ISOLATION, MOLECULAR STRUCTURAL ANALYSIS OF BERBERINE ALKALOID IN *MAHONIA LESCHENAULTII* - A TODA MEDICINAL PLANT BY USING IR, UV AND MS VALUES AND THEORETICAL COMPARISON .

T. Lawrence, S. Gunasekaran

ABSTRACT: The alkaloid berberine is isolated from the leaf of *Mahonia leschenaultii* with various organic polar solvents and subsequent IR, UV, and MS spectral analysis on the berberine was carried out and characterized. The theoretical values of IR, UV spectral data and possible structure of the compound are obtained by the Gaussian and ChemDoodle software. These theoretical values are good agreement with the experimental values and the structure of the alkaloid confirmed.

Keywords: Isolation, Alkaloid, *Mahonia leschenaultii*, IR, UV, MS values.

1 INTRODUCTION

The methanol extract of the root and root bark of *mahonia leschenaultti* has rich in alkaloids [1]. The alkaloids ware isolated including emetine, veratrine, strychnine, piperine, caffeine, quinine, berberine, coniine, atropine, etc., [2]. One of principal alkaloids in this plant is berberine (1.02%) [3,4]. Although a number of synthetic routes to the berberine alkaloid have been thoroughly investigated, all of those using readily available starting materials fail to give rise to the naturally occurring compounds without multistep reaction sequence [5]. The Todas, the Nilgiris tribe, Tamilnadu, India, are using the extract for postnatal treatment in women [6, 7]. Methanol extract of this plant is act as an anti fungal and anti bacterial medicine [8]. The berberine was isolated successively with various organic solvents [9, 10]. Biotechnological approaches have to be sought for the large scale production of the alkaloids such as berberine [11].

2 MATERIALS AND METHODS

2.1 Plant collection

Mahonia leschenaultii species of Berberidaceae family is a shrub with rough, grayish – brown, corky bark and leaves in circle present at the end of slender branches. Flowers are yellow, in long erect racemes [12, 13]. The plant leaves was collected from Parson's Valley forest area, Udagamandalam, The Nilgiris district, Tamilnadu, India in the month of December.

2.2 Sample Preparation

3 kg of *Mahonia leschenaultii* leaves were collected from healthy plant and cleaned, washed with water, removes the needles, dried for 3 days and mechanically powdered for obtained course powder. It is subjected to extraction in a Soxhlet condenser apparatus by methanol. For removing moistures such as gum, wax etc., 1.5 kg of the leaf powder is soaked in 2 liter petroleum ether for 72 hours by 3 times and the leaf separated and dried. Similarly by benzene the moistures were removed [14]. The petroleum ether and benzene extracts does not show the alkaloid presents while performing Mayer's and Dragen dorff's reagent alkaloid test. The leaf powder dried completely from benzene and is soaked in methanol for 96 hours. The methanol extract collected in air tight container. The menthol extract prepared for 2 times from the coarse powder. The collective methanol extract filtered multiple times by #1 waterman paper for removing of powder and sediments. A Soxhlet condenser arrangement is used for evaporating the excess methanol from methanol extract by mantel arrangement at 60 ^{0} C. The methanol evaporates up to its 1/20th volume (50 ml) from methanol extract and it is called methanol concentrate.

The methanol concentrate may contain tar, moisture, flavonoids, steroids etc. Hence, it is washed by petroleum ether and with few drops of dilute H_2SO_4. Oil like residue removed and the methanol concentrate filter by multilayered No.1 waterman paper. Petroleum ether and methanol were evaporates and the brownish past like filtrate obtained and it was again dissolves in to the pure ethanol Then it is called methanol filtrate.

2.3 Isolation of alkaloid

The methanol filtrate contains an alkaloid [15] was separated by column chromatography on basic silica mesh 400, in petroleum ether. It is quick physical method for separation of individuals from mixing of compounds. This is a separation which works well for tropane alkaloids such as atropine, cocaine, and scopolamine berberine [16]. The methanol filtrate was chromatographed (SiO2, CHCl3: MeOH = 10:1) for isolate pure alkaloids. The column was packed by using the suspension of silica gel in petroleum ether. All the fractions of elutes were collected and kept in separate containers. Petroleum ether and methanol were evaporated out and all samples dissolve in pure nHexane and were named as S3/C1, S3/C2, S3/C3, S3/C5 and S4/C5.

2.4 Alakaloid Test

Mayer's reagent [17] and Dragen dorff's reagent [18] are used for testing the sample fractions S3/C1, S3/C2, S3/C3, S3/C4 and S4/C5. Few drops of Mayer's regent added with 3 ml for

all the samples. S3/C2 gives while precipitate and it shows that the presence of alkaloid and again S3/C2 confirms the alkaloid by Dragen dorff's regent, obtaining brown precipitate.

3 Characterization of the alkaloid

The purity of S3/C2 was checked by Thin Layer Chromatography Merck silica gel coated glass slab kept slanting in 20ml nHexane containing 100ml closed beaker and a small dot of S3/C2 placed at mid of the plate. The paper chromatography as #1 waterman paper hanged in 20ml nHexane containing 100ml closed beaker and a small dot of S3/C2 placed at mid of the paper. TLC & PLC shows the movements of single dot. Hence S3/C2 was a single compound.

3.1 Experimental Section

UV spectra were recorded on a SHIMADZU spectrometer at. IR spectra of samples liquid base were also recorded the SHIMADZU Fourier transform infrared spectrometer using NaCl cells. MS spectral data were recorded by JEOL GC Mate instrument at IITM, Chennai.

4 Result and discussion

The Gaussian software (V3) was used for obtaining IR, UV spectrum and was good agreement with the most possible molecular structure of the given IUPAC named compound of molecular diagram. The IUPAC name of berberine was obtained by ChemDoodle software .The IUPAC name of the berberine is 18,19-Dimethoxy-6.8-diolxa-i-azapentacyclohenicosa -1,3,5,10,13,15,17,19- octaene and the molecular formula is $C_{20}H_{18}NO_4$.Theoretical values are compared with experimental result and are listed bellow.

4.1 Berberine

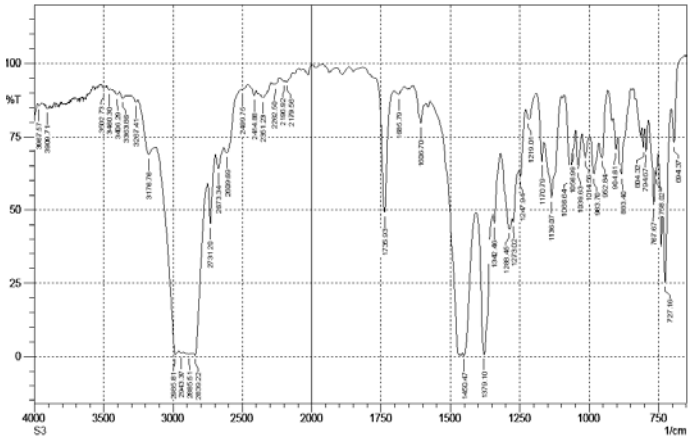
IR(cm^{-1}) υ_{max} , N-H stretch 3460, 3406, 3363, 3267, C-H stretch (aromatic functional groups) 3176. C-H stretch (alkanes) 2985, 2943, 2885, 2839. C-H stretch 2731, C (triple bond) N stretch (alkynes) 2196, 2179, C=O stretch (saturated aliphatic) 1736. C=O stretch (aldehydes, ketones) 1685, C-C stretch (in ring, aromatic) 1606, 1450, C-H rock (alkanes) 1379, C-O stretch (esters) and C-N stretch (aromatic amines) 1288, 1273, 1247, O-H bend (carboxylic acids) 983,952, 904, C-H "OOP" 883,794,767,756.

4.2UV - λ, 669.50, 605.50, 406.50, 307.00.

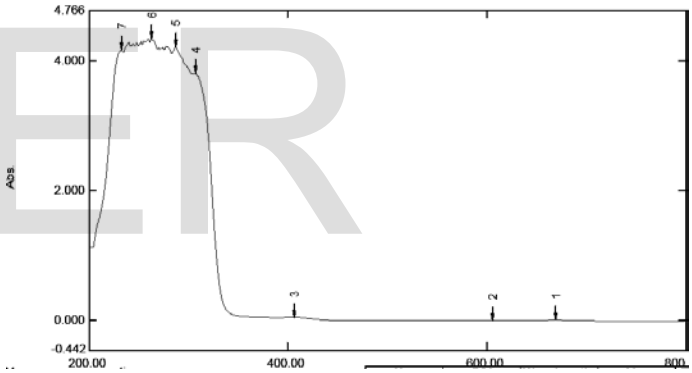
4.3 Gaussian software output

IR(cm^{-1}) υ_{max} , C-H Stretch 3170, 3162, 3193, 3175, 3193,3175, 3216 , 3250, 3081,3033, 3081, 3033, N-C Stretch 1664,1482 , 1387. C-C Stretch 1677 ,1461, 1686 ,1516,1413, 1686, 1664 ,1550 ,1387 ,1177 ,1004 , N-C Stretch 1305 ,1225, C-C Stretch 1640, 1640,1567, 1664 , 1334 1295 , 1413, 1461,1305 ,O-C Stretch 1516,1295,1115, 841, 1072,995, 983, C-C-C Bending 855, 610, C-O-R Bending 1072, 983,746, H-O-C Bending 1305,1225, 1213, 1177, H-C-N Bending 1431,1334, H-C-C Bending 1664,1225,1213,1207, H-C-C Bending 1334,1207, 1461,1305,1225,1153, 1516,1251, 1213, C-N-C Bending 486, 417, C-C-C Bending 704,506, C-C-N Bending 1004, Bending O-C-C 506.

5 IR Spectrum of Berberine alkaloid

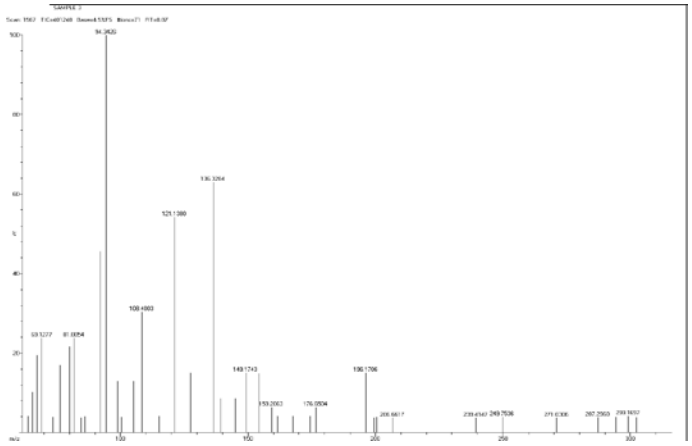


5.1 UV Spectrum of Berberine

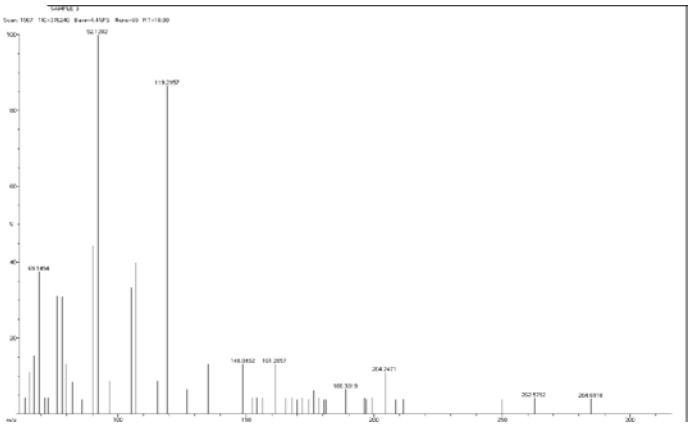


5.2 Mass Spectral data

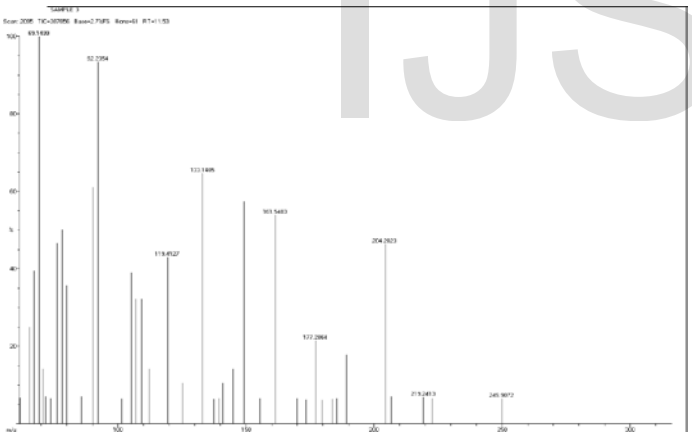
Scan 1567, TIC=401248, Base:4.5%FS, #ion=71, RT=8.87, Peaks m/z: 69.12 (C_5H_{11}), 81.88 (C_5H_5O), 94.34(C_6H_6O C_5H_5O), 108.4, 121.13 (C_8H_9O), 136.32, 149.17 (dialkyl phthalates), 159.20 ($C_{10}H_{10}O$), 176.75, 196.17, 206.66,239.41, 249.75, 271.03, 287.29,299.16.



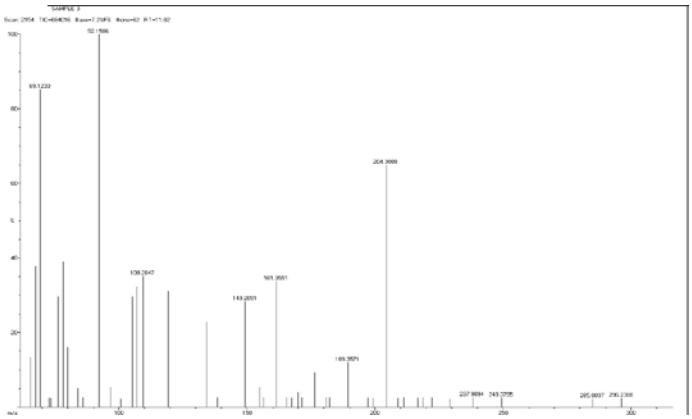
5.3 Scan1967, TIC=376240, Base: 4.4%FS, #ions=65, RT=10.88, m/z: 69.14(C_5H_9), 92.12 (C_6H_6N), 119.21, 148.84 (dialkyl phthalates), 161.61 ($C_{10}H_{10}NO$), 188.93($C_{11}H_{21}NO_2$), 204.24, 262.57, 284.63.



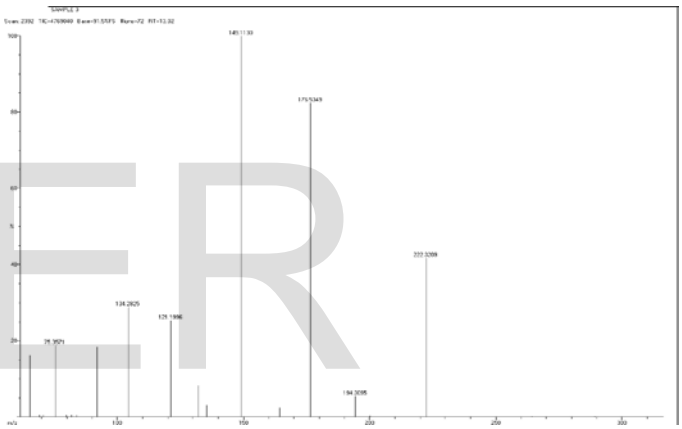
5.4 Scan 2095, TIC=387856, Base: 2.7%FS, #ions=61, RT=11.53, m/z: 69.14(C_5H_9), 92.23 (C_6H_6N), 119.41, 133.14, 161.54 ($C_{10}H_{10}NO$), 177.28, 204.28,219. 24,249.90.



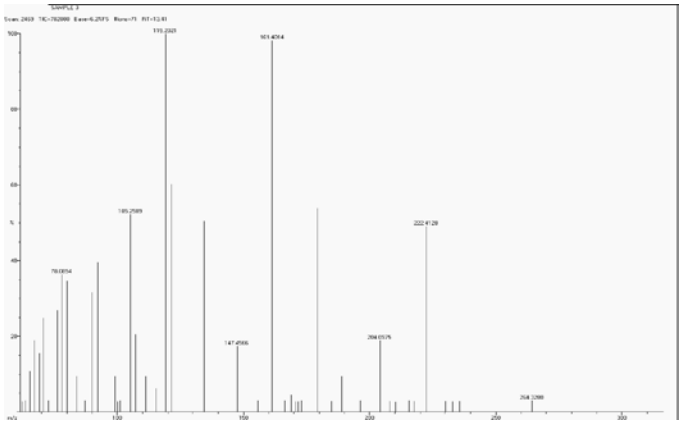
5.5 Scan 2154, TIC=684016, Base 7.2%FS, #ions=62, RT=11.82, m/z: 62.12, 92.15(C_6H_6N), 109.28, 149.28(dialkyl phthalates), 161.35 ($C_{10}H_{10}NO$), 189.35($C_{11}H_{21}NO_2$), 204.30, 237.98, 249.31, 285.0, 293.23.



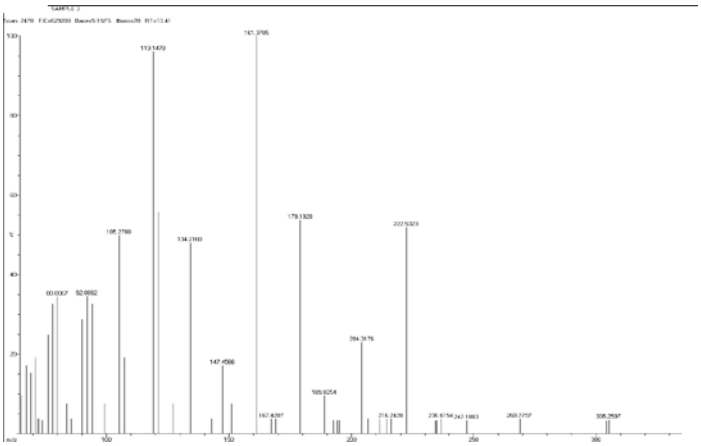
5.6 Scan 2392, TIC=4769040, Base 9.5%FS, #ions=72, RT=13.02, m/z: 75.35, 104.28, 121.19 (C9H9O), 149.11 (dialkyl phthalates), 176.53, 194.30 ($C_{11}H_{21}NO_2$), 222.32.



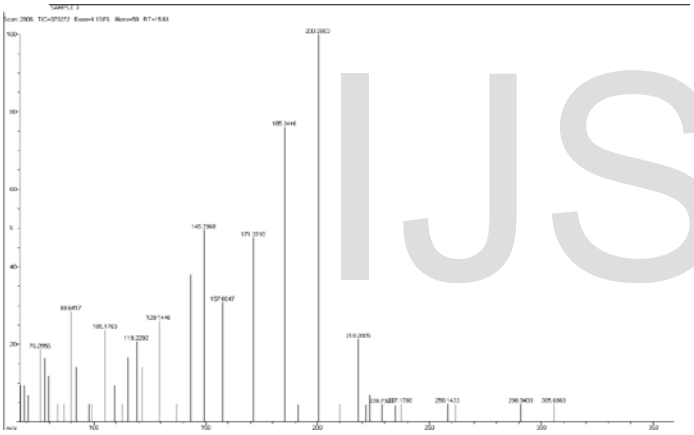
5.7 Scan 2469, TIC=702080, Base: 6.2%FS, #ions=71, RT=13.41, m/z: 78.08(C_5H_4N some pyridines), 105.25, 119.20, 147.45, 161.40 ($C_{10}H_{10}NO$), 204.09, 222.41, 164.32.



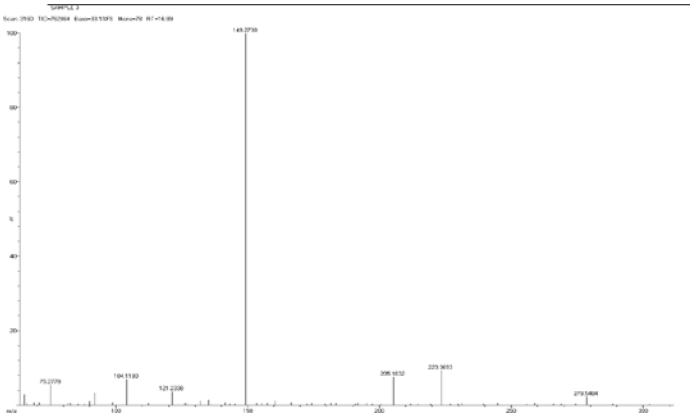
5.8 Scan 2470, TIC=629280, Base: 5.1%FS, #ions=78, RT=13.41 m/z: 80.0(C_5H_5O Pyrroles), 92.08(C_6H_6N), 105.27, 119.14, 134.2, 147.45, 161.37($C_{10}H_{10}NO$), 167.42, 179.19, 189.02 ($C_{11}H_{21}NO_2$), 204.31, 222.53, 236.61, 247.18, 268.77, 305.25.



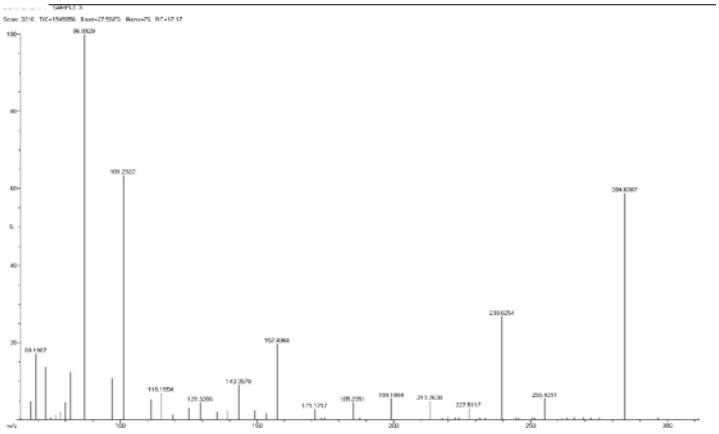
5.9 Scan 2906, TIC=370272, Base: 4.1%FS, #ions=59, RT=15.61. m/z: 76.25, 89.84 (C_7H_5 Heterocyclics containing N and O), 105.17, 119.22, 129.14, 149.29 (dialkyl phthalate), 157.60, 171.39, 185.34, 200.28, 218.28, 228.73, 258.14, 290.94,305.



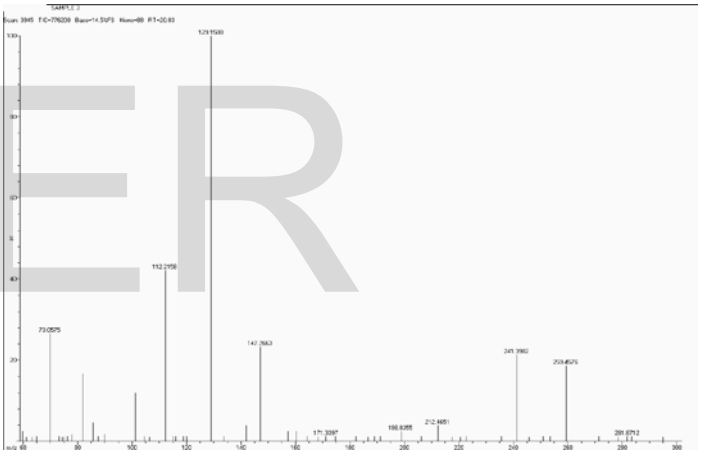
5.10 Scan 3160, TIC=762864, Base 33.1%FS, #ions=78, RT=16.89, m/z:75.27,104.11,121.23 (C9H9O), 149.27 (Dialkyl phthalate), 205.18, 223.36, 278.54.



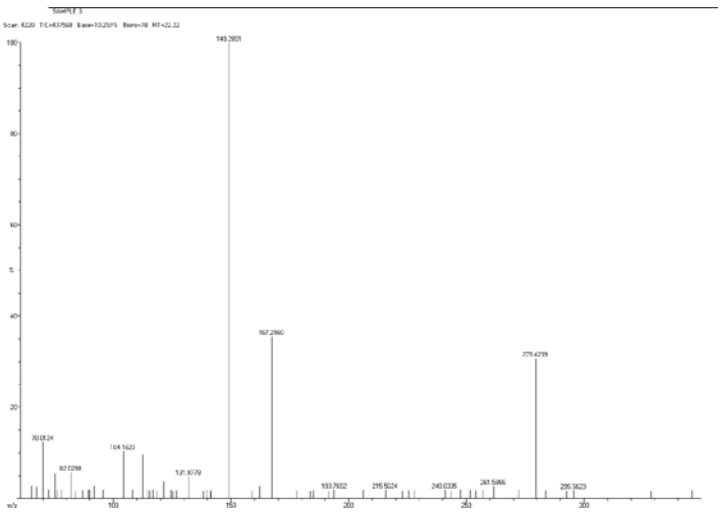
5.12 Scan 3216, TIC=1545856, Base 27.5%FS, #ions=76, RT=17.17, m/z: 69.19, 86.89(C_7H_5 Heterocyclics containing N and O), 101.20, 115.15, 129.32, 143.55, 157.49, 171.17, 185.23, 199.18, 213.26, 227.51, 239.62, 255.42, 264.60.



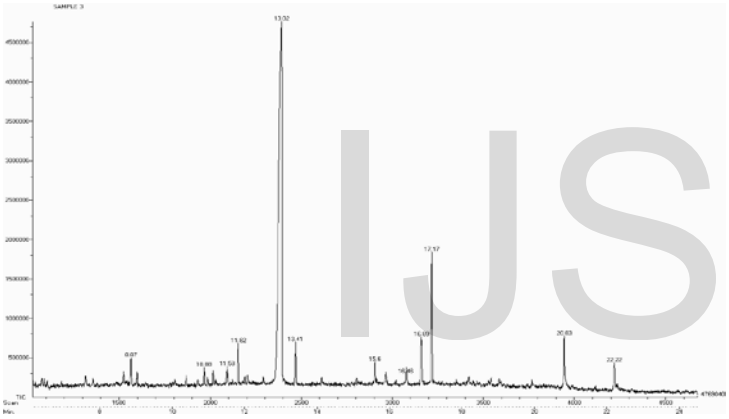
5.12 Scan 3945, TIC=776208, Base 14.5%FS, #ions=88, RT=20.83, m/z: 70.05, 112.21, 129.15, 147.26, 171.30, 198.83, 212.46, 241.39, 259.45, and 281.87.



5.13 Scan 4220, TIC=437568, Base 10.2%FS, #ions=78, RT=22.22, m/z: 70.01 (C_5H_{10} Hydrocarbon), 82.02, 104.16, 131.87, 149.29 (Dialkyl phthalate), 167.24, 193.76 ($C_{11}H_{21}NO_2$), 215.56, 240.83, 261.58, 279.42, 295.56.

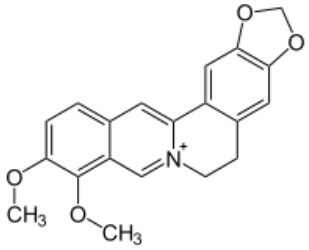


5.14 TIC min-8.81, 10.88, 11.53, 11.82, 13.41, 15.6, 16.89, 17.17, 20.83, 22.22.



6 Conclusion.

The berberine alkaloid isolated at 60^0C from leaf of the *Mahonia leschenaultii* plant. The experimental values of IR, UV and MS are used to predict the molecular structure, atomic stretching, possible molecular functional group, etc., for the confirmation of berberine alkaloid present in the *Mahonia leschenaultii* plant and are confirmed. The theoretical values are good agreement with the experimental values. The molecular structure is found by Gaussian software (V3) and also IUPAC name by ChemDoodle software and is represented as.



6.1**Molecular structure of berberine alkaloid**

6.2 IUPAC Name of berberine

18, 19-Dimethoxy-6.8-diolxa-i-azapentacyclohenicosa -1, 3, 5, 10, 13, 15, 17, 19- octane and the molecular formula is $C_{20}H_{18}NO_4$.

7 Acknowledgments

I would express my sincere thanks to Mr. Govindasamy, Dr. Janarthanan and Mr. Shanmugam for their valuable suggestions, motivation and help for the betterment of my original research manuscript preparations.

8 References.

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About author.

- **Lawrence T.,**
  Research scholar, Department of Physics, Karpagam University, Coimbatore,Tamilnadu, India. – 641021'
Email. .tlaran@rediffmail.com,

- Dr. S. Gunasekaran D.Sc.,
  Former Registrar, Periyar University, Salem.
  Head (Rtd), PG and Research Dept. of Physics, Pachayappa's College, Chennai.
  Dean, Research and Development, St. Peter's University, Avadi, Chennai, Tamilnadu, India – 600054.
Email. sethugunasekaran@rediffmail.com.