NOVEL BENZYLISOQUINOLINES FROM AN ENDEMIC PLANT OF MADAGASCAR CISSAMPELOS SP (MENISPERMACEAE)

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ABSTRACT

*Cissampelos sp is an endemic plant of Madagascar. It is used locally to treat several diseases. A chemical investigation was done. The genus Cissampelos is recognized to be rich in Alkaloids compounds. Chromatographical technics were used to isolate compounds. Five Alkaloids were obtained from the Alkaloid extract. Two new benzylisoquinolines named 11-(amino(hydroxy)methyl)-6,7-dimethoxy-1,2,3,4,7,8,9,10-octahydro-6H-2,10-epiminobenzo[f][12]oxacyclododecin-13-one **1** and 4-(3-(3-amino-3-oxopropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)butanoic acid **2** were identified. Their structures were elucidated by analysis of their spectroscopically data including 1D, 2D NMR, ESI-MS and in comparison with model compounds data. The alkaloid extract showed an antioxidant activity.*

Keywords: *Cissampelos, Menispermaceae, Benzylisoquinoline, NMR, ESI-MS*

1. INTRODUCTION

The Menispermaceae family is rich in alkaloids compounds such as isoquinoline alkaloids [1].Isoquinoline, tropoloisoquinoline, and bisbenzylisoquinoline alkaloids were found in this family and the isolation of krukovine and limacine, which exhibit anti-plasmodial activity has been mentioned [2]. Reports in literature show that alkaloids are the main constituents of *Cissampelos* genus and its biological activities are due to this compound's family [3].The traditional medicine in Madagascar mentioned that *Cissampelos sp* is used against several diseases like diarrhoea, dysentery, ulcers, colic, intestinal worms and urogenital problems like menstrual problems, infertility, uterine bleeding and skin infection [4] [5].This work reports the first chemical study about *Cissampelos sp*.

2. MATERIAL AND METHODS

2.1 GENERAL EXPERIMENTAL PROCEDURE

Melting points were measured with an Electrothermal melting point apparatus and value was not corrected. NMR spectral were recorded with Brüker AV-300 and AV-500 using a cryoprobe for1H, BBD, APT, HSQC, and HMBC experiment. Chemical shift values are given in δ(ppm) using the peak signals of the solvent DMSO (δH:2.50-3.49; and δC:39.52) as reference, and coupling constants are reported in Hz. ESI-MS data were measured with a Finnigan MAT95 spectrometer (70eV). Column chromatography was performed on silica gel HF60 (6.3-20 μm) (Merck, Darmstadt, Germany). Normal-phase silica gel 60 TLC plates (w/UV 254) were used for fraction detection. The chromatograms were visualised using UV light at 254 nm and sprayed with Draggendorf reagent.

2.2 PLANT MATERIAL

The aerial parts of Cissampelos sp were collected in august 2012 in one village next to Moramanga at the Eastern rainforest of Madagascar. The plant was authenticated at the botanical and zoological park of Tsimbazaza Antananarivo Madagascar where a voucher specimen is deposited.

2.3 EXTRACTION AND ISOLATION

130g of the aerial parts powder were extracted by aqueous hydrochloric acid 0,1N to prepare the acidic extracts, that is neutralized with NaOH 0,1N. The neutralized solution was extracted three times with chloroform. The organic phase containing the alkaloids were collected. 3,75 g was obtained. Column chromatographical technic using silica gel was performed with the system $CHCL_3$/MeOH/water as eluant. Five pure compounds were isolated from the total alkaloids extract. The structural elucidation of the compounds noted 1 and 2 was established using spectroscopic methods including 1D, 2D-NM and ESI-MS.

3. RESULTS AND DISCUSSIONS

3.1 IDENTIFICATION OF COMPOUND 1

Compound **1** (Fig.3) was obtained as brown powder with a molecular formula $C_{18}H_{24}N_2O_5$ on the basis of accurate mass measurement (ESI-MS, m/z 356(M+6H). The analysis of ^1H and ^{13}C and DEPT NMR data shows resonances for 11 carbons corresponding to a derivate of 6,7-methoxy-1,2,3,4-tetrahydroisoquinoline system [6]. The ^1H and ^{13}C NMR spectra shows two O-methyl, five methylene, six methine groups among them two are aromatic, two oxygenated aromatic quaternary carbons, one carboxyl at 173.4 ppm, one aminomethanol at 78.9 ppm and two quaternary aromatic carbons. The ^1H NMR spectrum exhibited two resonance peaks at δ_H= 3.70 and δ_H=3.63 for two methoxy protons attached to the aromatic quaternary carbons C-6 and C-7 resonating respectively δ_C= 147.6 and δ_C=146.7. The O-methyl's positions are confirmed by HMBC correlations.
The aspect of the spectra ^{13}C BB NMR of compound **1** allows maintaining that its structure would be closed to 6,7-diméthoxy-1,2,3,4-tetrahydroisoquinoline **5**. The ^1H NMR spectrum also showed two singlets aromatic protons H-5 and H-8 having HMBC correlations respectively with C-8a, C-7, C-4 and C-4a, C-6, C-1.

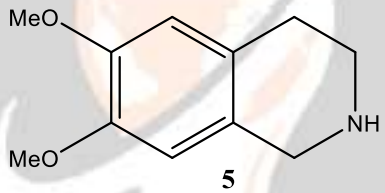

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Fig-1: 6,7- diméthoxy-1,2,3,4-tetrahydroisoquinoline

The NMR shift value of both carbons C-1 and C-3 implicate that the isoquinoline moiety would be substituted at these positions. By means of HMBC analysis, the correlations (H-4; C-9) allowed to confirm that C-3 is linked to the tail of 1-amino-1-hydroxypropan of 1-amino-1-hydroxypropan-2-yl butyrate. The HMBC correlation between H-15 and C-1 confirm that C-1 is linked with the tail part of the butyrate of 1-amino-1-hydroxypropan-2-yl butyrate 6.

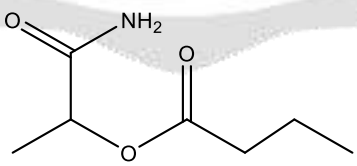

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Fig-2: 1-amino-1-hydroxypropan-2-yl butyrate

The ^1H NMR spectra also showed characteristic signals of methylene group H-4 at δ_H= 2.25 and δ_H=2.50 (ddd). They have HMBC correlations with C-3, C-5, C-9, C-4a and C-8a. The ^{13}C DEPT 135 spectrum confirms that C-4 is a methylene group resonating at δ_C= 32.9. For H-3, the ^1H NMR spectrum exhibited resonance of methine group at δ_H= 2.91 (d) and its carbon chemical shift at δ_C= 56.2 corresponding to the carbon adjacent of nitrogen. H-3 has HMBC correlations to the carbon C-4a, C-1 and C-10.
The high down field shift of H-10 at δ_H= 6.20 indicates that H-10 is attached to an oxygenated carbon. This

carbon C-10 resonating δ_C= 79.0 is consistent with a sp^3 carbon bonded with an oxygen of the ester moiety of 1-amino-1-oxopropan-2-yl butyrate.
The ^1H and ^{13}C NMR spectra showed the presence of methylene group C-15 (24.2, 4.66) at the β-position of the carboxyl group(C-13) having a shift value δ_C= 173.9. Their position is confirmed by HMBC correlations.
The ^{13}C NMR spectrum showed a resonance peak at δ_C= 56.3 (C-1) which is characteristic of a methine group of the isoquinoline unit. This position is confirmed by its HMBC by means of its correlation with H-3 and H-15(δ_H= 2.91, 1.73-1.75). This observation affirms that C-1 is substituted with the other part of 1-amino-1-hydroxypropan-2-yl butyrate.

The ESI-MS shows a molecular mass at M+6H =356. This one comes from two main fragments: the 6,7-dimethoxy-1,2,3,4-tetrahydro isoquinoline (m/z=191), and the 1-amino-1-hydroxypropane-2-yl butyrate (m/z=159) .

From the results above, compound **1** was identified as 11-(amino(hydroxy)methyl)-6,7-dimethoxy-1,2,3,4,7,8,9,10-octahydro-6*H*-2,10-epiminobenzo[*f*][12]oxacyclododecin-13-one.

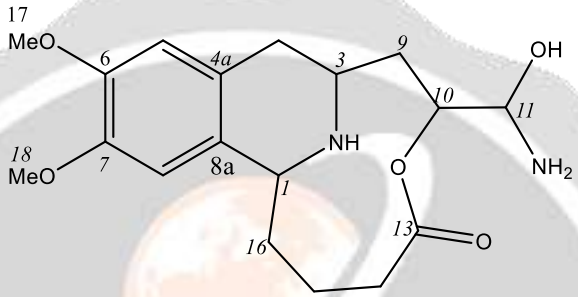

Fig-3: Carbon numbering of compound 1

Table 1: ^{13}C BB, 1H and HMBC data of compound 1

Carbon numbering	Compound 1		
	δ ^{13}C	**δ ^{1}H**	**HMBC**
1	56.3	4.48, 1H (d)	56.2, 110.3, 123.8
3	56,2	2.91, 1H (m)	56.3,123.8
4	32,9	2.50, 2.25, 2H (d)	32.9,111.5, 130.0
4a	123.8	-	-
5	111.5	6.50, 1H (s)	32.9, 130.0, 146.7
6	147.6	-	-
7	146.7	-	-
8	110.3	6.80, 1H (s)	56.2, 123.8, 147.6
8a	130.0	-	-
9	32.9	1.26, 1.21, 2H (dd)	32.8
10	79.0	6.20, 1H (d)	56.2
11	78.9	-	-
13	173.9		
14	32.8	2.18-2.28, (m,m)	32.8
15	24.2	1.73-1.75, 2H, (m,m)	56.3, 173.9
16	32.7	1.79,2H (q)	130.0, 32.9
6-OMe(17)	56.2	3.75, 1H, (s)	146.7
7-OMe(18)	56.3	3.70, 1H (s)	147,6

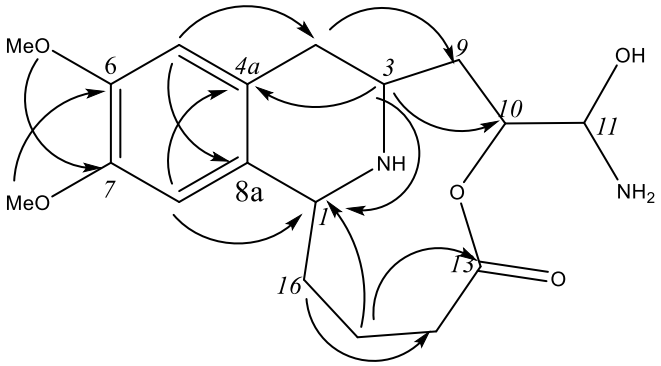

Fig-4: HMBC correlations arrows for compound 1

3.2 IDENTIFICATION OF COMPOUND 2

Compound **2** was isolated in the same way used for compound **1.** The spectral data of NMR and ESI-MS allows suggesting that compound **2** resembles to compound **1**. However, the absence of the carbon C-10 (79.0 ppm) of compound **2** maintains that the oxygen linked to this carbon has been detached. In addition, the absence of the carbon C-11 (78.9 ppm) of compound **2** shows that the hydroxyl linked to this carbon was oxidized to be a ketone there. These transformations contributed to the biogenesis of compound **2** (C-10, 32.0 ppm and C-11, 174.4 ppm) according to the diagram shown in Figure 3. Compound **2** presents a free carboxylic acid terminal and a free amid terminal. Compound **2** is named 4-(3-(3-amino-3-oxopropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)butanoic acid.

Fig-3: Biogenesis of compound 2

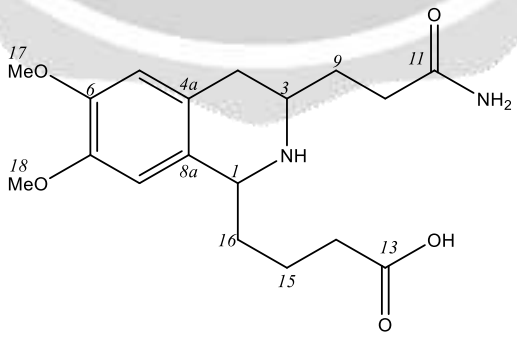

Fig-4: Structure and carbon numbering of Compound 2

Table 2: ^{13}C BB, ^{1}H and HMBC data of compound 2

Carbon numbering	Compound 2		
	δ ^{13}C	**δ ^{1}H**	**HMBC**
1	54.2	3.81, 1H (d)	60.1; 110.2; 125.4; 130.1
3	60.1	2.97, 1H (m)	24.5, 60.1, 125.4
4	32,0	2.71, 2H (d)	37.1, 112.2, 130.1
4a	125.4	-	-
5	112.2	6.88, 1H (s)	32.0, 130.1, 146.9
6	146.9	-	-
7	147.1	-	-
8	110.2	6.94, 1H (s)	54.2, 125.4, 147.1
8a	130.1	-	-
9	32.0	1.78,1H (d)	176.8, 32.0
10	21.4	1.45, 2H (t)	60.1
11	174.4	-	-
13	176.8	-	-
14	32.0	1.97, 2H, (m)	30.1
15	27.4	2.38, (m,m)	54.2, 174.4
16	30.1	2.05, 2H, (t)	32.0, 130.1
6-OMe (17)	56.2	3.81, 1H, (s)	147,1
7-OMe (18)	56.1	3.82, 3H (s)	146.9

The NMR spectral data and ESI-MS analysis confirms the presence of two novels isoquinolines isolated for the first time from *Cissampelos sp.* of Menispermaceae family.

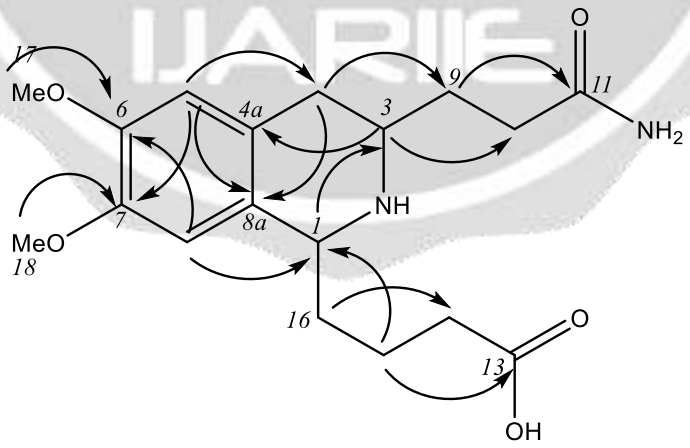

Fig-5: HMBC correlations arrows for compound 2

4. ANTIOXYDANT ACTIVITY

Qualitative antioxidant assay was performed by the standard TLC- DPPH method [4]. The compounds 1, 2 and alkaloids crude extract were spotted on a TLC plate and air dried, then plates were sprayed with 0.002% ethanolic DPPH (2, 2-Diphenyl-1-picrylhydrazyl) solutions using an atomizer. Positive activity was detected

with all tested compounds by the pale yellow spots on a reddish-purple background due to the decolorization of DPPH by the antioxidant. Ascorbic acid was used as the positive control [7].

5. NMR AND ESI-MASS DATA OF COMPOUNDS 1 AND 2

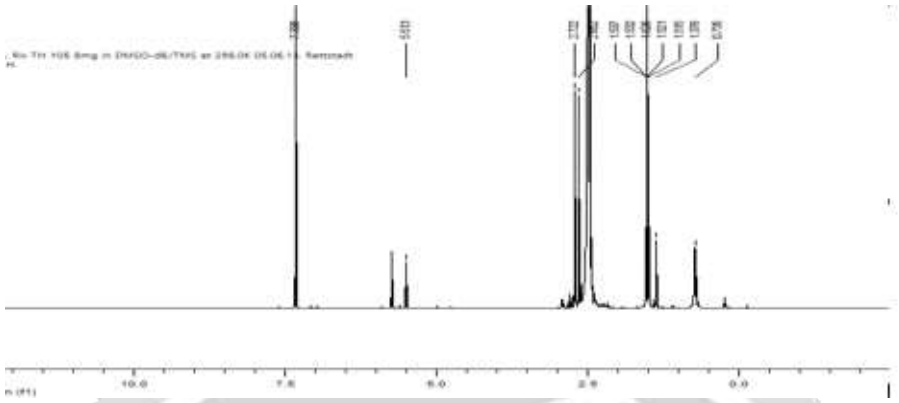

Fig-6: ^{1}H NMR data of Compound 1

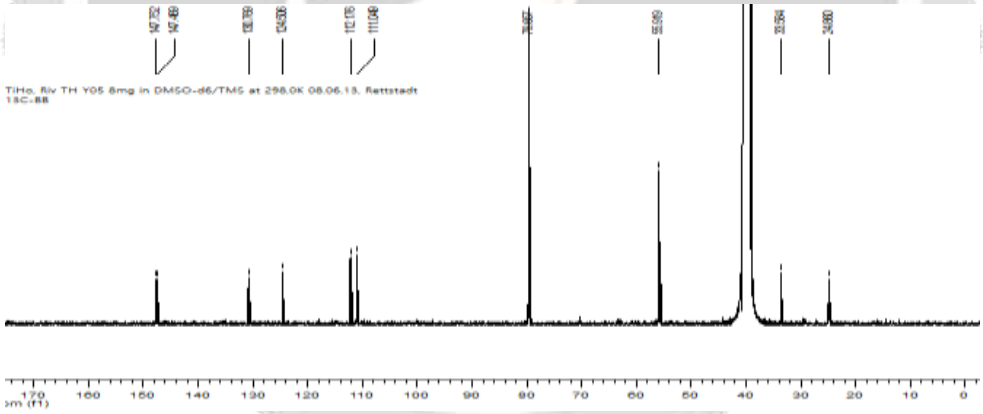

Fig-7: ^{13}C NMR data of Compound 1

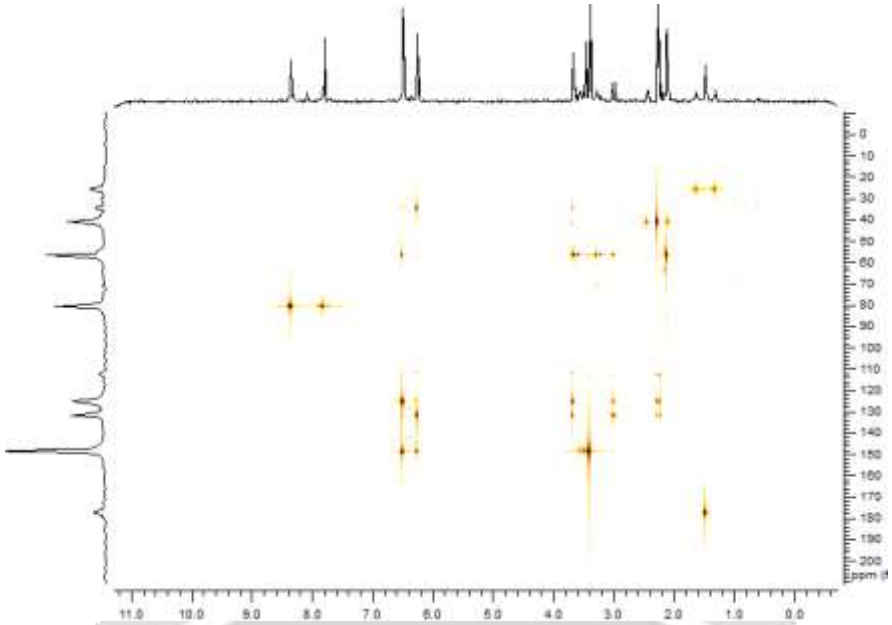

Fig-8: HMBC NMR data of Compound 1

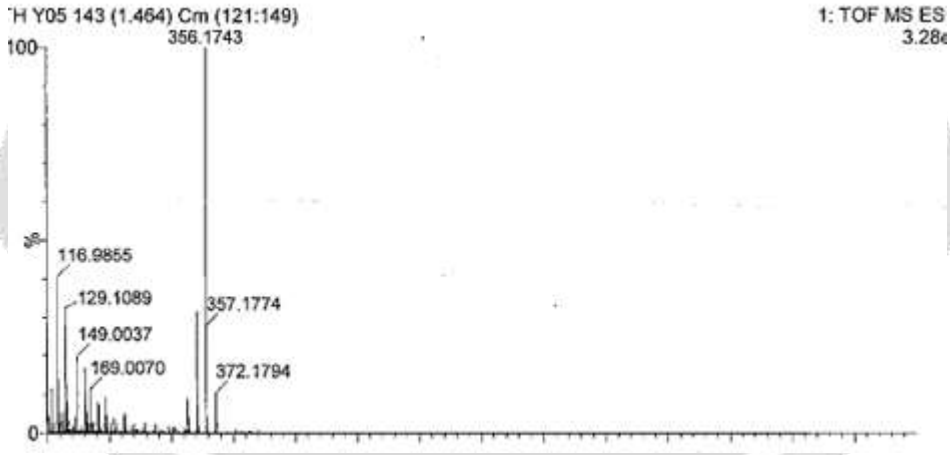

Fig-9: ESI-MS of Compound 1 (348+8H)

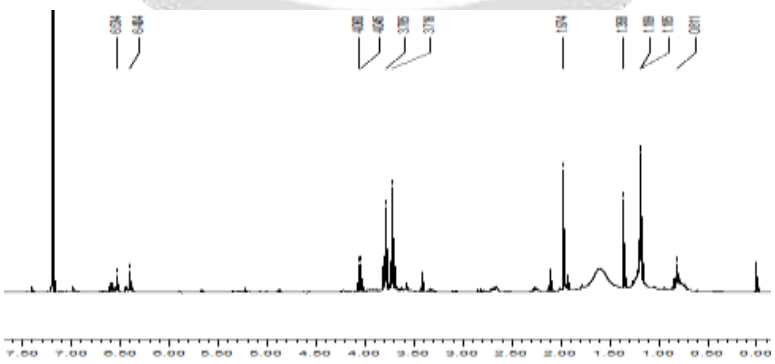

Fig-10: ^{1}H NMR data of Compound 2

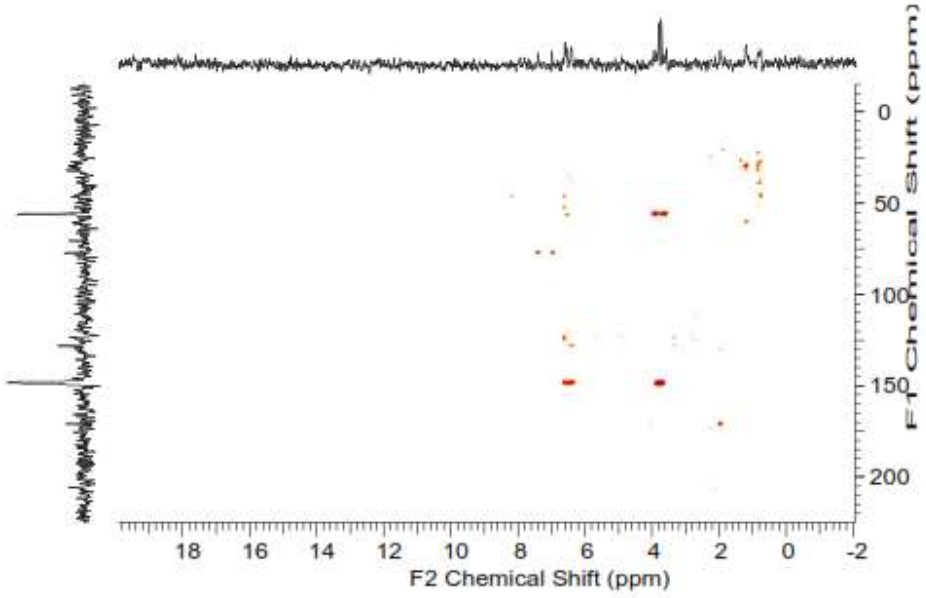

Fig-11: ^{13}C NMR data of Compound 2

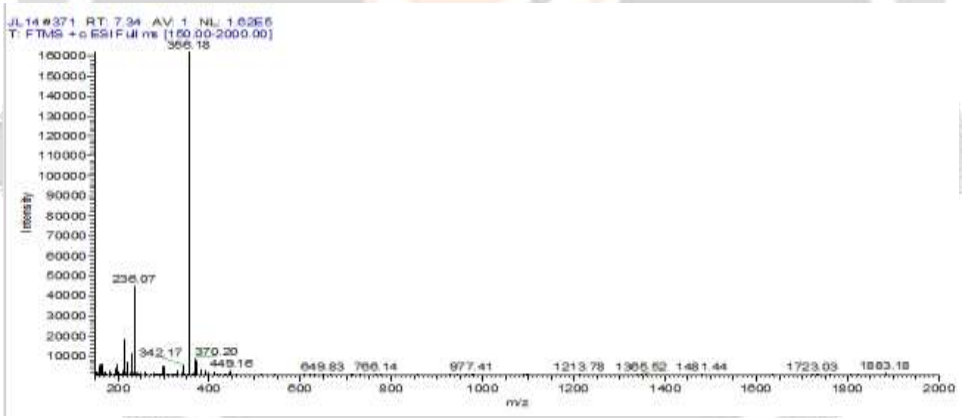

Fig-12: ESI-MS of Compound 2

6. ACKNOWLEDGEMENT

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7. REFERENCES

[1]. P. Grenand, C. Moretti, 2004. Pharmacope´ es traditionnelles de Guyane. IRD e´ di- tions, Paris, p. 464.
[2]. J.C.P.Steele, M.S.J. Simmonds, N.C. Veitch, D.C.Warhurst, 1999. Evaluation of the anti-plasmodial activity of bisbenzylisoquinoline alkaloids from *Abuta* grand- ifolia. Planta Med. 65, p413–416.
[3].W.S.M.Johnatan, E.M.C.Walmir, F.G.R.Fábio, R.S.Edilbero, D.P.L.Roberta, Roberta, G.M.C. José, 2020. *Cissampelos* genus: biological activities, ethnobotanical and phytochemical aspect. Phytochemistry Reviews,

Vol.19, p.955-982.
[4].G.Amresh, G.D.Reddy,C.V. Raoand P.N.Shing, 2007. Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. Journal of Ethnopharmacology; 110; p526 -531.
[5]. C. Bullough and al. 1982**.** Herbal medicines used by traditional birth attendants in Malawi. Trop. Geograph. Med. 34; p81-85.
[6]. C. Y. Chen, F. R. Chang, W. B. Pan, and Y. C. Wu, 2001, Phytochemistry, 56, 753
[7]. T. Atsumi, I. Iwakura, Y. Kashiwagi, S. Fujisawa, T. Ueha. 1999. Free radical scavenging activity in the non- enzymatic fraction of human saliva: a simple DPPH assay showing the effect of physical exercise. Antioxid Redox Signal; 1:537–546.