

## **Electronic Supplementary Information (ESI)**

### **Expedient preparative isolation, quantification and characterization of limonoids from Neem fruits**

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### General Information:

Neem fruits were collected from Aurangabad region in Maharashtra during March to May. Chemicals were procured from Sigma. For the extraction purpose technical grade solvents were purchased locally and distilled prior to use. For MPLC and HPLC purpose HPLC grade solvents (from Sigma) were used. For LC-ESI-MS experiments, LC-MS grade solvents were purchased from JT Baker. For HPLC and LC-ESI-MS runs, high purity water was collected from Milli-Q water purification system made by Millipore. Automated MPLC system from Teledyne, Isco (model: Combiflash Rf 200) was used for the preparative purification of the extracts. HPLC was performed on a Waters HPLC system (Delta 600 pump and controller) coupled with Waters 2489 UV/Visible detector. LC-ESI-MS/MS<sup>2</sup> runs were performed on Q Exactive Orbitrap associated with Accela 1250 pump made by Thermo Scientific. NMR data (<sup>1</sup>H, <sup>13</sup>C, DEPT-135) were recorded on Varian INOVA spectrophotometer (400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C) in CDCl<sub>3</sub> and the residual solvent or TMS signals were labeled as the reference. Thin layer chromatography was carried out on silica gel G-precoated plates (0.25 mm) from Merck and developed in 40 % ethyl acetate in hexane. Spots were visualized by spraying with a solution of 3.0 % anisaldehyde, 2.8 % H<sub>2</sub>SO<sub>4</sub>, 2 % acetic acid in ethanol followed by heating.

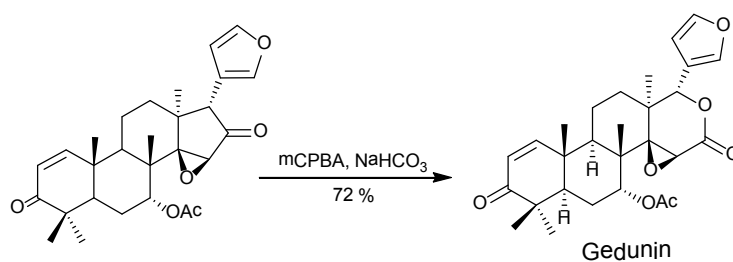
### HPLC conditions:

HPLC runs were carried out on analytical XBridge C<sub>18</sub> column (4.6x100 mm, 5 μm) with a gradient solvent program of 35 min (0 min, 40 % methanol/water; 10 min, 60 % methanol/water; 25 min, 90 % methanol/water; 28 min, 60 % methanol/water; 30 min, 40 % methanol/water; 35 min, 40 % methanol/water) and detection at 230 nm with a flow rate 1 mL/min. Samples were dissolved in HPLC grade methanol and 20 μL of it was injected after filtration.

### LC-ESI-MS and MS/MS conditions:

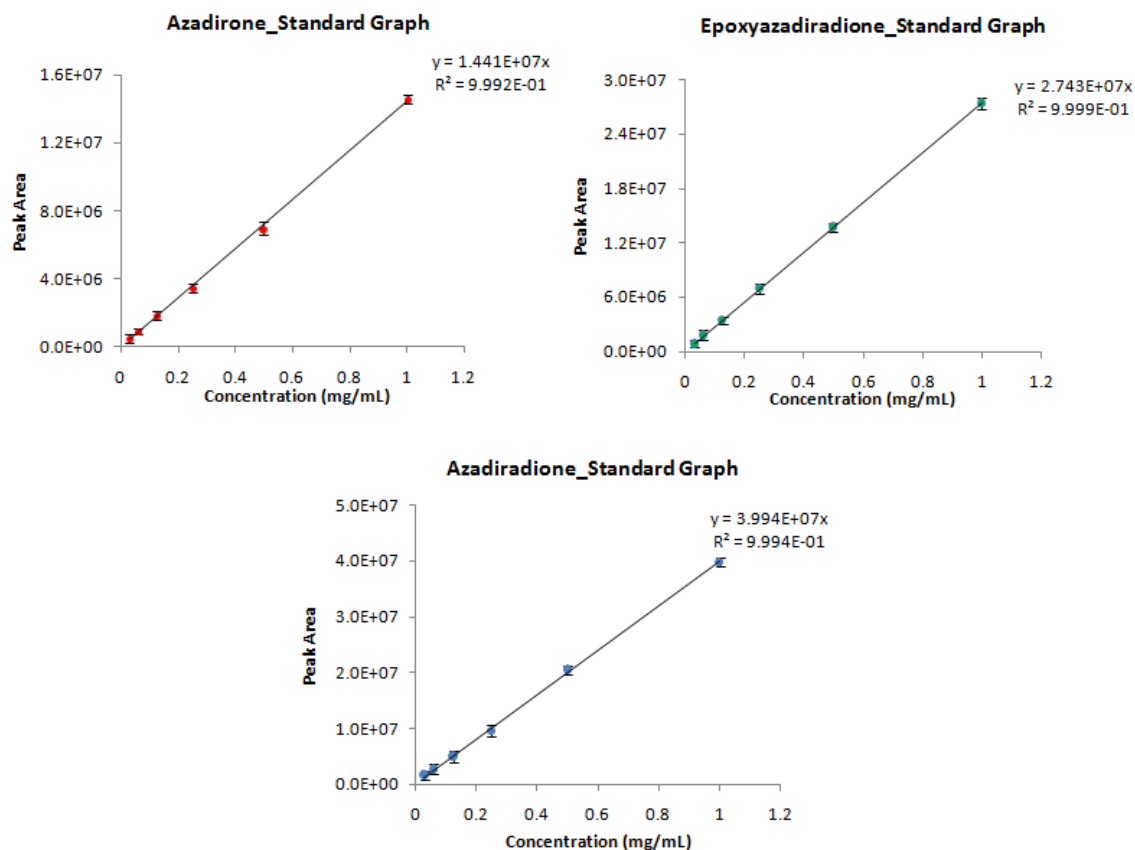
Samples were resolved through Thermo Scientific Hypersil Gold column of particle size 5 μm with a flow rate of 0.5 mL/min and gradient solvent program of 30 min (0.0 min, 10 % methanol/water; 0.5 min, 10.0 % methanol/water; 3.0 min, 45 % methanol/water; 6.0 min, 60 % methanol/water; 20.0 min, 90 % methanol/water; 27.0 min, 90 % methanol/water; 27.5 min, 10 % methanol/water; 30.0 min, 10 % methanol/water). 0.1 % LC-MS grade formic acid was also added to the solvents methanol and water. MS and MS/MS experiments were performed in ESI-positive ion mode using the tune method as followed: sheath gas flow rate 45, auxiliary gas flow rate 10, sweep gas flow rate 2, spray voltage (|KV|) 3.60, spray current (μA) 3.70, capillary temperature (°C) 320, s-lens RF level 50, heater temperature (°C) 350. ESI-MS data were recorded in full scan mode within the mass range *m/z* 100 to 1000 whereas the ESI-MS/MS experiments were performed in data dependent t-*ms*<sup>2</sup> mode using inclusion input of desired *R<sub>t</sub>* and *m/z* to be processed for MS/MS. In specific, *R<sub>t</sub>* values for azadirone, epoxyazadiradione and azadiradione were observed to be 24.0, 20.4 and 16.7 min respectively in full scan ESI-MS run. Further [M+H]<sup>+</sup> ions as observed in individual ESI-MS spectra corresponding to *m/z* 437.2, 467.2 and 451.2 respectively were subjected to MS/MS experiments restricting the range of *R<sub>t</sub>* in the inclusion mode as 23.5-25.5 min, 19.0-21.5 min and 15.5-17.7 min respectively. Each MS/MS experiment was performed in six different stepped normalized collision energies (NCE); 15, 20, 25, 30, 35 and 40 % with stepped value 20 %.

### Semi-synthesis of gedunin:

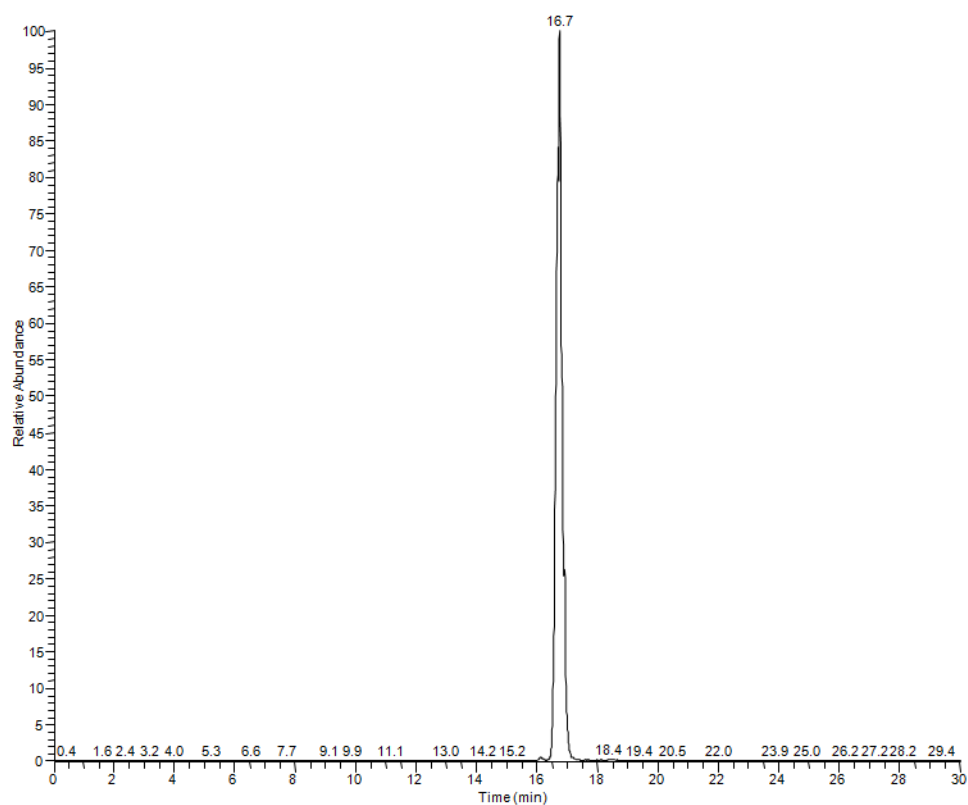


1.50 g (3.21 mmol) epoxyazadiradione taken in 30 mL of anhydrous dichloromethane in an inert atmosphere was cooled to 0 °C. Then 1.84 g (6.42 mmol) of 60 % m-chloroperbenzoic acid was added in stirring condition followed by 0.54 g sodium bicarbonate. After 5 min the reaction vessel was shifted to room temperature and stirred for another 1 h. Further, the reaction mixture was concentrated under reduced pressure and taken in 30 mL of ethyl acetate. The ethyl acetate layer was washed thrice with saturated aqueous solution of sodium bicarbonate (3 x 100 mL). Then ethyl acetate layer was concentrated and the white crude obtained was purified over silica gel (100-220 mesh) column using 0.3 % methanol in dichloromethane as the eluent to furnish 1.21 g (2.32 mmol, yield ~ 72.2 %) pure gedunin.

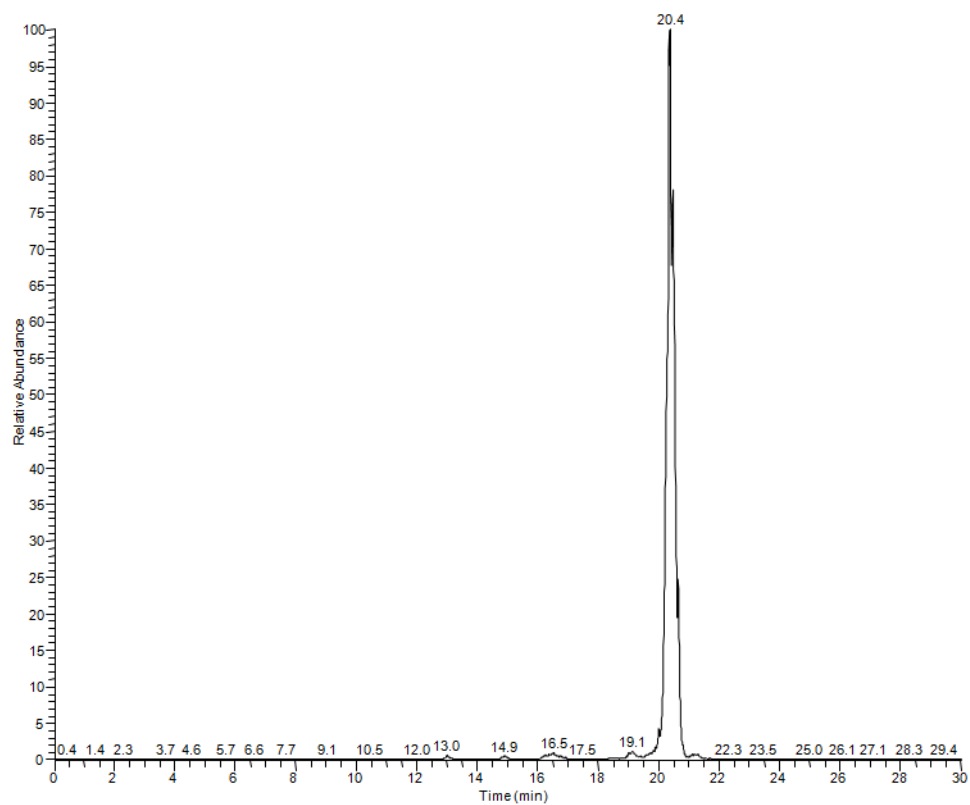
### Standard plots:



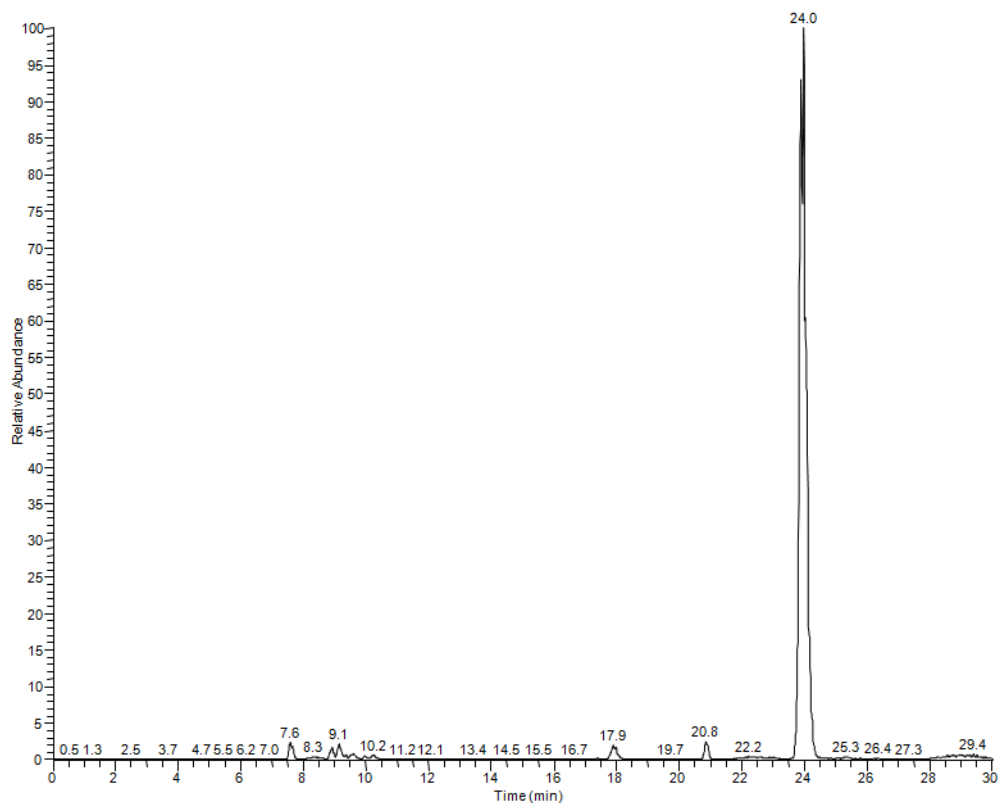
**LC-ESI-MS chromatograms:**



**Fig. S1** LC-ESI-MS chromatogram of azadiradione (III).

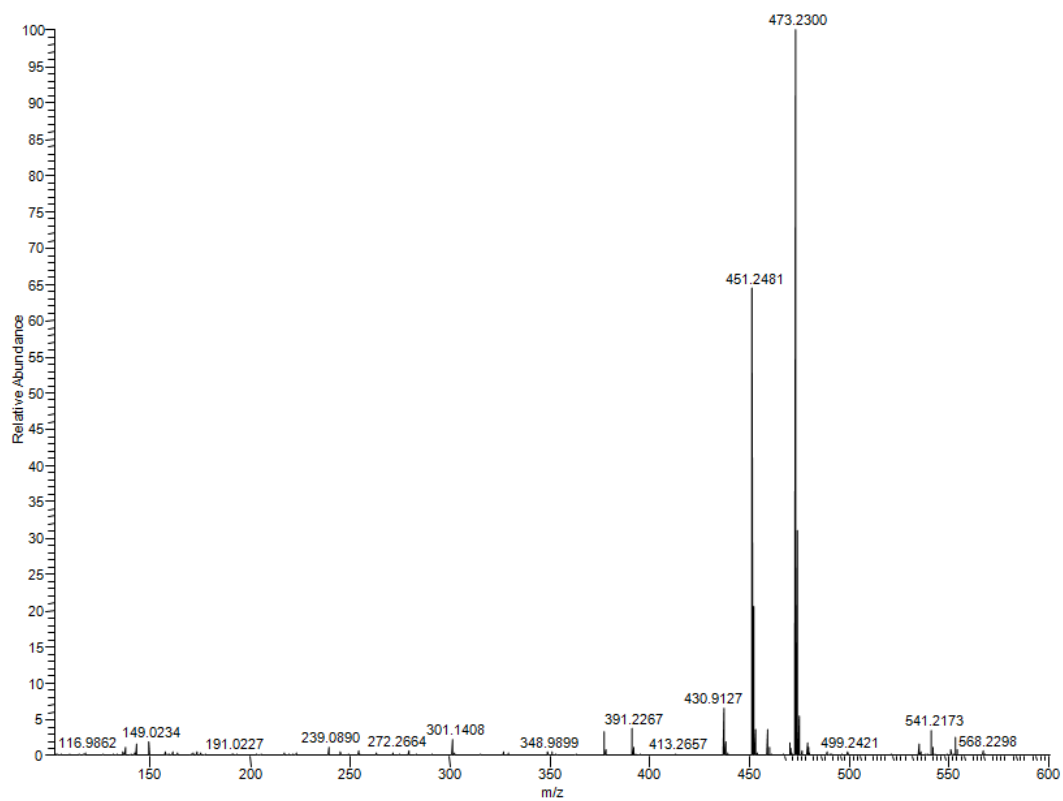


**Fig. S2** LC-ESI-MS chromatogram of epoxyazadiradione (II).

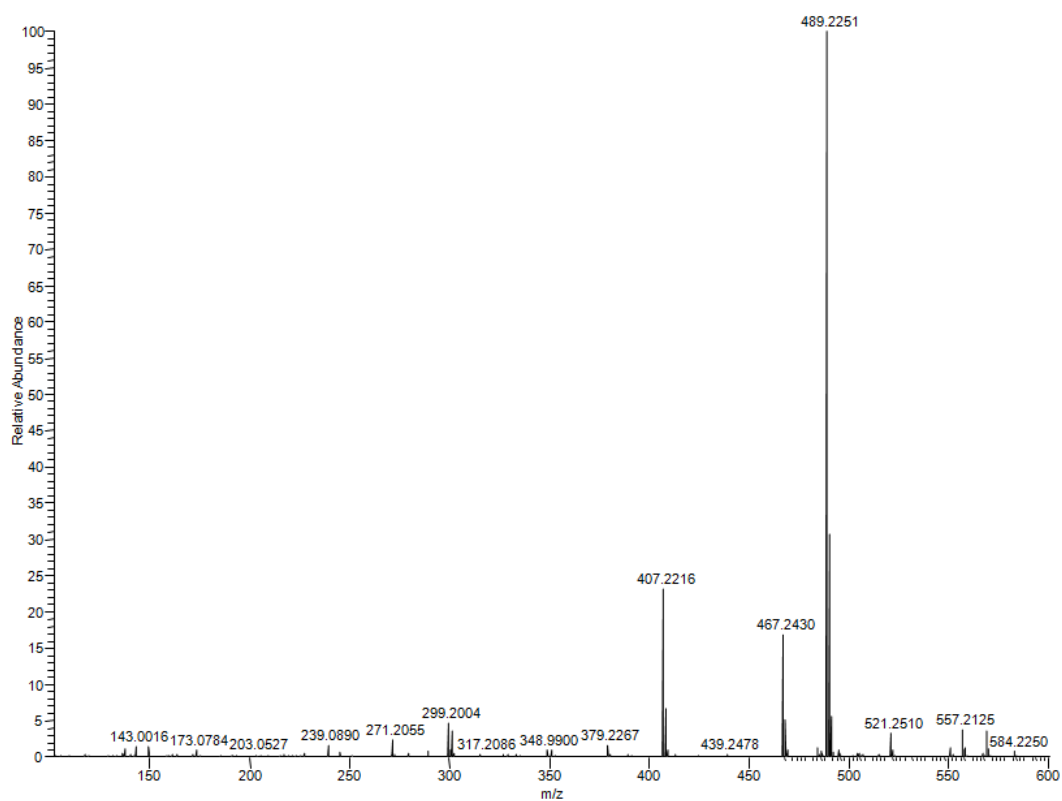


**Fig. S3** LC-ESI-MS chromatogram of azadirone (**I**).

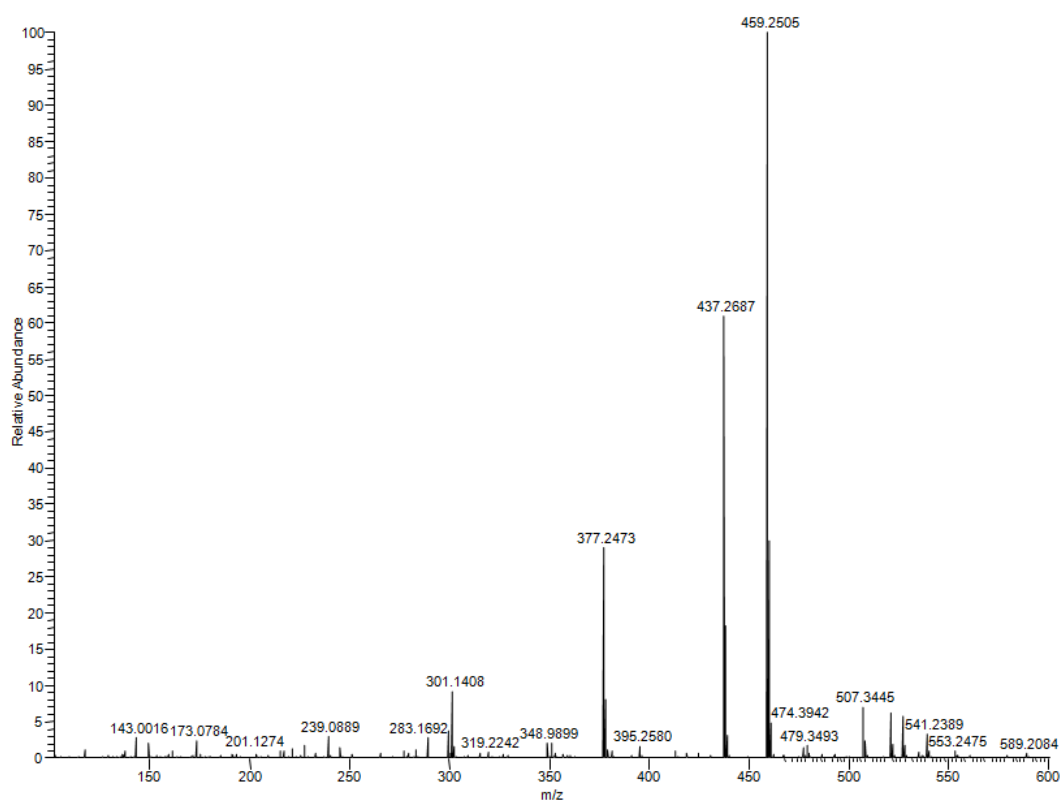
**ESI-MS Full Scan Spectra:**



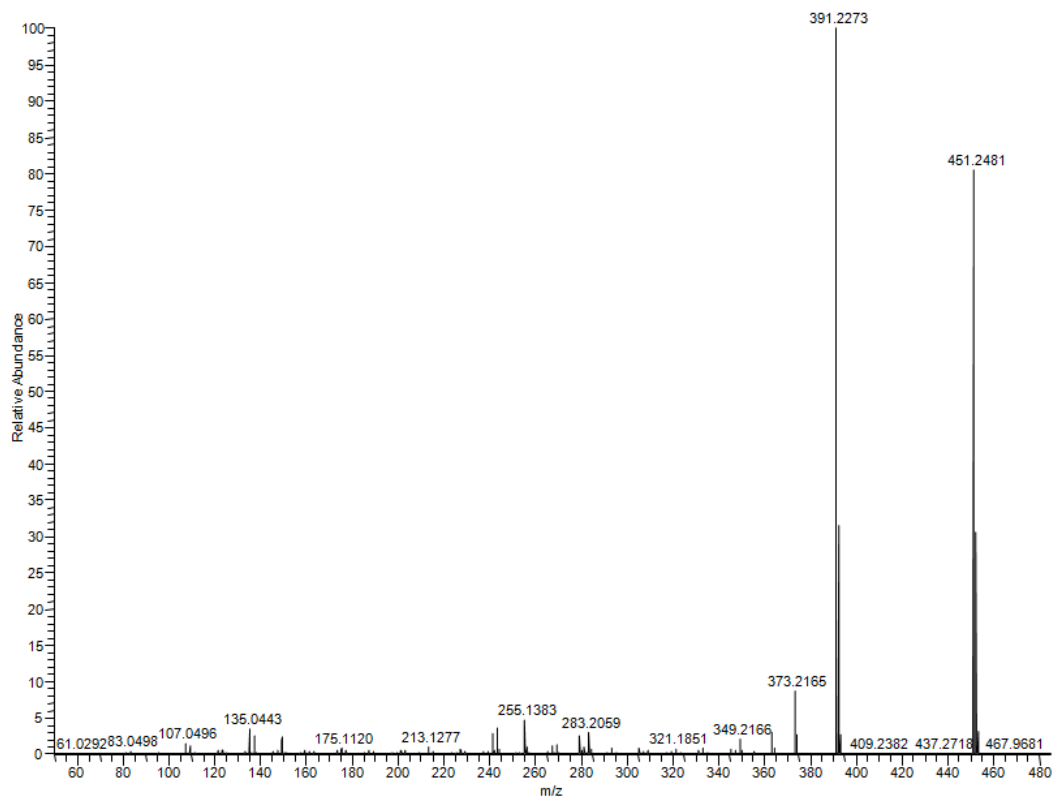
**Fig. S4** ESI-MS spectrum of azadiradione (**III**).



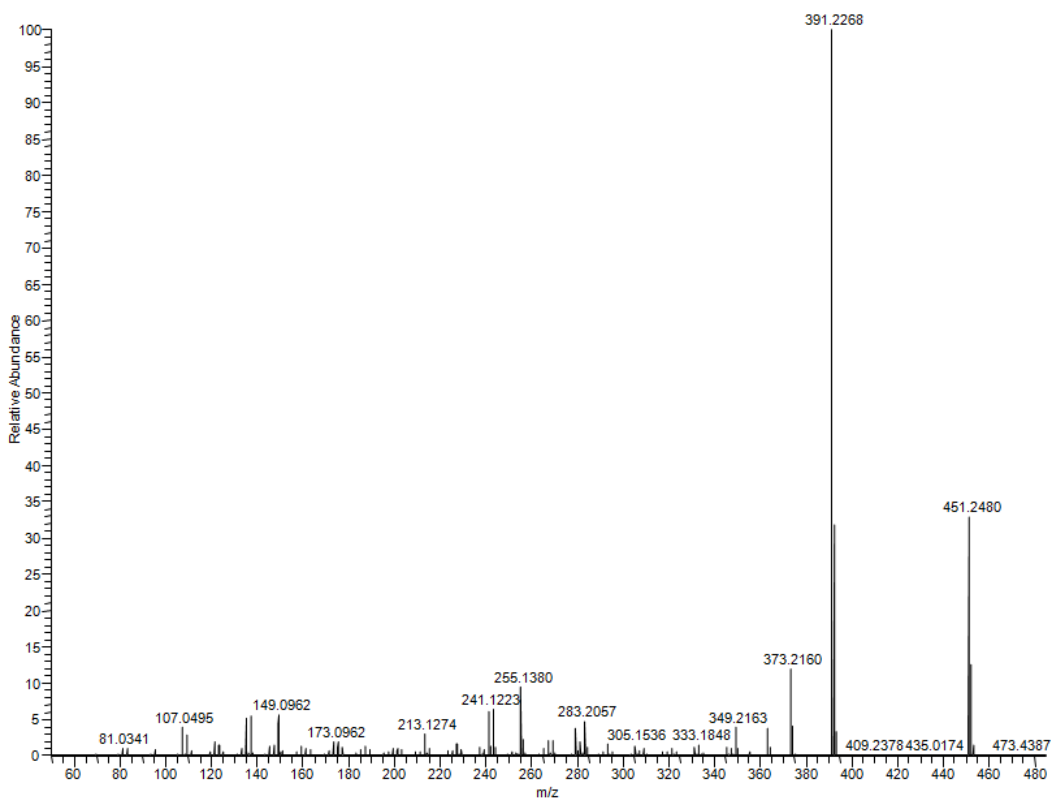
**Fig. S5** ESI-MS spectrum of epoxyazadiradione (II).



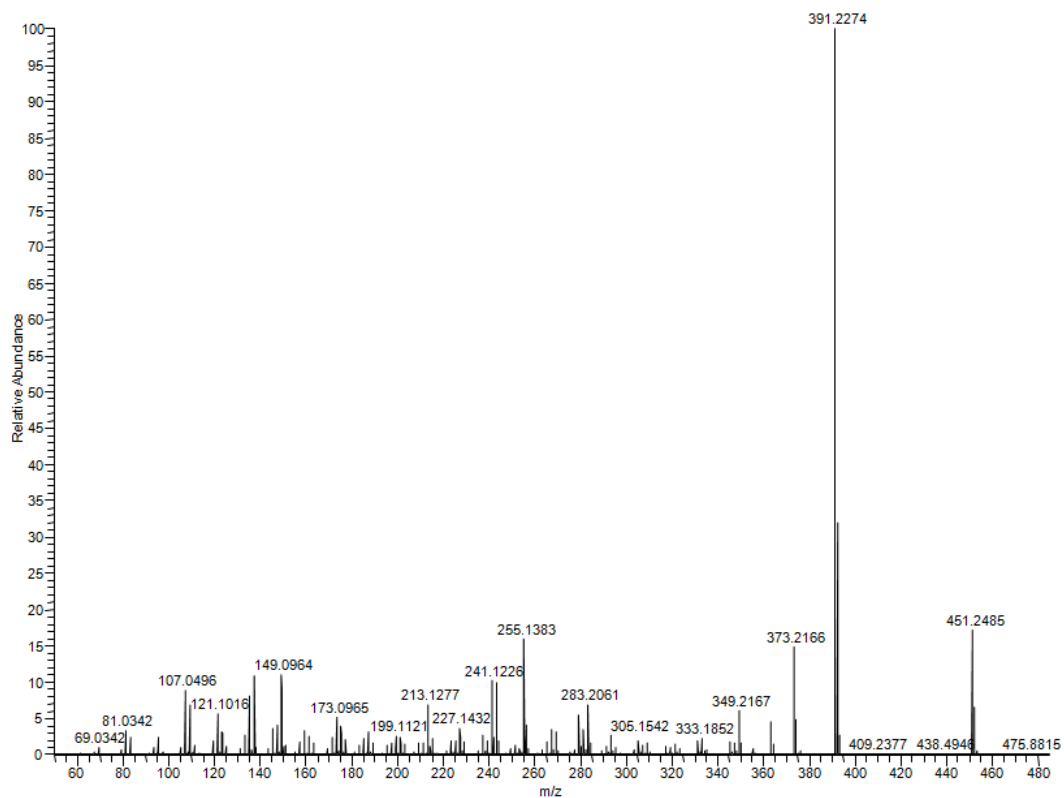
**Fig. S6** ESI-MS spectrum of azadirone (I).



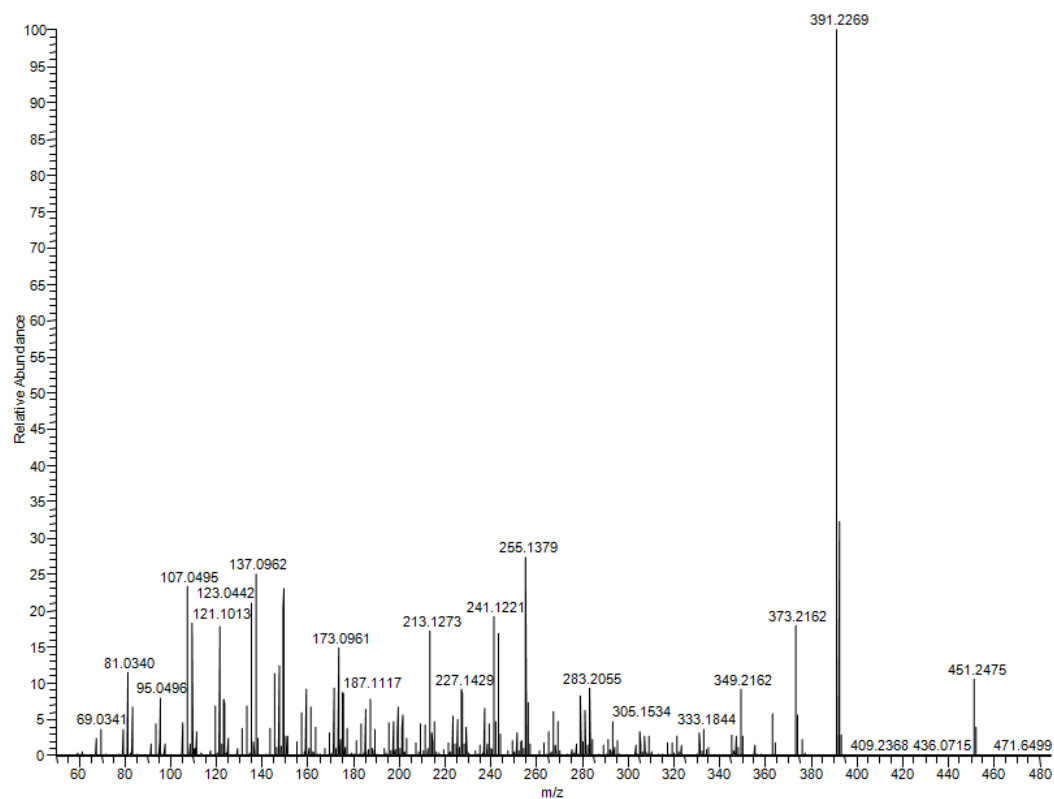
**Fig. S7** ESI-MS/MS spectrum of azadiradione (III) of  $m/z$  451.2 in NCE = 15 %.



**Fig. S8** ESI-MS/MS spectrum of azadiradione (III) of  $m/z$  451.2 in NCE = 20 %.

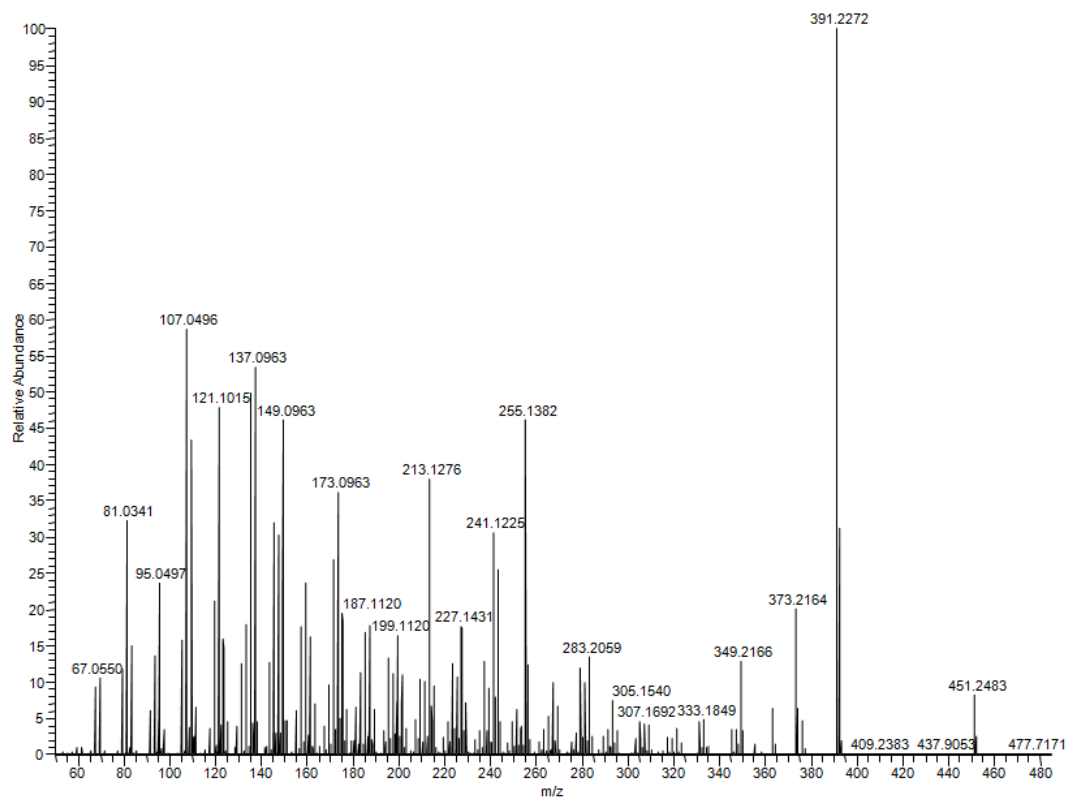


**Fig. S9** ESI-MS/MS spectrum of azadiradione (III) of  $m/z$  451.2 in NCE = 25 %.

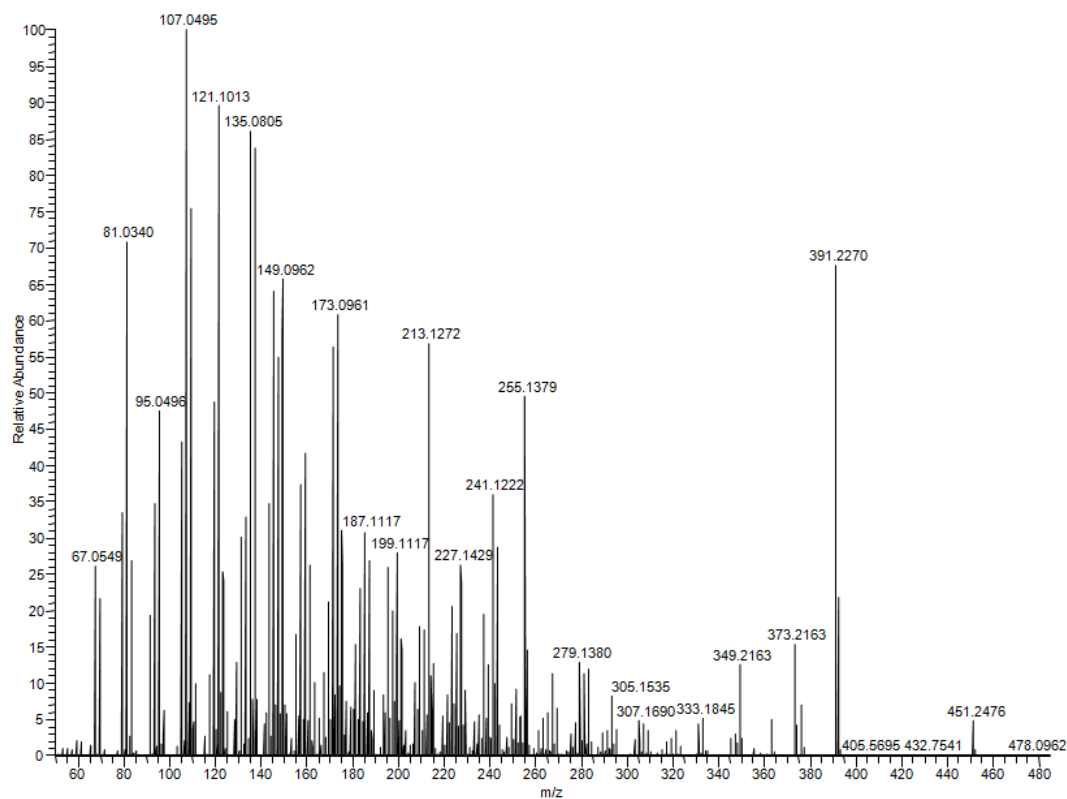


**Fig. S10** ESI-MS/MS spectrum of azadiradione (III) of  $m/z$  451.2 in NCE = 30 %.

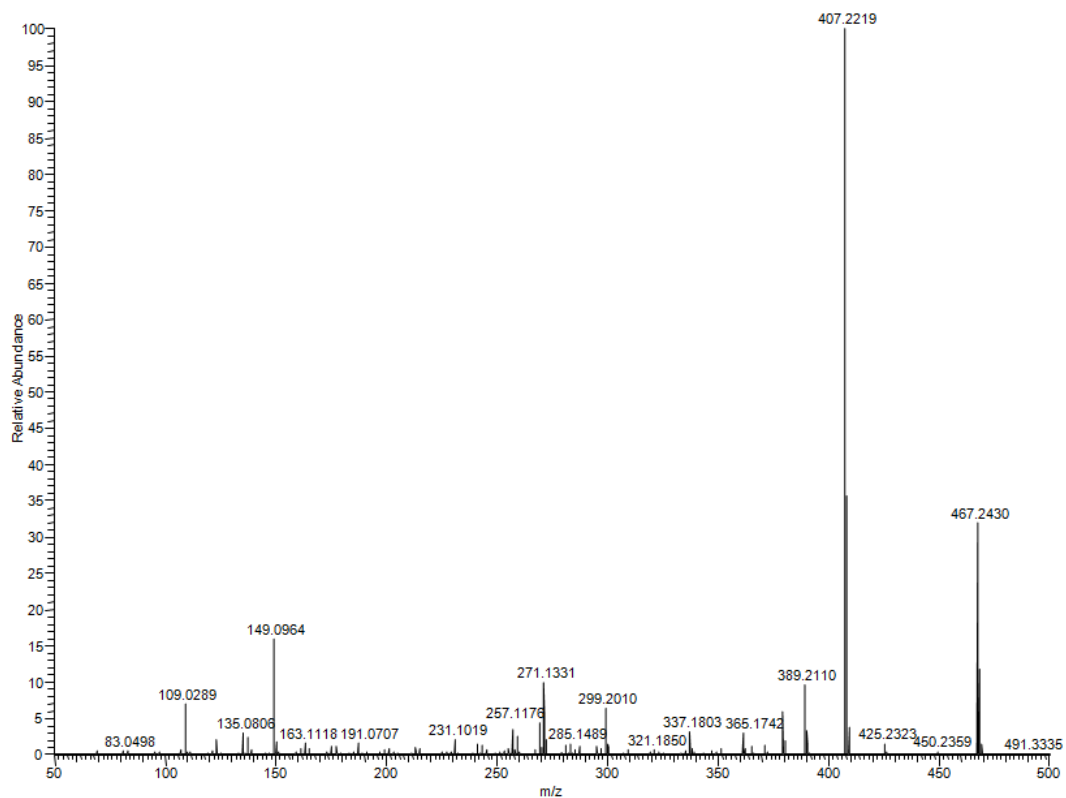




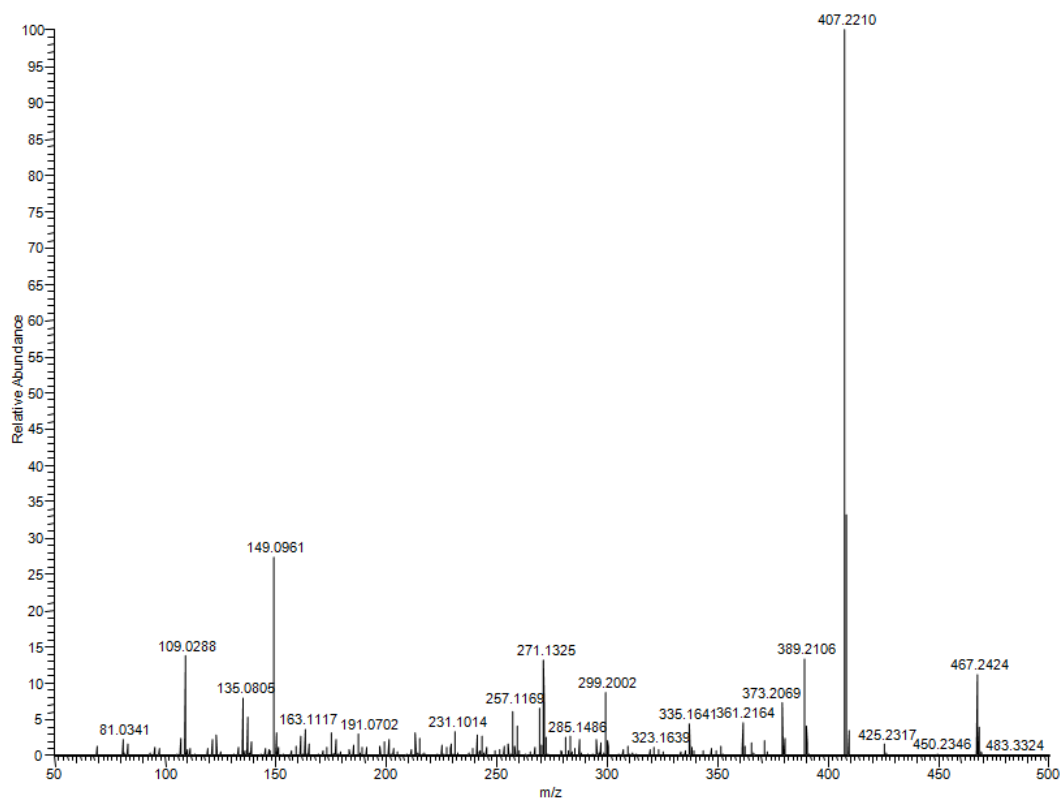
**Fig. S11** ESI-MS/MS spectrum of azadiradione (III) of  $m/z$  451.2 in NCE = 35 %.



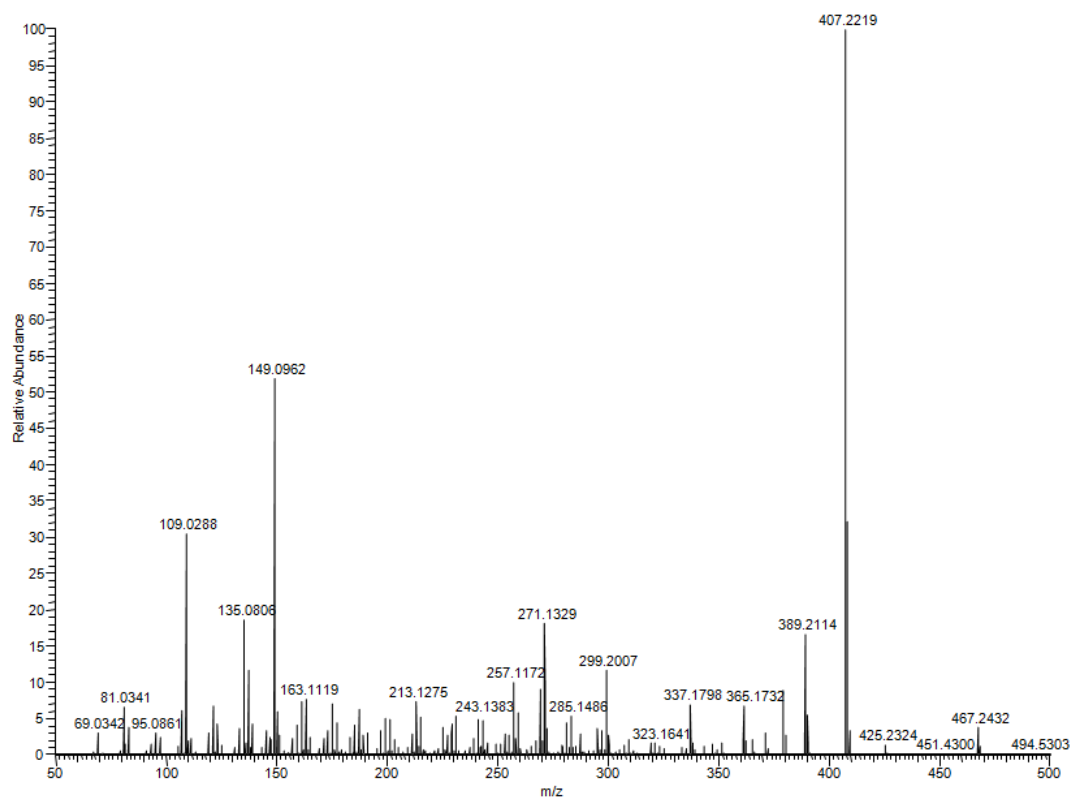
**Fig. S12** ESI-MS/MS spectrum of azadiradione (III) of  $m/z$  451.2 in NCE = 40 %.



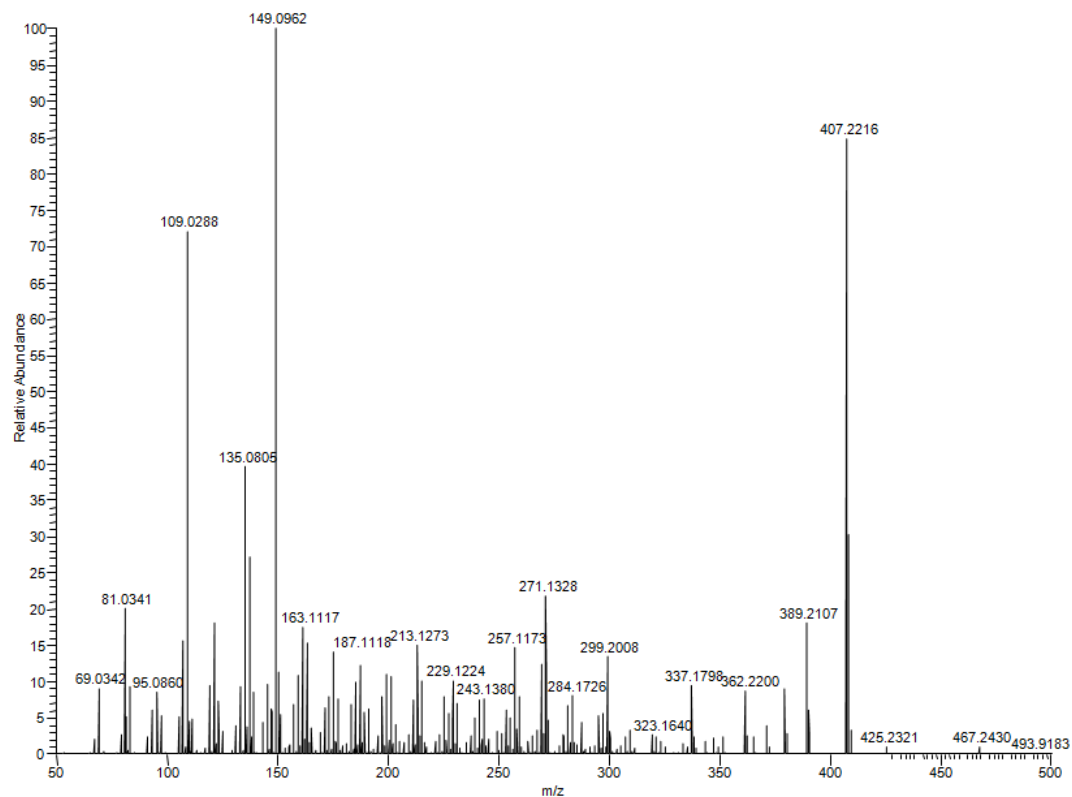
**Fig. S13** ESI-MS/MS spectrum of epoxyazadiradione (II) of  $m/z$  467.2 in NCE = 15 %.



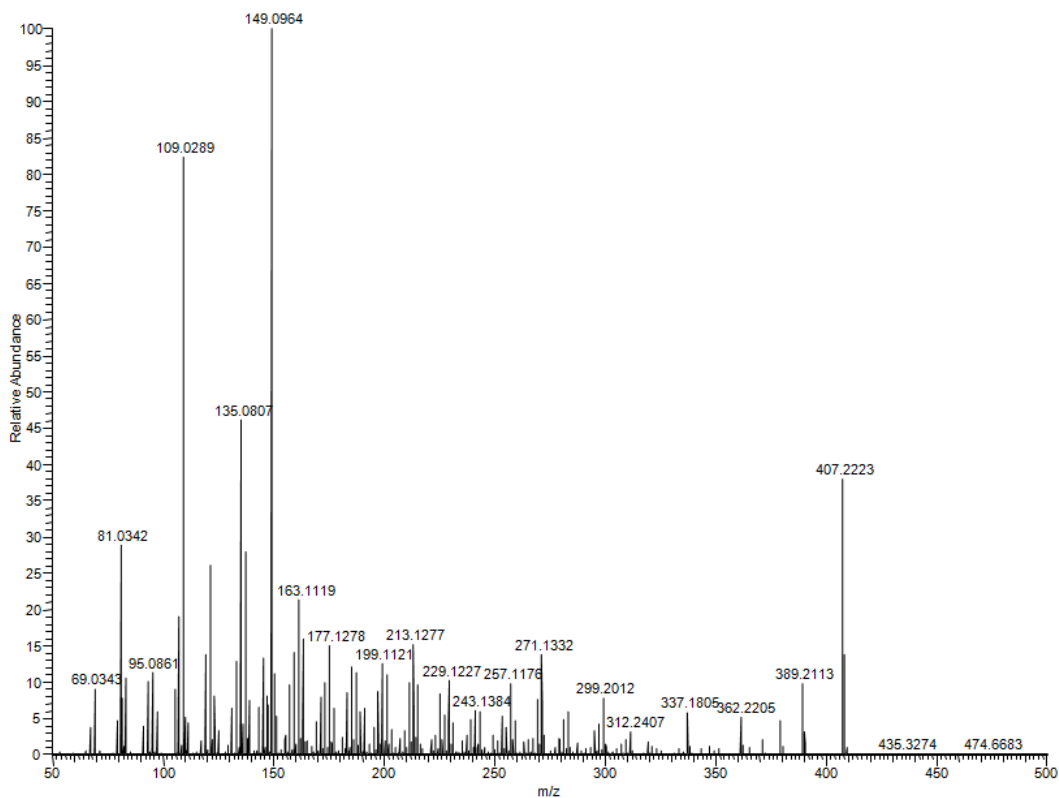
**Fig. S14** ESI-MS/MS spectrum of epoxyazadiradione (II) of  $m/z$  467.2 in NCE = 20 %.



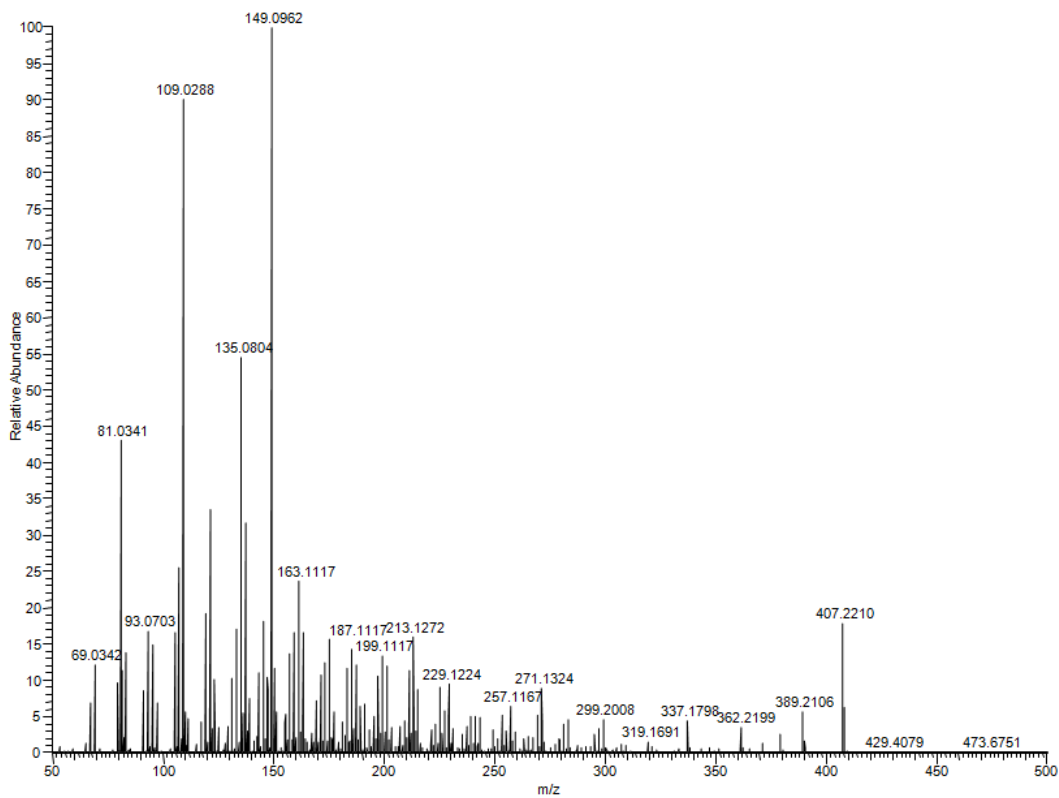
**Fig. S15** ESI-MS/MS spectrum of epoxyazadiradione (II) of  $m/z$  467.2 in NCE = 25 %.



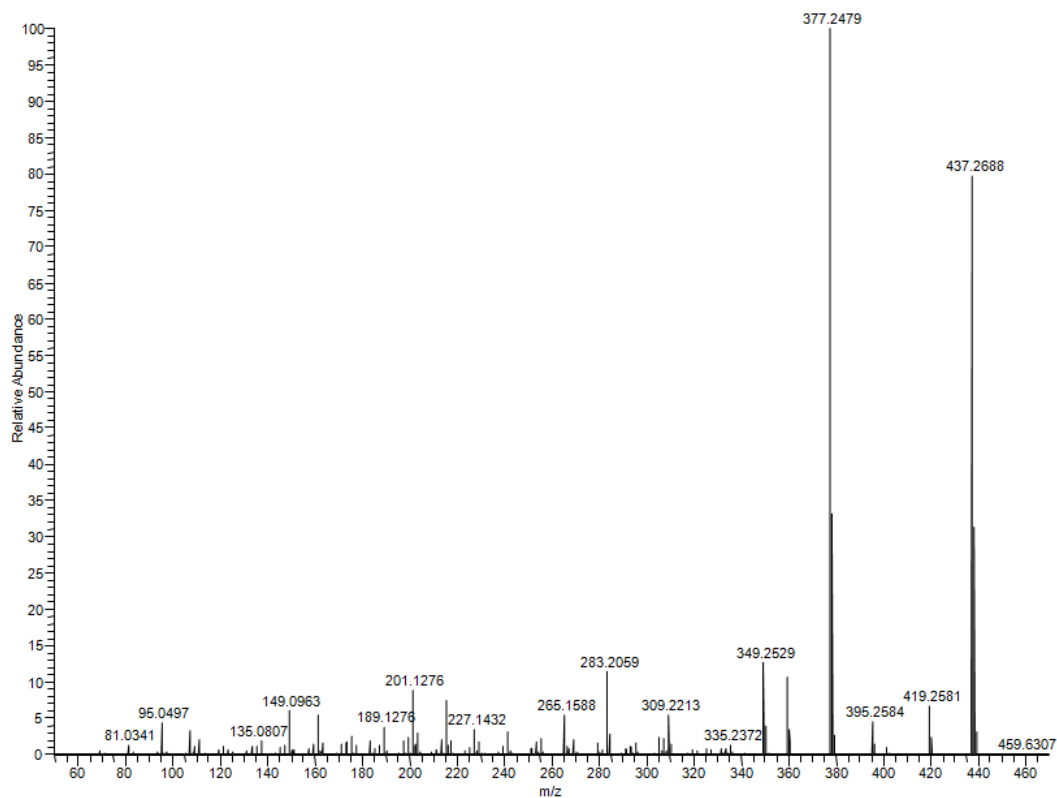
**Fig. S16** ESI-MS/MS spectrum of epoxyazadiradione (II) of  $m/z$  467.2 in NCE = 30 %.



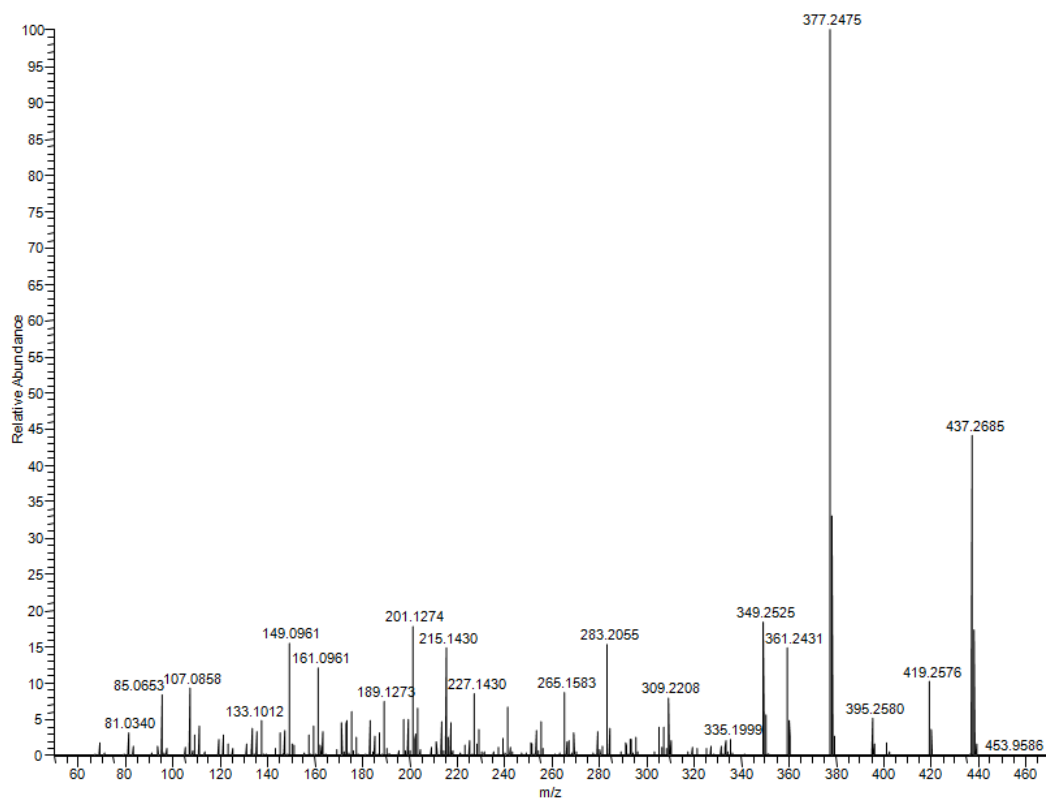
**Fig. S17** ESI-MS/MS spectrum of epoxyazadiradione (II) of  $m/z$  467.2 in NCE = 35 %.



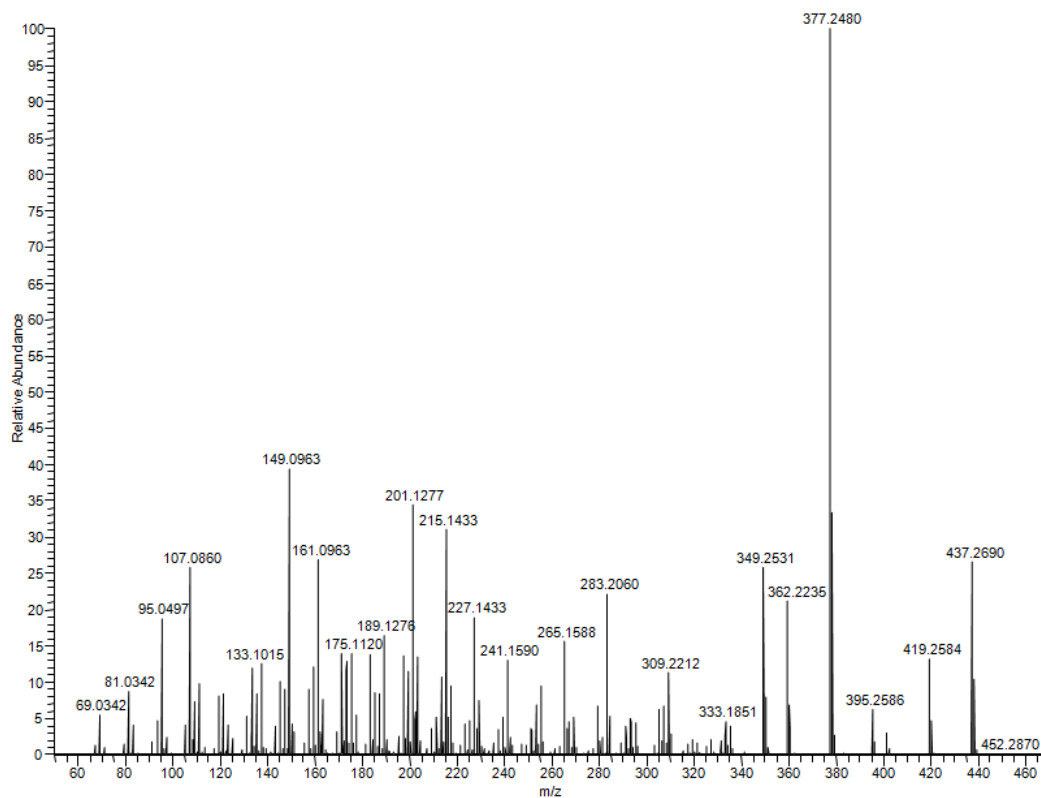
**Fig. S18** ESI-MS/MS spectrum of epoxyazadiradione (II) of  $m/z$  467.2 in NCE = 40 %.



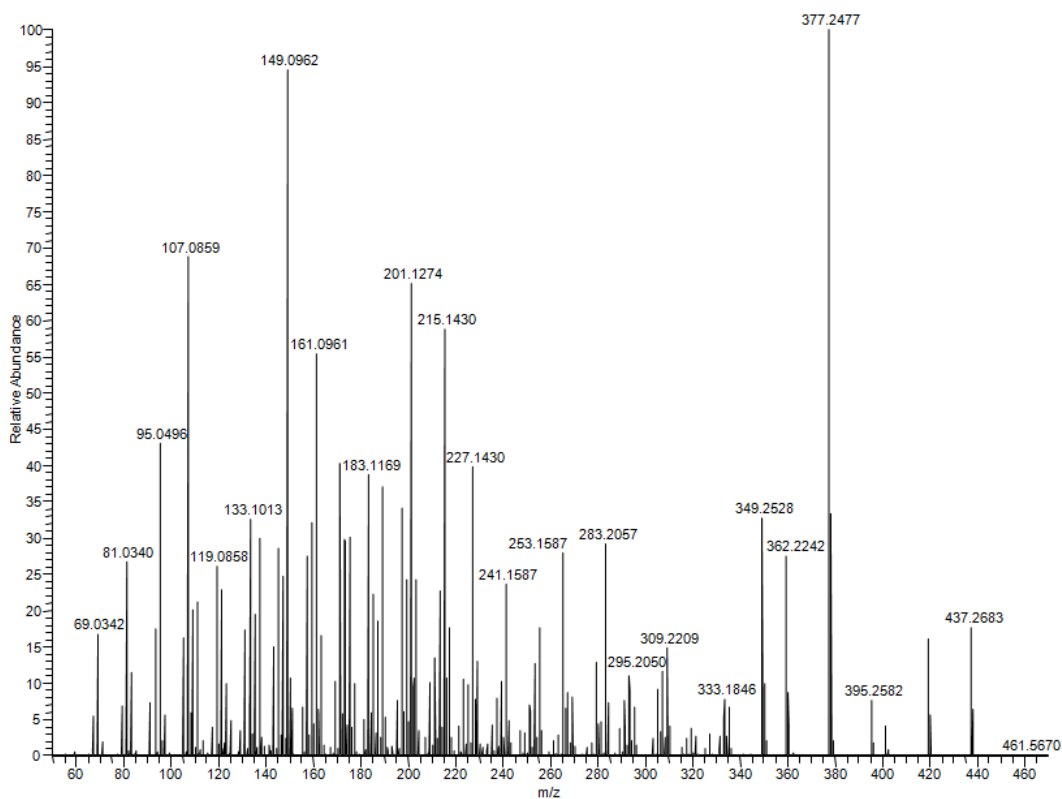
**Fig. S19** ESI-MS/MS spectrum of azadirone (I) of  $m/z$  437.2 in NCE = 15 %.



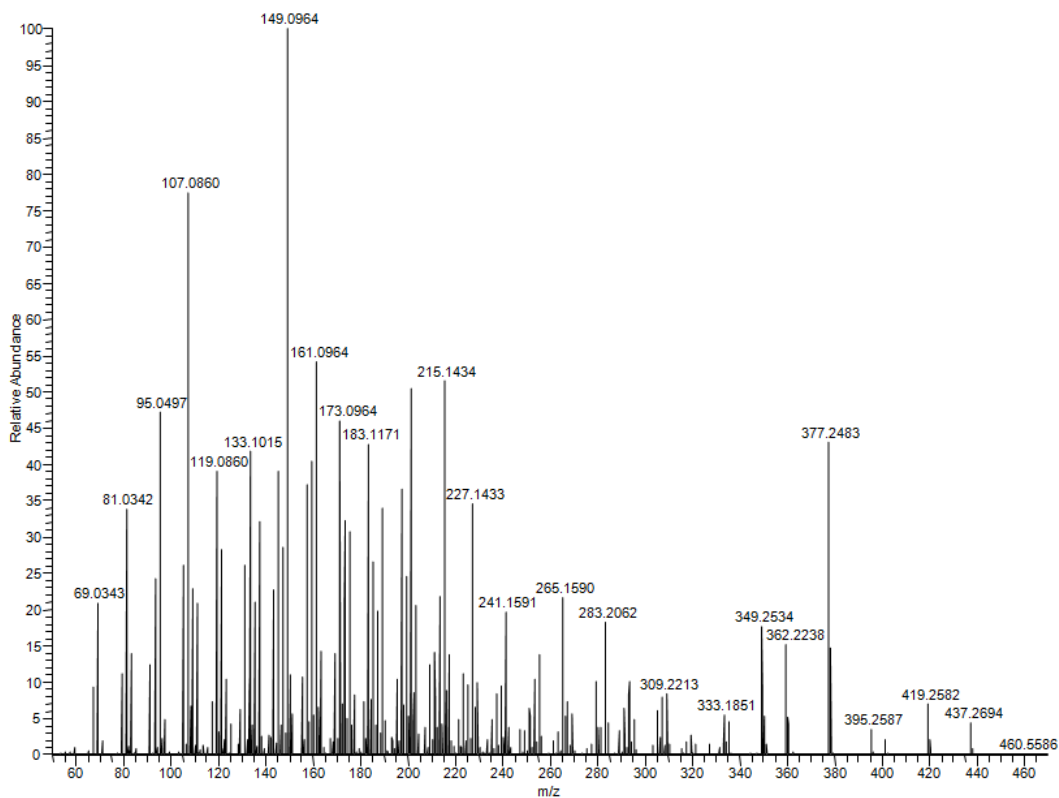
**Fig. S20** ESI-MS/MS spectrum of azadirone (I) of  $m/z$  437.2 in NCE = 20 %.



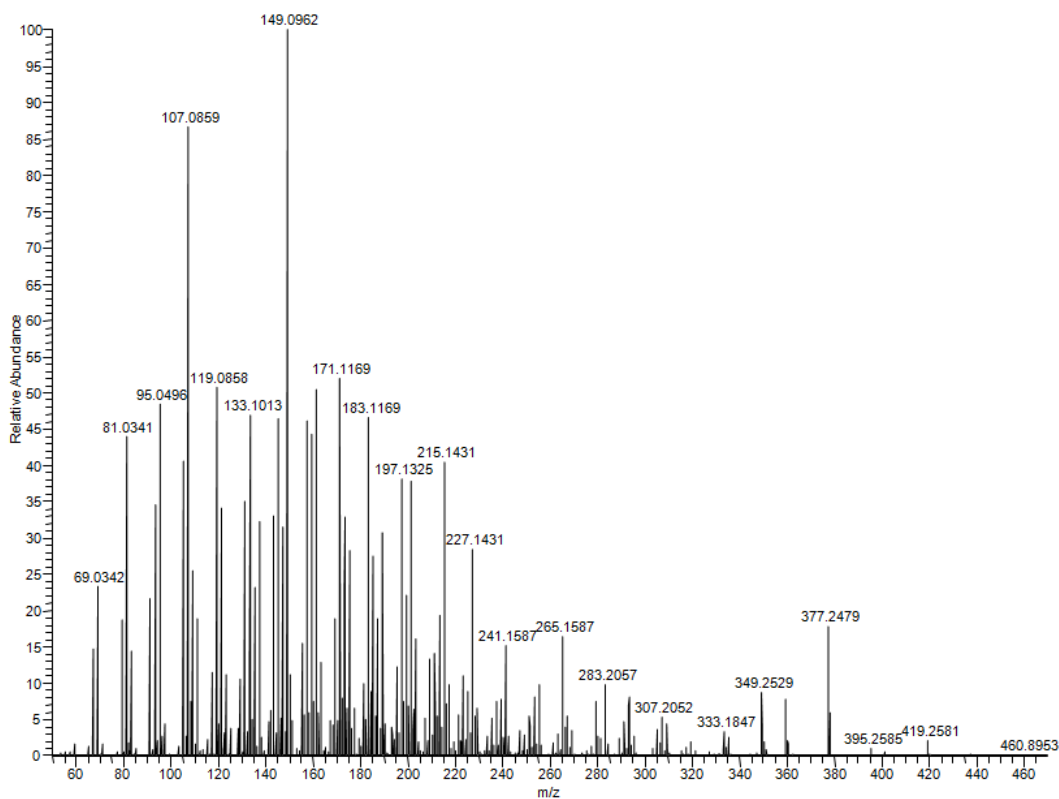
**Fig. S21** ESI-MS/MS spectrum of azadirone (I) of  $m/z$  437.2 in NCE = 25 %.



**Fig. S22** ESI-MS/MS spectrum of azadirone (I) of  $m/z$  437.2 in NCE = 30 %.



**Fig. S23** ESI-MS/MS spectrum of azadirone (I) of  $m/z$  437.2 in NCE = 35 %.



**Fig. S24** ESI-MS/MS spectrum of azadirone (I) of  $m/z$  437.2 in NCE = 40 %.