Santoni, Dewanti Suci, Yola Marta, Mai Efdi and Sanusi Ibrahim*

Chemistry Department, Andalas University, Padang, Indonesia

ABSTRACT

A coumarine, 7-ethylamino-4,6-dimethyl coumarin was isolated from Polyalthia longifolia bark. The structure of isolated compound was determined on UV, IR, gas chromatography, mass chromatography, ^{1}H NMR, and ^{13}C NMR spectral data.

Keywords: 7-ethylamino-4,6-dimethyl coumarin, *Polyalthia longifolia*

INTRODUCTION

In the previous research, three clerodanediterpenes, kolavenic acid, polyalthialdoic acid, and 16α-hydroxy-cleroda-3,13(14)*Z*-dien-15,16-olide, have been isolated from *Polyalthia longifolia* leaves. These three compounds were tested against human leukemia (HL-60) cells. Polyalthialdoicacidand16α-hydroxy-cleroda-3,13(14)*Z*-dien-15,16-olide showed high potency as anticancer agent. Now, we will reporta coumarin from *Polyalthia longifolia*stem bark.

EXPERIMENTAL SECTION

Plant Material

Polyalthya longifolia stembark was taken from the natives of first Marunggi, South District Pariaman, West Sumatra, Indonesia. Identification was held in Herbarium Universitas Andalas (ANDA), Departement of Biological Science, Padang, Indonesia.

Chemicals

The chemicals used were silica gel 70 – 230 mesh (Merck),*n*-hexane, ethyl acetate, methanol, ethanol, acetone, cloroform, dicloromethane, aquadest, sephadex LH-20. They were purchased from Wako pure Chemical Industry Ltd and Sigma Aldrich Company.

Instruments

The equipments used were glassware, a set of solvent distilationtools, rotary evaporator heidolp WB, 2000,oven, thin layer chromatography (TLC)silica gel 60 F254, Merck KGaA Darmstadt Germany, melting point apparatus (John Fisher), UV-Vis spectrophotometer series 1700, Perkin Elmer 1600, IR spectrophotometer series, GC-MS QP 2010 plus shimadzu, UV lamps (GL-58 UV 254 and 365 nm), JEOL Spectrophotometer 400 MHz 1H- JNM-ECS 400 NMR, 13C NMR and 2D- NMR (HMQC and HMBC)

Procedures

Polyalthya longifolia bark (3 kg) was grounded and macerated successively with n-hexane, ethyl acetate, and methanol. This maceration process yielded n-hexane extract (77 gr),ethyl acetat extract (35 gr), and methanol extract (20 gr). Ethyl acetat extract (5gr) was subjected to column chromatography with eluents hexane to methanol with increasing polarity to yield some fractions. Each fraction was monitored with Thin Layer Chromatography. The same

patterns were combined to produce 7 fractions (F1, F2, F3, F4, F5, F6,F7). Fraction F5 (115 mg) was then fractionated with Column Chromatography silica gel with eluents dichloromethane: ethyl acetate (7:3). This process yielded a pure compound (39 mg). Compound obtained was identified by spectrometric methods.

RESULTS AND DISCUSSION

This compound had melting point of 170 - 171°C . Testing by thin layer chromatography gave a yellow stain with Rf 0,71 with eluent a mixture of methanol: ethyl acetate (1: 9). Gas chromatography showed one peak at Rt 34,461.

UV spectrum showedmaximum absorption at 210, 237 and 366 nm. UV spectrum absorption peaks indicated a conjugated double bond because it absorbed UV at λ> 200 nm, which indicated conjugated double bonds chromophore system (-C = C-C = C-) or on the aromatic ring. There was also a chromophore which gave the transition from n to π *. This indicated a weak transition of the free electron pairs of the oxygen in a ketone (-C = O) or indicated conjugation system of piron heteroatoms in the ring which absorbs at λ> 300 nm.

IR spectrum gave absorption bandsat702, 743, 808, 836, 870, 921, 1009, 1036, 1074, 1125, 1174, 1219, 1278, 1340, 1381, 1401, 1430, 1477, 1551, 1616, 1693, 2851, 2971 and 3364 cm^{-1}. Peak at 1616.51 cm-1 indicated the presence of C = Cin aromatic ring, at 1693.95 cm^{-1} indicated the signal of C = O and strain C (O) -O at 1174.17 cm^{-1}. Their wave numbers 3364.81 cm^{-1} which indicated NH amine group attached to the compound, as well as the visible strain aliphatic CH peak at 2971.06 $cm^{-1.}$

Furthermore ^{13}C NMR indicated a purified compound containing 13 C atoms with chemical shifts 14.5242; 17.4430; 18.6925; 38.9710; 96.1629; 108.3815; 110.3650; 121.2482; 126.5139; 152.2383; 156.2253; 156.6545; 165.1246 ppm.

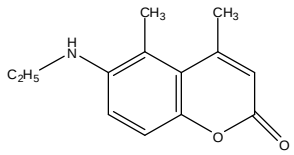
From DEPT, at 135 MHz indicated there were three primary carbons at chemical shifts 14.5242; 17.4430; 18.6025 ppm. There was one signal appeared facing down at 38.9710 and a group of secondary carbon. Furthermore, there were 3 tertiary carbons at 96.1629; 108.3815; and 126.5139 ppm. So, there were 6 quaternary carbons with the chemical shift 110.3650; 121.2482; 152.2283; 156.2253; 156.6545; and 165.1246 ppm.

^{1} H NMR indicated 15 hydrogens with chemical shifts as follow: 1.2952 (3H); 2.1889 (3H); 2.3835 (3H); 3.3096 (2H); 4.8921 (1H); 5.9233 (1H); 6.4317 (1H); 7.3397 ppm (1H). ^{1} H NMR indicated the existence of 3 CH_3; 1 CH_2 and 4 H in the form of a proton bound to N (NH) and three protons bound to the aromatic core (H-aromatic).

GC chromatogram showed a single peak with retention time (Rt) 34.461.

MS provided mass spectra fragmentation patterns with m/e of 41.1; 65.0; 86.5; 115.1;145.1;174.1;202.1; and220.0. GC-MS data is similar to a data base of 7- etilamino-4,6- dimethyl coumarin at a price of RT (retention time) 34.464 and fragmentation patterns at 39.0; 65.0; 87.0; 115.0; 145.0; 174.0; 202.0; and 221.0. Based on these data, the isolated compound is assigned as 7 ethyl amino-4.6- dimethyl-coumarin.

We have isolated a coumarine from the bark of *Polyalthia longifolia.* Based on spectroscopic analysis (UV, IR, GC-MS, ^{1}H NMR, and ^{13}CNMR), the coumarine structure is 7 ethyl amino-4.6- dimethyl-coumarin



Acknowledgement
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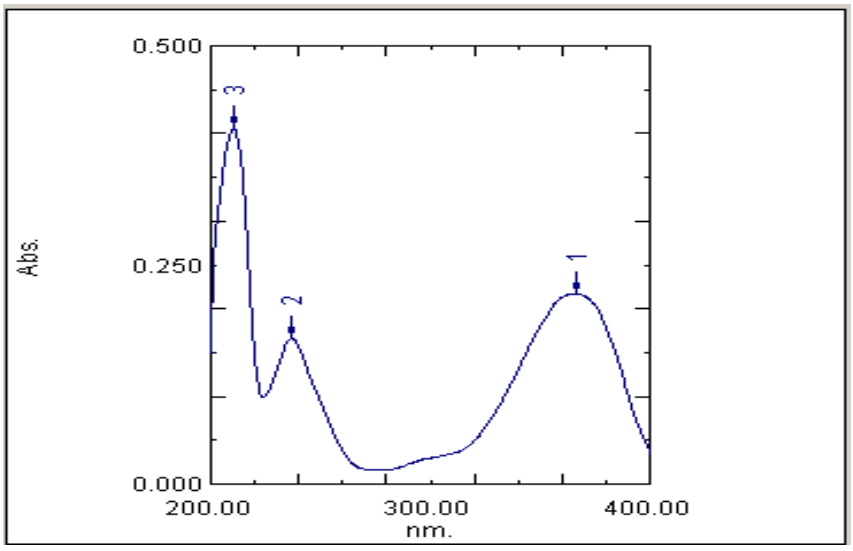
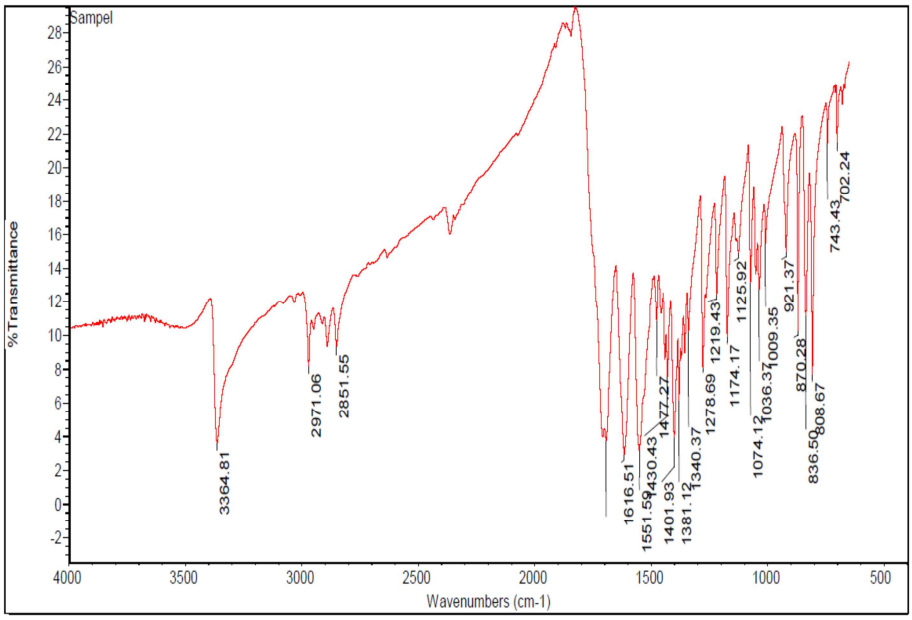
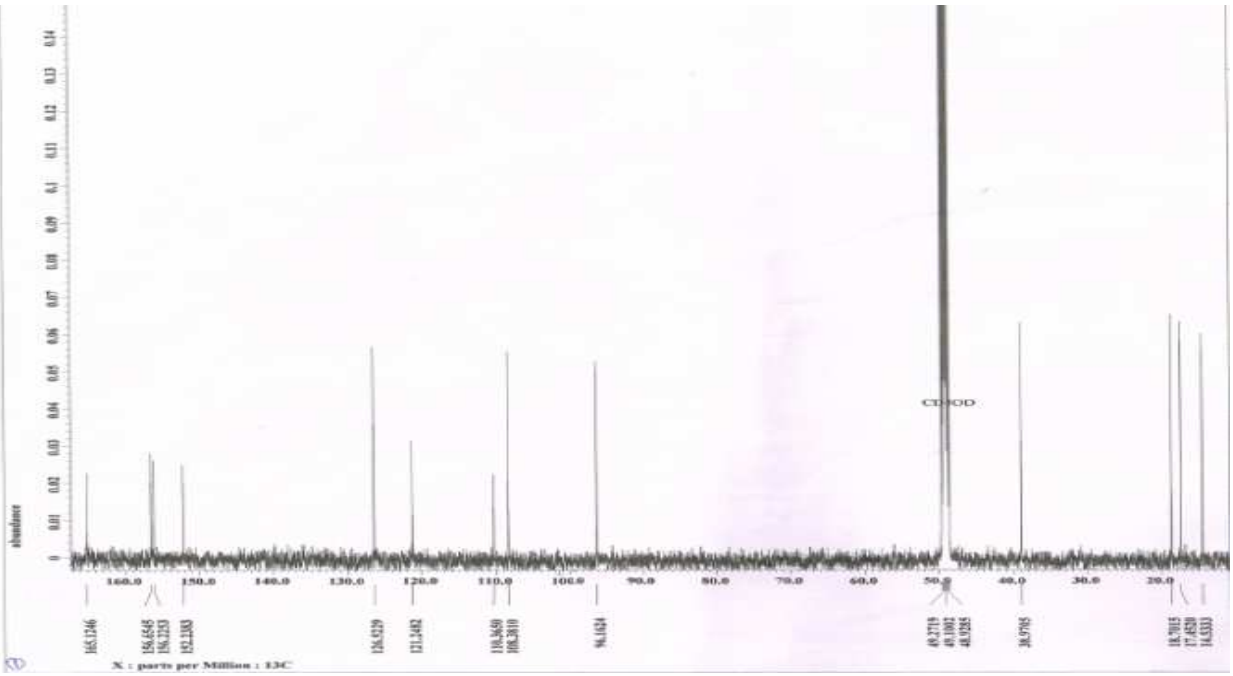
Spectrum UV,IR, NMR and GC-MS



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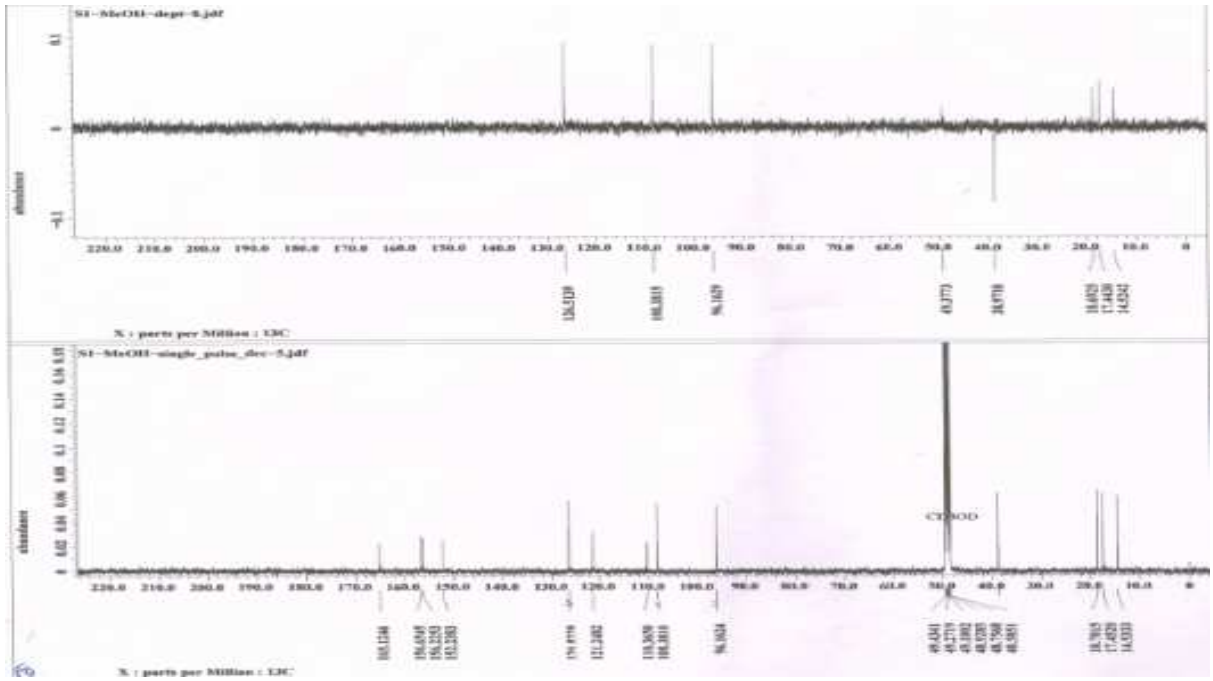
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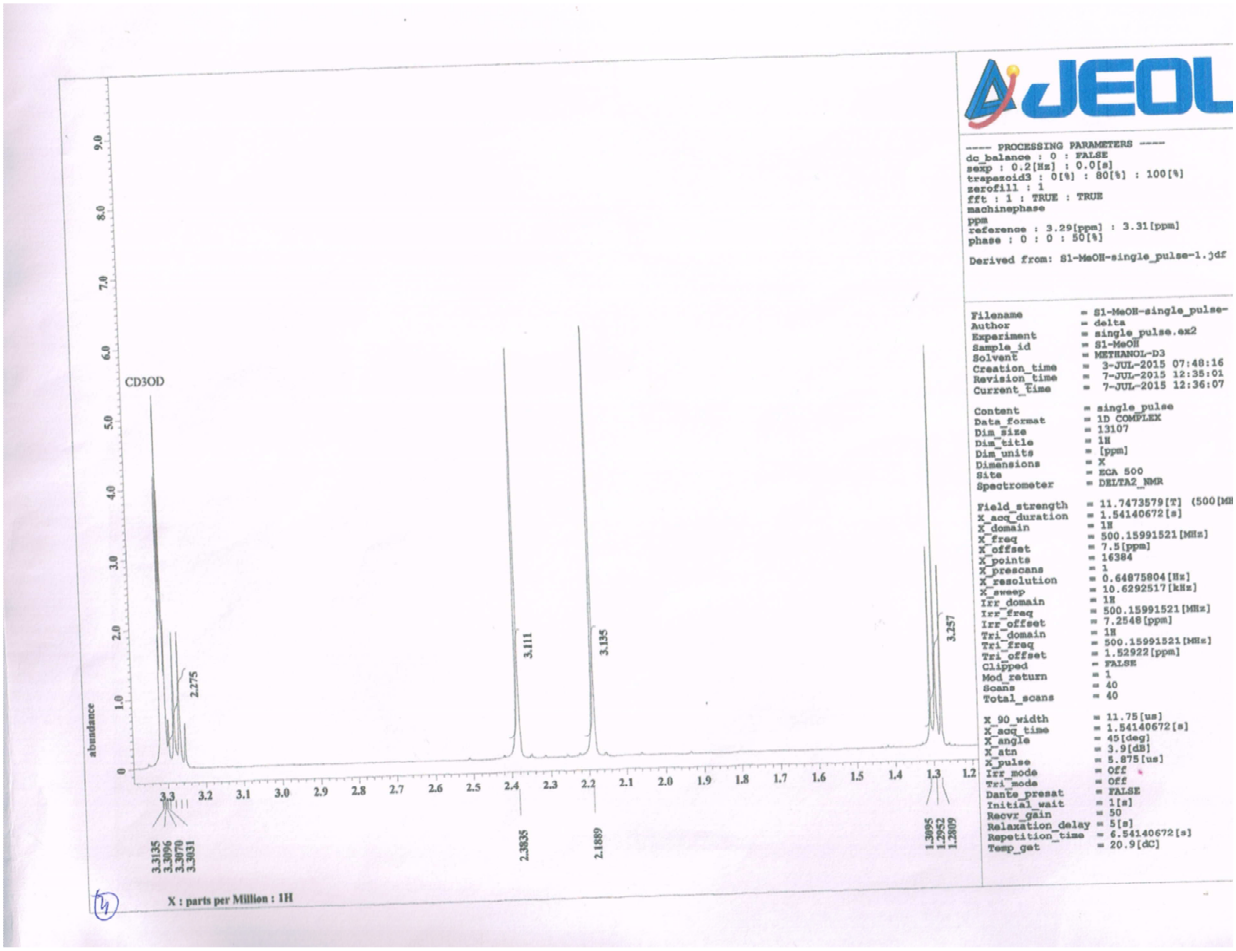
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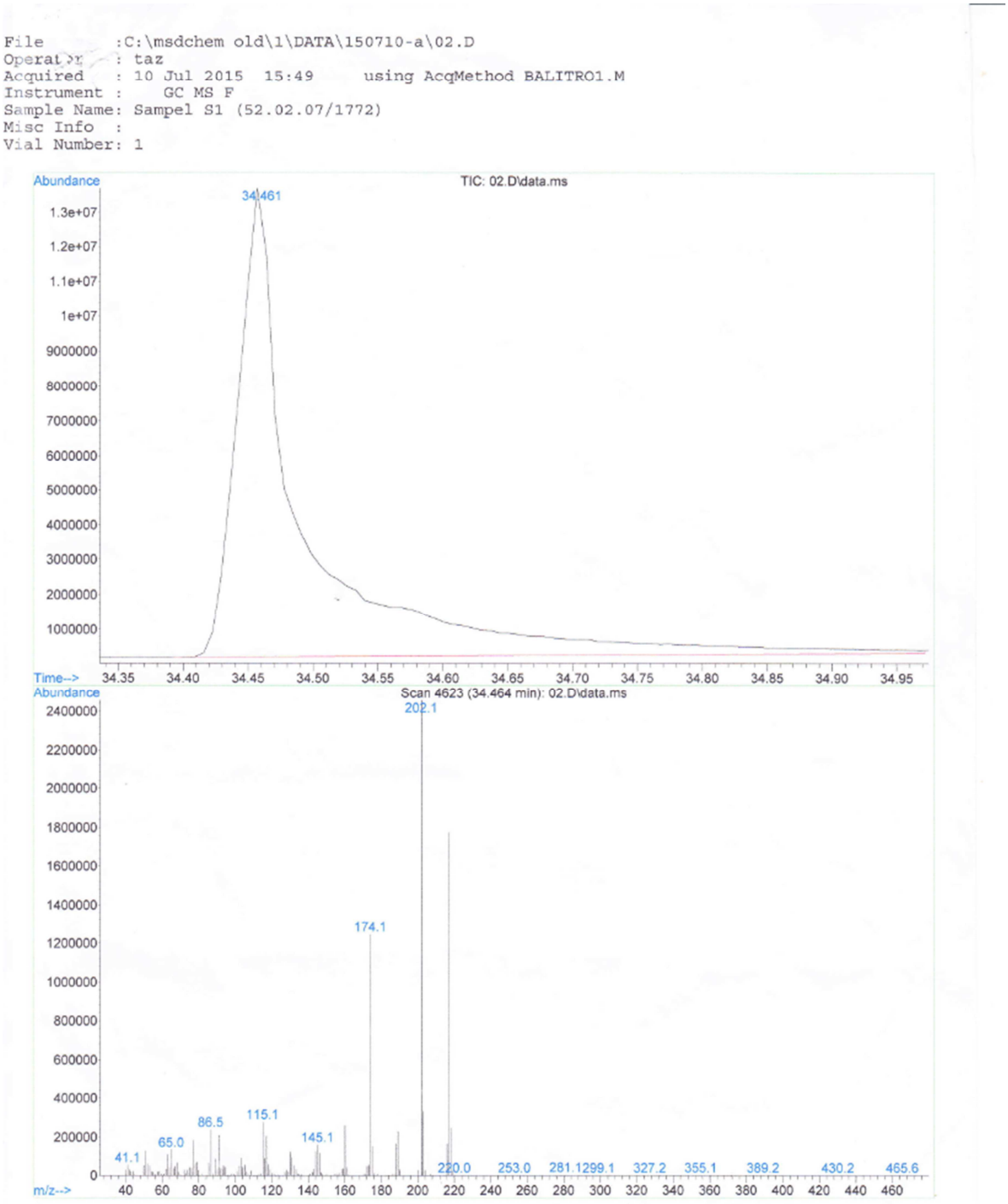
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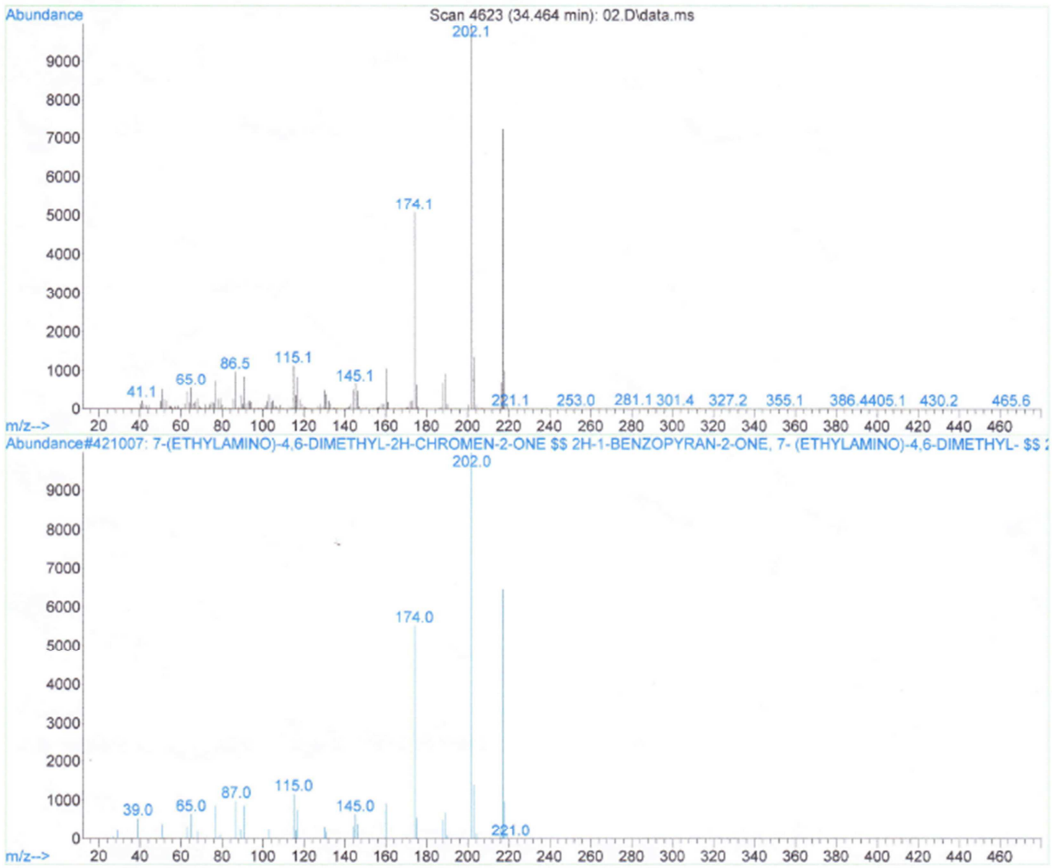

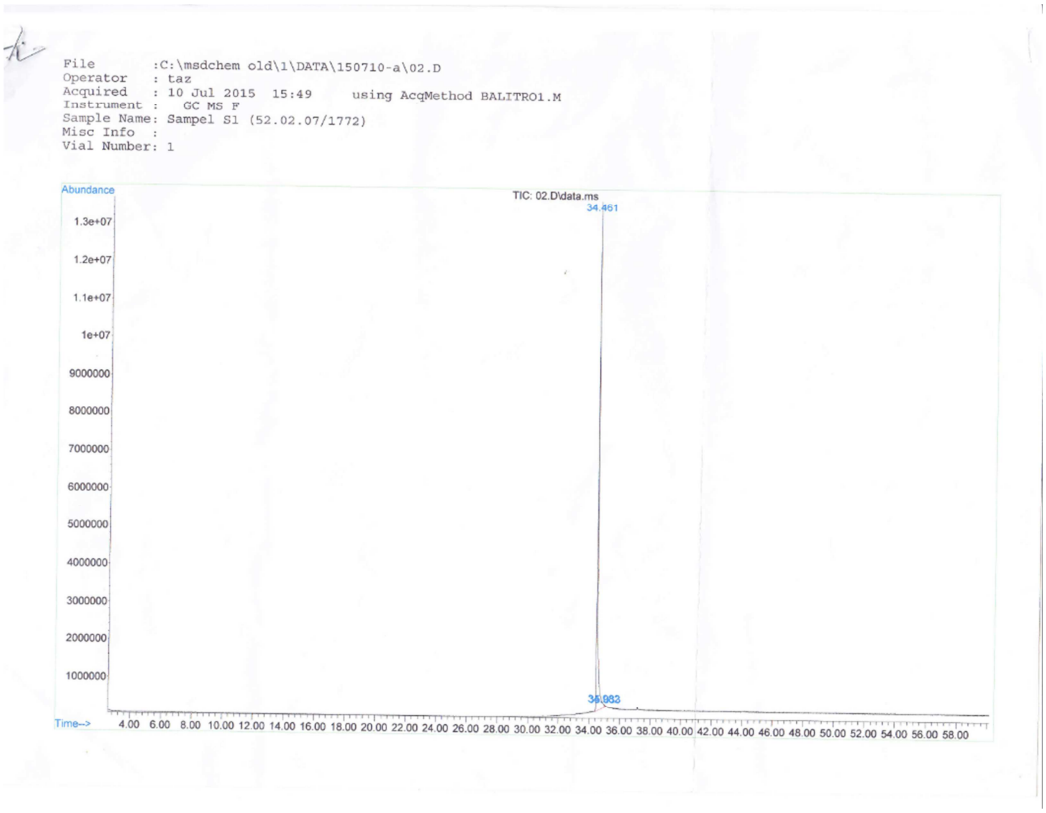
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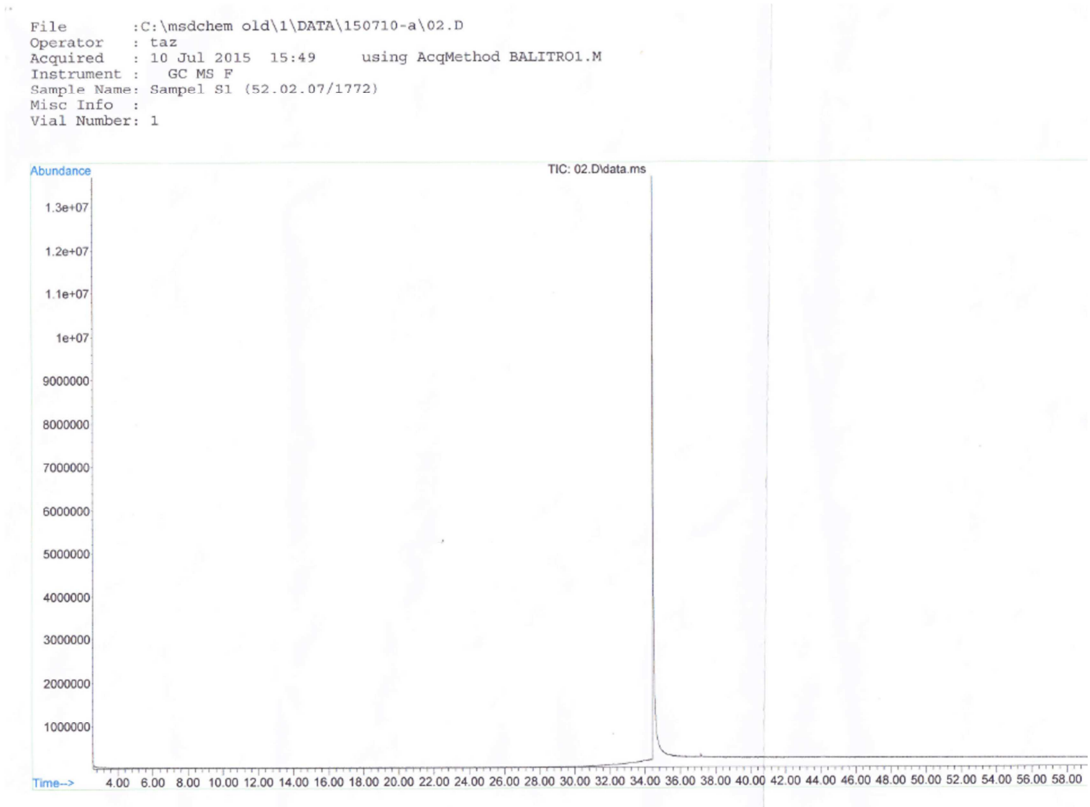
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