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# Allelopathic effect of *Wedelia trilobata* L., on the germination and growth of *Cicer arietinum*, *Vigna unguiculata*, and *Vigna radiata* seedlings

S. Shahena, Maya Rajan, Vinaya Chandran, Linu Mathew*

School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.

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**ABSTRACT**

Allelopathy describes a natural process in biotic communities, wherein a plant species suppresses the growth and establishment of neighboring flora. Such plants can be used as sources of allelochemicals to control weeds. The proposed work evaluated the allelopathic activity of *Wedelia trilobata* L., against the seed germination and seedling growth of *Cicer arietinum* (chickpea), *Vigna unguiculata* (cowpea), and *Vigna radiata* (green gram) by the leaf extracts of *W. trilobata* L., by Petri dish bioassay. Inhibition in shoot and root growth and fresh and dry weights was tested by one-way analysis of variance. The aqueous extract of Wedelia significantly inhibited the growth of pulse seedlings with a more pronounced effect at higher concentrations. Further, bioassay-guided fractionation yielded the petroleum ether extract showing maximum growth inhibition indicating the non-polar nature of the allelopathic compound. This extract was further fractionated, and the allelo-compounds were identified by HR-LCMS- MSMS-QTOF analysis as 9-amino-nonanoic acid, colforsin, artelinic acid, Osthol, 4-Nonylphenol, Lagochilin, and Ophiobolin A. The present study suggested a promising tool for controlling weeds through allelopathic compounds isolated from Wedelia, which can be used as natural herbicides that are less disruptive to the ecosystem.

## 1. INTRODUCTION

Higher plants mediate plant to plant eco-physiological interactions by synthesizing secondary metabolites called allelochemicals. When released into the environment from the host plant, they influence the development and growth of its neighbors [1]. These compounds do not hamper the primary metabolism that is essential for the host plants for their survival [2]. They are present in almost all plant tissues of the hosts [3].

Weeds hamper human activity in both crop and non-crop areas. They fight with the standing crop for nutrition and other resources and hinder the overall crop growth and development. Eventually, this causes a qualitative and quantitative reduction in the crop yield [2]. A successful invasion of a weed in any ecosystem is the result of enhanced growth and reproductive potential, adaptive character, and other interferences due to the drop in available resources and, by allelopathy [4,5].

Allelopathy is essential in the plant incursion process. The absence of co-evolved tolerance to new chemicals produced by the aggressors by the existing native vegetation allows these invaders to dominate natural ecosystems [6]. The chemicals or secondary metabolites produced by the host plant responsible for the allelopathic effects are termed as allelochemicals. The allelochemicals may enhance the successful establishment and reproduction of the beneficiaries [7,8]. Root exudation, volatilization, the decay of the plant matter, and leaching of plant foliage are the processes which discharge these allelochemicals to the environment [9,10]. Allelochemicals are bio-communicators since living organisms use them to exchange growth signals [11].

The most important pathway of releasing allele-chemicals is by root exudation. The root exudates determine the bioavailability and phytotoxicity of allelochemicals; by interacting with the inorganic and organic soil components and soil microorganisms [12]. The phytotoxic levels of these compounds in the soil are determined by the prevailing soil conditions and the physical, chemical, and biological transformations of allelochemicals in the soil environment [13-15].

Allelopathy supplies a less laborious and effective biological alternative to chemical and mechanical methods of weed control without affecting the environment adversely [16]. It has presented an alternative for developing eco-friendly agricultural practices, enhancing crop productivity, and maintaining ecosystem stability [10]. Moreover, allelopathic interactions may be significant in ecosystems by influencing weed control and crop productivity [17]. Modern agricultural systems depend on synthetic herbicides to provide protection against weeds which results in

*Corresponding Author: Linu Mathew,
*School of Biosciences, Mahatma Gandhi University,*
*Kottayam - 686 560, Kerala, India.*
*E-mail: linumathew@mgu.ac.in*

severe environmental hazards, including the production of herbicide-resistant plants [18]. Thus, allelopathy can be used as an alternative natural strategy which protects crops against competing plants, and allelochemicals can be used as natural weedicides which are safe to the ecosystems [19].

*Wedelia trilobata* (L.) Hitchc (*Sphagneticola trilobata*) is a perennial evergreen creeper of the family Asteraceae [20]. This plant is a harmful invasive weed with deleterious effects on surrounding plants. The harmful effect of allelopathic compounds of *W. trilobata* to crop plants is due to the presence of various bioactive compounds with extensive biological activities [21]. It was introduced as an ornamental garden plant, and its invasion has now caused significant damage to the natural ecosystem and biodiversity in South India. IUCN (2001) [22] listed *W. trilobata* as one of the worst invasive alien weed. The successful incursion of *W. trilobata* is due to its high reproduction, broad eco-physiological adaptations, and more often, due to its allelopathic effects on adjacent native plants also [23].

The most common procedure of evaluating allelopathy is by studying the inhibition of growth and germination of seedlings of crop plants and weeds by aqueous, alcoholic, or other extracts of the allelopathic plant [24]. Hence, the present study was designed as a preliminary donor-receiver bioassay to decide the possible allelopathic effect of different extracts of leaves of *W. trilobata* against the growth of pulses such as *Cicer arietinum* (chickpea), *V. unguiculata* (cowpea), and *Vigna radiata* (green gram) through growth inhibition and to isolate allelo-compounds from this plant.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Donor Plant

The weed *W. trilobata* was selected as a donor plant. The fresh plants of *W. trilobata* were collected from the Mahatma Gandhi University campus, Kottayam, Kerala, India. The plant was identified, and a voucher specimen (Accession no: 7637) was deposited at Regional Herbarium, Kerala, at St. Berchmans College, Changanassery, Kerala, India.

### 2.2. Selection of Receiver Plant

*C. arietinum* (Chickpea)**,** *V. unguiculata* L. (Cowpea), and *V. radiata* L. (Green gram) were selected as receiver plants. The seeds of these plants were sourced from the local market of Kottayam, Kerala, India.

### 2.3. Preparation of Plant Extract

#### *2.3.1. Aqueous extract for checking the allelopathic effect*

The aqueous extract, prepared from the fresh leaves of the donor plants, was used for the present analysis. After washing thoroughly with tap water, 100 g of the fresh leaves of the *W. trilobata* were hand crushed and soaked in 100 ml distilled water. After keeping at room temperature for 24 h, the extract was filtered through a sieve and considered as 100% stock for further analysis. By diluting the stock with distilled water, other concentrations such as 25%, 50%, and 75% were prepared. Distilled water without the plant extract was used as the control.

#### *2.3.2. Preparation of donor seedlings*

The fresh seeds were washed thoroughly and soaked in tap water overnight at room temperature. Seeds showing signs of germination were used for the analysis.

#### *2.3.3. Germination by Petri-dish bioassay*

Sterile Petri plates (12 cm diameter) were used for the bioassay. A Whatman No.1 filter paper kept on the Petri dish was moistened with the plant extract according to the required concentration. A control moistened with distilled water was also kept. Ten germinating seeds were placed over the Petri plate and grown for 7 days. The plates were wetted daily with the same concentration of plant extract for getting the favorable moisture content for the growing seeds. Only those seeds with complete radical emergence are considered to be fully germinated. The experiments were repeated thrice with the daily recording of the data.

#### *2.3.4. Growth inhibition by organic solvent extracts*

Different solvent extracts were prepared by sequential cold extraction of the dried leaf powder for 48 h. As per Ma *et al*., 2014 [25], growth inhibition assay of germinated seeds by petroleum ether, chloroform, ethyl acetate, and methanol extracts and column chromatographic fractions was done. Briefly, 50 µl of extracts or fractions dissolved in acetone was mixed with 1.0 mL, 0.5% agar gel. The controls contained the same amount of acetone in 0.5% agar gel. Ten germinated seeds of pulses were placed in the agar media and grown in an illumination incubator at 27°C with a photoperiod of 12:12 h for 3–4 days. Treatments were repeated thrice.

### 2.4. Germination Percentage

The percentage of the germination was calculated as follows.

$$\text{Germination \%} = \text{No. of seeds germinated}/\text{ Total no. of seeds sown} \times 100$$

### 2.5. Growth of the Seedlings

The growth of the seedlings was estimated by measuring the shoot and root length of 5 randomly selected plants, and the average values were taken as cm/plant.

### 2.6. Fresh and Dry Weights

Five randomly collected seedlings from the control and treatment groups after 7 days of incubation were assayed for fresh and dry weight. The fresh weight of each sample was taken with the help of an analytical weighing balance and recorded in g/plant. Then, they were placed in a hot air oven at 80°C until the constant dry weight was obtained, which was recorded as g/plant.

### 2.7. Statistical Analysis

The experiments were carried out thrice. The means were expressed as mean ± SE. One-way analysis of variance was done using IBM SPSS Statistics, Version 20. The mean comparison was made by the least significant difference (LSD) at $p \leq 0.05$.

### 2.8. Extraction of Active Allelo-compounds

The petroleum ether extract was subjected to column fractionation with silica gel as the stationary phase and hexane:ethyl acetate (3:1) as mobile phase. The fractions (C1to C-13) were further subjected to seed germination bioassay. The fraction showing the highest inhibition was analyzed by HR LCMS- MSMS- QTOF analysis (Syncronis C18 100 × 2.1, particle size 1.7 u).

## 3. RESULTS AND DISCUSSION

Allelopathy plays a fundamental part in the maintenance of biodiversity and the natural resource base of vulnerable ecosystems. Furthermore,

it has roles in sustainable agriculture by expanding the range of weed management practices.

The Petri dish bioassay of the aqueous leaf extract of *W. trilobata* showed that the highly invasive weed *W. trilobata* is a promising allelopathic plant as they inhibit seed germination in selected pulse varieties such as *C. arietinum*, *V. unguiculata,* and *V. radiata*. *W. trilobata* extracts significantly inhibited seed germination [Figure 1-3]. They also reduced shoot and root length and fresh and dry weight of the pulse seedlings, which was comparable to the inhibition by the extracts of *Eucalyptus* [26] and *Tridax procumbens* L. [2]. Allelopathy is positively associated with other environmental stresses such as pests and diseases, extremes of temperature, nutrient starvation, and water stress, and stress due to radiation and herbicides. Such stressed circumstances frequently boost the production of allelochemicals and potential for allelopathic interference. This makes the host plant more successful in a stressed environment [27].

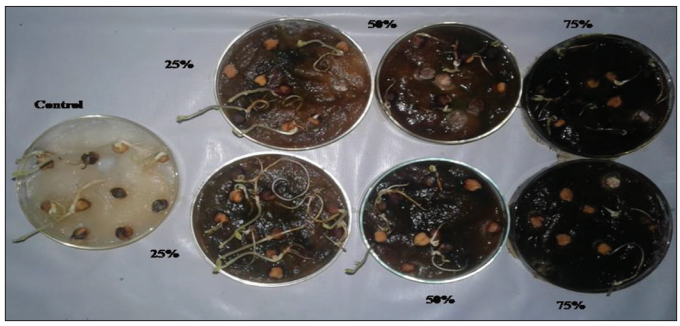



**Figure 1:** Allelopathic effect of *Wedelia trilobata* on seed germination of *Cicer arietinum*.

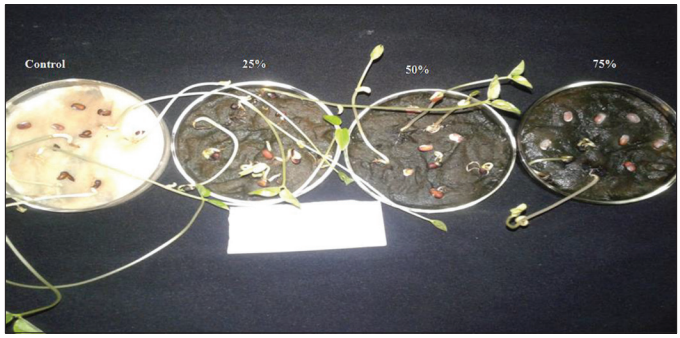



**Figure 2:** Allelopathic effect of *Wedelia trilobata* on seed germination of *Vigna unguiculata*.

### 3.1. Seed Germination

The germination of the *C. arietinum*, *V. unguiculata,* and *V. radiata* was significantly affected by the *W. trilobata* extract with more pronounced inhibition at high concentrations. Among the three varieties, the germination of the *C. arietinum* seeds showed the highest inhibition against the water extract in every tested concentration [Figure 4]. *V. unguiculata* showed a moderate level of inhibition, and *V. radiata* was the least inhibited as compared to other varieties in a concentration-dependent manner. A steady decrease in germination percentage in the presence of *W. trilobata* extracts was noticed from low to high concentrations in comparison to control, indicating the concentration-dependent nature of inhibition.

Germination percentage is considered to be an excellent indicator for the detection of allelopathic potential [28]. It is also used for the assessment of the effects of chemical compounds in the laboratory or the field [29]. The process of seed germination is complex and involves sequential changes in biochemistry, physiology, and morphology of the seedlings. Disruption of these changes results in hampering proper germination and growth. The presence or absence of some chemical compounds mediates this inhibition [28]. The leaf extracts of *Conocarpus lancifolius* (Engl.) held up germination and growth of *Vigna sinensis* L. and *Zea mays* L. in a concentration-dependent manner [30]. Furthermore, the leaf extracts of *Brassica nigra* [31], *Eucalyptus camaldulensis* [32], *Acacia auriculiformis* [33], and alfalfa [34] inhibited the germination and growth of some crops.

The strength of the aqueous extract had a significant role in declining water uptake essential for seed germination [30]. The osmotic stress imparted by the leaf extracts might lead to the differences in water uptake by the seedlings [35]. Germination was high in the control and treatments with low extract concentrations. Hence, water uptake might have played a role in lessening the germination inhibition due to the low osmotic potential of the extracts at low concentrations. A great many metabolic and physiological changes during seed germination could be related to water uptake [36]. Even though the biochemical processes inhibiting the germination were not clearly defined, allelochemicals in the leaf extract might have a role in increasing respiration and hydrolytic enzyme activity leading to low cell division and embryo enlargement [37].

### 3.2 Shoot and Root Length

The fresh leaf extracts of *W. trilobata* L. significantly affected the shoot and root lengths of *C. arietinum, V. unguiculata*, and *V. radiata* seedlings [Table 1]. The lowest shoot and root lengths were recorded in

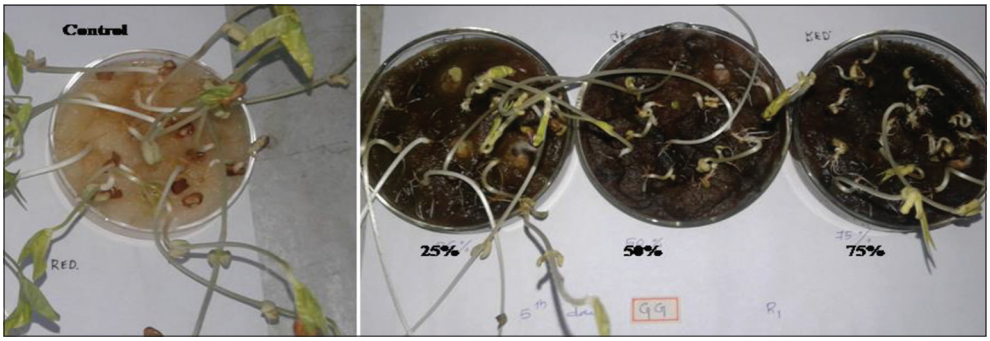



**Figure 3:** Allelopathic effect of *Wedelia trilobata* on seed germination of *Vigna radiate*.

*C. arietinum,* and the highest values were in *V. radiata*. The inhibition was found to be purely in a concentration-dependent manner. Moreover, the aqueous leaf extracts of *Tectona grandis* L. inhibited the seed germination and seedling growth of *Vigna mungo* (L.) [38]. Here, it is postulated that the aqueous Wedelia leaf extract might have affected the root and root related metabolic activities, leading to a decrease in the healthy shoot growth by denying water and nutrients. Furthermore, it might have restricted the photosynthetic activities, with a resultant lack of resources and energy needed for normal growth. Moreover, leaf etiolation and nanism were reported in plants treated with *W. trilobata* extracts [39].

The treated pulse seedlings possessed characteristic variations in their root length as compared to the control plants. Some seedlings showed a high specific root length in comparison to the shoot length, and some others had stunted roots. Reports suggested that these are characteristics of plants growing in sterile environments or being exposed to environmental stress [40].

### 3.3. Fresh and Dry Weight

After 7 days of treatment, the extracts from the fresh leaves of the *W. trilobata* L. decreased the fresh and dry weight of *C. arietinum*, *V. unguiculata,* and *V. radiata* [Table 2]. Even though significant reductions were found in all tested concentrations, the effect was concentration and species-dependent. The highest inhibition was shown by 75% of the extract concentration, and the least inhibition was at 25%. Growth inhibition was the least in *V. radiata*. The inhibition was found to be the highest in *C. arietinum* and *V. unguiculata* showed a moderate level of inhibition. The reduction in metabolic activities of the aerial and underground plant parts might have caused a substantial drop in the fresh and dry weights of the treated seedlings. The leaf extracts of *Tephrosia purpurea*, *Albizia amara,* and *Delonix regia* caused a decrease in the biomass of maize [41], and the aqueous extracts of the leaves of *T. grandis* caused a concentration-dependent reduction in the biomass of *V. mungo* [38].

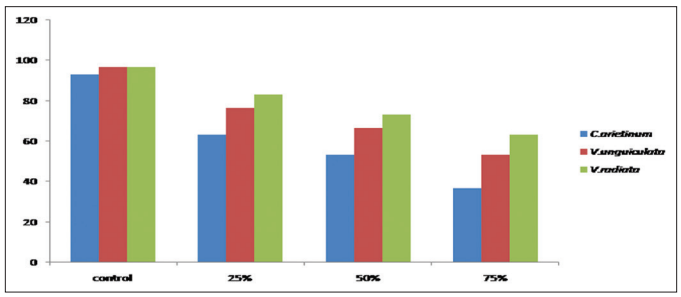



**Figure 4:** Graph bars represent allelopathic effect of different concentrations of *Wedelia trilobata* extract on the percentage of seed germination in *Cicer arietinum, Vigna unguiculata,* and *Vigna radiata.*

The germination and growth are considered as the outcome of cell division. Flavonoids and phenolic compounds in the *Zygophyllum album* had an inhibitory effect on cell division and held-up mitosis [42]. Moreover, the water extracts of *Z. simplex* L. suppressed the production of DNA and nucleoproteins of *Vicia faba* L. [43]. Furthermore, high concentrations of *Aloe vera* L. extract significantly altered the cell division and growth rate of *Allium cepa* L. [44]. Here also, we can surmise that Wedelia extract might have inhibited the growth of pulse seedlings by suppressing mitosis in the donor plant.

### 3.4. Extraction of Active Allelo-compounds

High metabolic rate is the characteristic feature of seedling growth; therefore, it is highly susceptible to allelopathy [45,46]. Petroleum ether extract significantly reduced germination percentage and the growth rate of the *C. arietinum* as compared to other extracts. All the tested parameters such as shoot and root lengths and fresh and dry weights were affected by the petroleum ether extract [Figures 5-8]. The rate of inhibition was in the order petroleum ether < chloroform < ethyl acetate < methanol. The non-polar nature of the inhibitor was evidenced by the high inhibition shown by the petroleum ether and chloroform extracts [47].

The allelopathic activities of *Ampelocissus latifolia* (Roxb.) Planch. leaf extracts correlated qualitatively and quantitatively with the useful phytochemicals and the solvents used for the extraction [46]. Here, the column fraction C3 was inhibitory as compared to other fractions in seed germination bioassay

**Table 1**: The effects of aqueous leaf extract of *Wedelia trilobata* on shoot length and root length of *C. arietinum, V. unguiculata,* and *V. radiata.*

| Treatment | *C. arietinum* | | *V. unguiculata* | | *V. radiate* | |
|---|---|---|---|---|---|---|
| | Shoot length (cm) Mean± Std. Error | Root length (cm) Mean± Std. Error | Shoot length (cm) Mean± Std. Error | Root length (cm) Mean± Std. Error | Shoot length (cm) Mean± Std. Error | Root length (cm) Mean± Std. Error |
| Control | 4.2200±0.5054$^{a}$ | 2.6400±0.3171$^{a}$ | 17.3400±2.0114$^{a}$ | 4.9000±0.3066$^{a}$ | 23.3000±0.8602$^{a}$ | 6.1800±0.2615$^{a}$ |
| | 4.2200±0.5054$^{a}$ | 3.1200±0.2245$^{a}$ | 18.4600±1.9472$^{a}$ | 5.0600±0.4833$^{a}$ | 21.600±0.7218$^{a}$ | 5.1600±0.5046$^{a}$ |
| | 4.9000±0.1975$^{a}$ | 2.5000±0.2000$^{a}$ | 15.3600±1.5481$^{a}$ | 6.3400±0.5409$^{a}$ | 19.5800±1.5907$^{a}$ | 6.8400±1.0755$^{a}$ |
| 25% | 2.2800±0.4212$^{bc}$ | 1.2000±0.2214$^{bc}$ | 10.6800±1.9787$^{bc}$ | 3.2600±0.1030$^{b}$ | 15.9000±0.6626$^{b}$ | 3.8600±.12884$^{b}$ |
| | 2.1800±0.2478$^{b}$ | 1.8000±0.3507$^{b}$ | 13.3400±1.7348$^{b}$ | 3.3200±0.2782$^{b}$ | 14.3600±0.8358$^{b}$ | 3.7000±0.5550$^{b}$ |
| | 2.0400±0.1600$^{b}$ | 1.5600±0.1600$^{b}$ | 10.0200±1.0538$^{b}$ | 3.7800±0.5953$^{b}$ | 13.9000±1.5959$^{b}$ | 4.6600±0.4885$^{b}$ |
| 50% | 1.2400±0.2874$^{c}$ | 0.8000±0.1414$^{cd}$ | 6.5000±1.4188$^{c}$ | 2.1000±0.0707$^{c}$ | 10.9800±0.5142$^{c}$ | 2.6000±0.1140$^{c}$ |
| | 1.0400±0.0510$^{c}$ | 0.9800±0.2177$^{c}$ | 8.2000±0.9940$^{c}$ | 1.9000±0.2569$^{c}$ | 8.2000±0.9940$^{c}$ | 1.9600±0.2839$^{c}$ |
| | 0.8800±0.0374$^{c}$ | 0.9200±0.0490$^{c}$ | 5.7000±0.8149$^{c}$ | 2.0600±0.3641$^{c}$ | 8.6400±1.0357$^{c}$ | 3.8200±0.2596$^{bc}$ |
| 75% | 0.4200±0.1241$^{cd}$ | 0.2000±0.0633$^{d}$ | 3.9800±1.0205$^{cd}$ | 1.1400±0.1749$^{d}$ | 7.6400±0.2874$^{d}$ | 1.3200±0.0800$^{d}$ |
| | 0.1800±0.0374$^{d}$ | 0.3400±0.0678$^{c}$ | 3.9000±0.4278$^{d}$ | 0.7800±0.0735$^{d}$ | 5.5000±0.2168$^{d}$ | 1.2000±0.1897$^{c}$ |
| | 0.2800±0.0860$^{d}$ | 0.2800±0.0583$^{d}$ | 1.9000±0.3317$^{d}$ | 0.9400±0.1691$^{c}$ | 3.8400±0.7243$^{d}$ | 2.1600±0.4226$^{c}$ |

The table represents mean of three replicates ± SE between the two treated groups at $P<0.05$ level. The mean with same letter is not significantly different according to Duncan's multiple range test ($P\leq0.05$). *C. arietinum: Cicer arietinum, V. unguiculata: Vigna unguiculata, V. radiata: Vigna radiata*

**Table 2**: The effects of aqueous leaf extract of *Wedelia trilobata* on fresh and dry weight of *C. arietinum, V. unguiculata,* and *V. radiata.*

| Treatment | *C. arietinum* | | *V. unguiculata* | | *V .radiata* | |
|---|---|---|---|---|---|---|
| | Fresh weight (g) Mean± Std. Error | Dry weight (g) Mean± Std. Error | Fresh weight (g) Mean± Std. Error | Dry weight (g) Mean± Std. Error | Fresh weight (g) Mean± Std. Error | Dry weight (g) Mean± Std. Error |
| Control | 1.0260±0.0906$^{a}$ | 0.1273±0.0297$^{a}$ | 1.4620±0.0679 $^{a}$ | 0.6995±0.1679 $^{a}$ | 1.4480±0.0652 $^{a}$ | 0.9367±0.0387 $^{a}$ |
| | 0.8440±0.1254$^{a}$ | 0.0531±0.0100$^{a}$ | 1.2980±0.0967$^{a}$ | 0.0876±0.0084 $^{a}$ | 1.6620±0.0590 $^{a}$ | 0.4035±0.1661 $^{a}$ |
| | 0.9760±0.0765$^{a}$ | 0.0871±0.0045$^{a}$ | 1.4440±0.1366$^{a}$ | 0.1174±0.0301$^{a}$ | 1.7580±0.0750$^{a}$ | 0.3702±0.1792$^{a}$ |
| 25% | 0.7100±0.0405$^{b}$ | 0.0555±0.0091$^{b}$ | 1.0040±0.0722$^{b}$ | 0.1080±0.0330$^{b}$ | 1.1400±0.0648$^{b}$ | 0.2415±0.0911$^{b}$ |
| | 0.4460±0.0686$^{bc}$ | 0.0325±0.0064$^{b}$ | 0.9620±0.1516$^{b}$ | 0.0535±0.0068$^{b}$ | 0.9160±0.1067$^{b}$ | 0.1035±0.0355$^{b}$ |
| | 0.6660±0.0378$^{b}$ | 0.0534±0.0032$^{b}$ | 0.6780±0.0524$^{b}$ | 0.0620±0.0070$^{b}$ | 0.8900±0.0939$^{b}$ | 0.0774±0.0169$^{b}$ |
| 50% | 0.5740±0.0573$^{b}$ | 0.0255±0.0038$^{b}$ | 0.7040±0.0689$^{c}$ | 0.0518±0.0096$^{b}$ | 0.8920±0.0273$^{c}$ | 0.1080±0.0535$^{bc}$ |
| | 0.2800±0.0709$^{c}$ | 0.0196±0.0020$^{bc}$ | 0.5560±0.1175$^{c}$ | 0.0363±0.0045$^{b}$ | 0.6500±0.0930$^{c}$ | 0.0459±0.0106$^{b}$ |
| | 0.3540±0.05432$^{c}$ | 0.0277±0.0058$^{c}$ | 0.5460±0.0665$^{b}$ | 0.0305±0.0090$^{b}$ | 0.5200±0.0478$^{c}$ | 0.0367±0.0145$^{b}$ |
| 75% | 0.3080±0.0570$^{c}$ | 0.0117±0.0008$^{b}$ | 0.4400±0.0559$^{d}$ | 0.0263±0.0052$^{b}$ | 0.6060±0.0480$^{d}$ | 0.0255±0.0073$^{cd}$ |
| | 0.0520±0.0180$^{cd}$ | 0.0133±0.0013$^{c}$ | 0.1700±0.0354$^{d}$ | 0.0159±0.0039$^{c}$ | 0.3320±0.0546$^{d}$ | 0.0139±0.0015$^{b}$ |
| | 0.1800±0.0164$^{d}$ | 0.0172±0.0043$^{c}$ | 0.2060±0.0223$^{c}$ | 0.0164±0.0027$^{b}$ | 0.4200±0.0966$^{c}$ | 0.0190±0.0007$^{b}$ |

The table represents mean of three replicates ± SE between the two treated groups at $P<0.05$ level. The mean with same letter is not significantly different according to Duncan's multiple range test ($P\leq0.05$). *C. arietinum: Cicer arietinum, V. unguiculata: Vigna unguiculata, V. radiata: Vigna radiata*

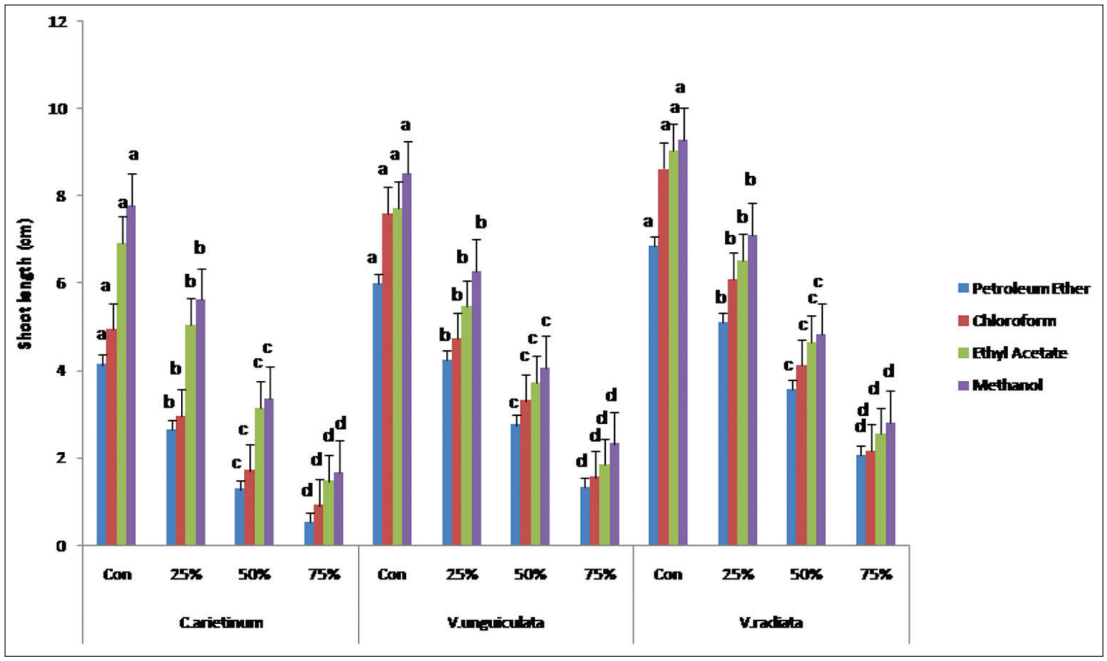



**Figure 5:** Graph bars represent allelopathic effect of concentrations of solvent extracts of *Wedelia trilobata* on shoot length of *Cicer arietinum*, *Vigna unguiculata,* and *Vigna radiata.* The figure represents mean of three replicates ± SE between the treated groups at $P < 0.05$ level. The mean with same letter is not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

[Figure 9]. HRLCMS- MSMS- QTOF analysis based metabolite profiling of petroleum ether column fraction C3 of *W. trilobata* detected around 100 compounds by positive ionization and 35 compounds by negative ionization with seven potentially active secondary metabolites with allelopathic effect [Supplementary Tables: Tables S1 and S2]. They include 9-amino-nonanoic acid, colforsin, artelinic acid, Osthol, 4-Nonylphenol (4-NP), Lagochilin, and Ophiobolin A [Table 3].

9-aminononanoic acid is an omega-amino fatty acid: Nonanoic acid substituted by an amino group at position 9. It is a metabolite derived from nonanoic acid. *Cleome viscosa* root exudates showed the presence of lactam nonanoic acid. The aqueous solution of this purified compound inhibited the growth of rice, gram, and mustard seeds in a concentration-dependent manner [48].

*Cistus ladanifer* is an allelopathic and autoallelopathic species due to the presence of flavonoids and diterpenes in the leaf exudates [49]. Colforsin is a water-soluble derivative of forskolin. Forskolin (coleonol), produced by the Indian Coleus plant (*Plectranthus barbatus*), is a labdane diterpene, and lagochilin is a bitter diterpene compound.

Artenilic acid is the semi-synthetic derivative of the natural compound artemisinin, which is used as a drug for the treatment

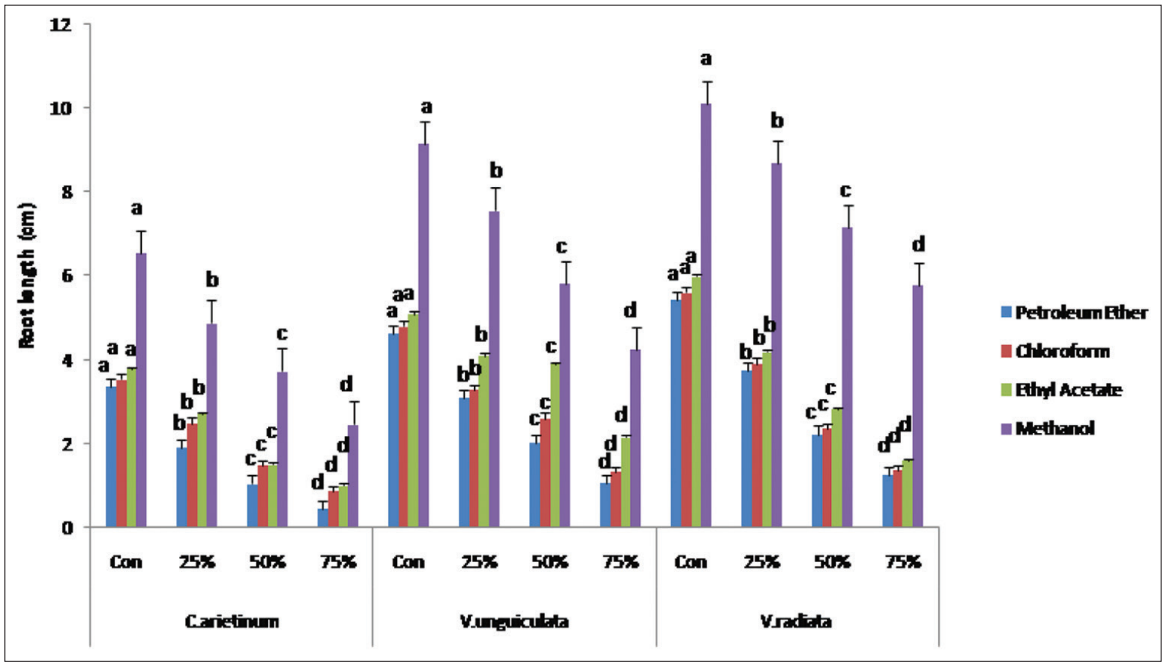



**Figure 6:** Graph bars represent allelopathic effect of concentrations of solvent extract of *Wedelia trilobata* on root length of *Cicer arietinum*, *Vigna unguiculata*, and *Vigna radiata.* The figure represents mean of three replicates ± SE between the treated groups at $P < 0.05$ level. The mean with same letter is not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

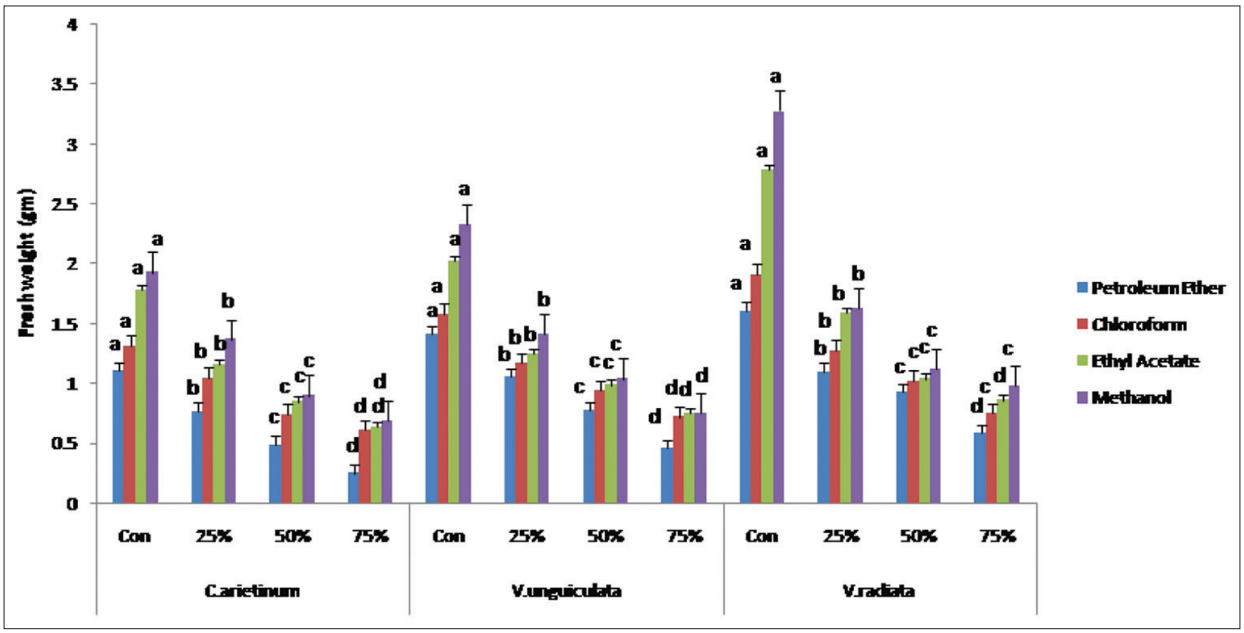



**Figure 7:** Graph bars represent allelopathic effect of concentrations of solvent extract of *Wedelia trilobata* on fresh weight of *Cicer arietinum*, *Vigna unguiculata,* and *Vigna radiata.* The figure represents mean of three replicates ± SE between the treated groups at $P < 0.05$ level. The mean with same letter is not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

of Malaria. It is obtained from *Artemisia annua* L. Large amounts of this antimalarial compound are released into soil ecosystems by rainwater leachate, root exudates, and decomposition of plant materials. It can inhibit plant growth around *A. annua* L. and microbial growth in soils [50].

Osthol is a derivative of coumarin. The leaf extracts of *Gliricidia sepium* (Fabaceae) inhibited the growth of lettuce (*Lactuca sativa*) radicles due to the presence of coumarins [51].

4-NP is an anthropogenic contaminant influencing the biotic and abiotic environmental factors [52].

Ophiobolin A found in the water extract and ethanolic extract of *W. trilobata* is a phytotoxic sesterpene ($C_{25}$) with potential as a natural herbicide against grass weeds [21,53].

The 9-amino-nonanoic acid, colforsin, artelinic acid, Osthol, 4-NP, Lagochilin, and Ophiobolin A in C3 column fraction of petroleum ether

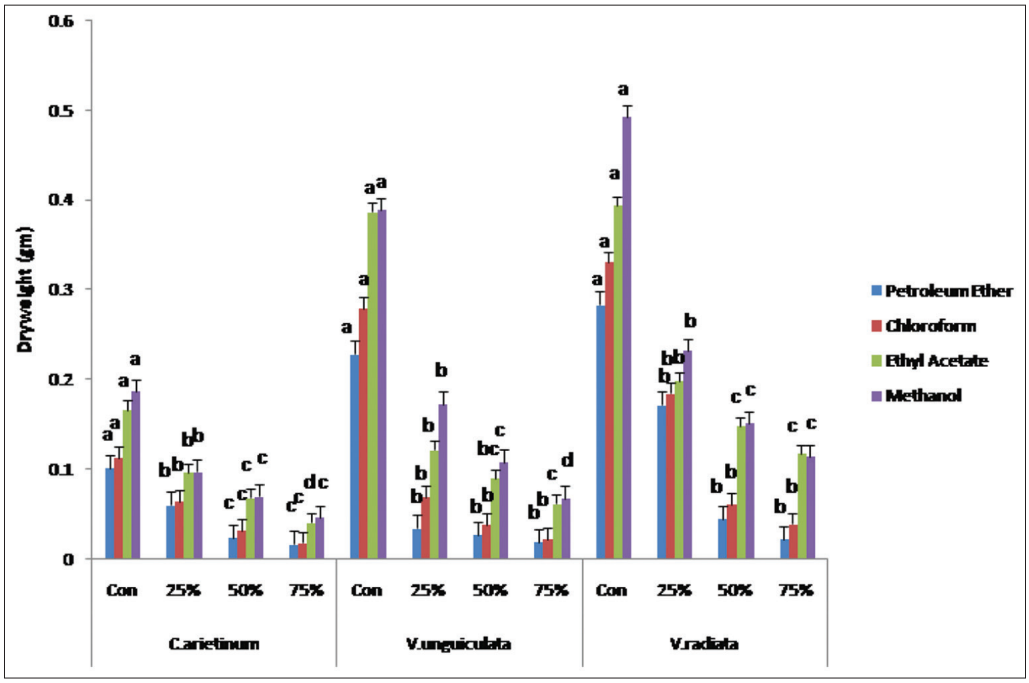



**Figure 8:** Graph bars represent allelopathic effect of concentrations of solvent extract of *Wedelia trilobata* on dry weight of *Vigna arietinum*, *Vigna unguiculata,* and *Vigna radiata.* The figure represents mean of three replicates ± SE between the treated groups at $P < 0.05$ level. The mean with same letter is not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

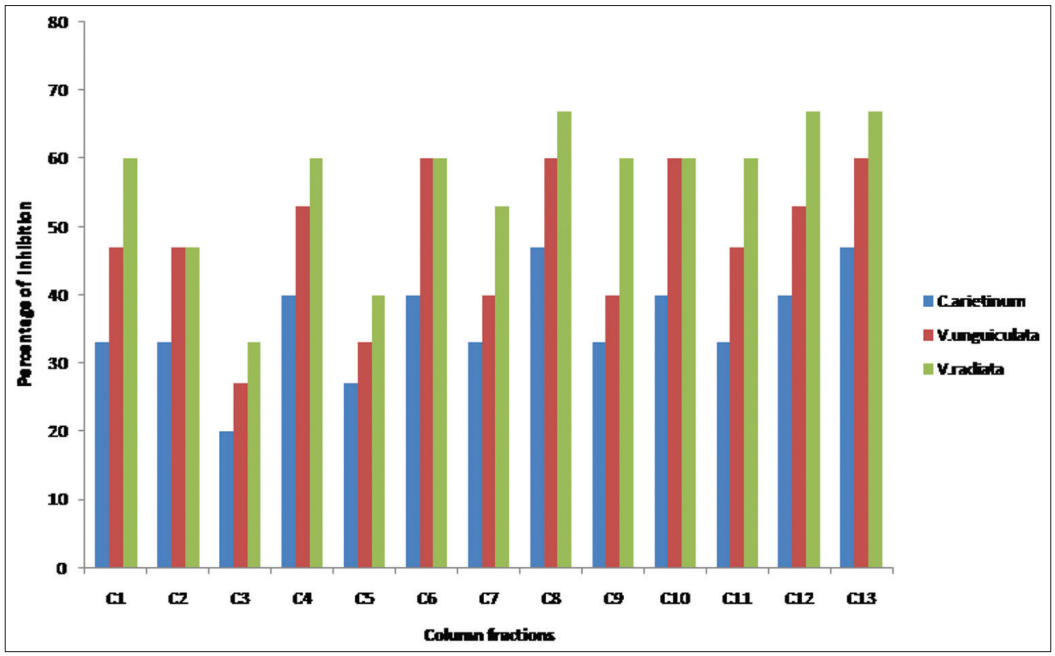
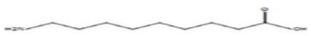
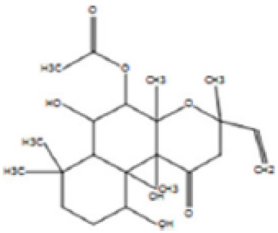
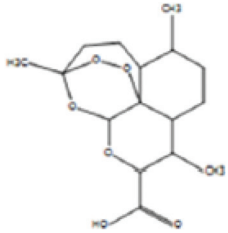
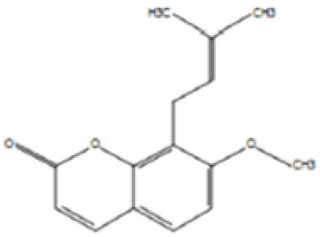
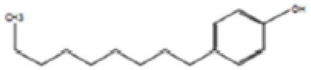
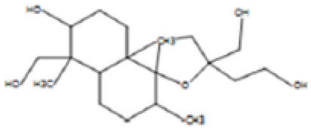
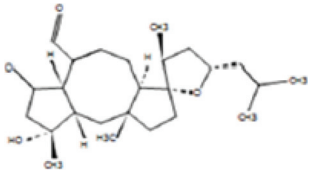



**Figure 9:** Graph bars represent allelopathic effect of column fractions of *Wedelia trilobata* extract on percentage of seed germination in *Cicer arietinum*, *Vigna unguiculata,* and *Vigna radiata.*

extract of *W. trilobata* reveals the presence of diterpenes, sesterpenes, coumarins, and various other metabolites and the presence and the combined effect of these compounds might be the reason for the allelopathy of *W. trilobata.*

The suppressive outcome of allelochemicals is remarkably reliant on their concentrations [54-56]. The contribution of chemical compounds in the allelopathy is prominent. Hence, the higher concentration of these chemical compounds results in a more pronounced effect on the target plant species. It is difficult to isolate the effect of a single chemical compound and blame it for growth hang-up as the allelopathy process is complicated [57]. Besides, this process may accompany other ecological influences like the microbial and chemical nature of the soil [58,59].

**Table 3**: Lists of compounds isolated by HR LCMS- MSMS- QTOF from C3 fraction of petroleum ether extract of *Wedelia trilobata.*

| Sl. No. | Compound name | Compound structure |
|---|---|---|
| 1. | 9-amino-nonanoic acid |  |
| 2. | Colforsin |  |
| 3. | Artelinic acid |  |
| 4. | Osthol |  |
| 5. | 4-Nonylphenol |  |
| 6. | Lagochilin |  |
| 7. | Ophiobolin A |  |

## 4. CONCLUSION

The present study indicated that even though all the tested pulse seedlings were inhibited by different *W. trilobata* extracts, they could bear stress up to a certain extent. Nevertheless, as the extract concentrations increased, a significant reduction in the tested parameters occurred in a concentration-dependent manner. The detrimental effects of the extracts indicated that the allelochemicals present in any concentration in the soil or as soil mulch could decrease the overall yield of the affected crops. The inhibitory effects are enhanced at high concentrations of the extracts, rather due to the combined effects of the compounds constituting the extract than a distinct component.

The presence of diterpene, sesterpenes, and coumarin derivatives along with sesquiterpene lactones was identified in the extracts of Wedelia leaves by HRLCMS- MS/MS- Q-TOF analysis. These compounds might have resulted in the allelopathic effect of *W. trilobata* L. These compounds can be used for monitoring allelopathic interactions of *W. trilobata* and the presence of Osthol, a coumarin derivative, is reported for the 1st time in the present study. Allelochemicals functions as biomarkers and they can be used for assessing allelopathic interactions. The study of the molecular mechanism of growth inhibition by these compounds is the prospect.

## 5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## 7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 9. PUBLISHER'S NOTE

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## SUPPLEMENTARY TABLES

**Table S1**: List of compounds obtained from positive ionization of HRLCMS- MSMS- QTOF analysis.

| Compound label | RT | Mass | Abund | Name | Formula | MFG Formula | DB Formula | DB Diff (pp) | Hits (DB) |
|---|---|---|---|---|---|---|---|---|---|
| 9-amino-nonanoic acid | 1.097 | 173.1404 | 1099110 | 9-amino-nonanoic acid | $C_9 H_{19} N O_2$ | $C_9 H_{19} N O_2$ | $C_9 H_{19} N O_2$ | 6.63 | 2 |
| N-Ethylaniline | 1.213 | 121.0884 | 1615922 | N-Ethylaniline | $C_8 H_{11} N$ | $C_8 H_{11} N$ | $C_8 H_{11} N$ | 5.89 | 4 |
| Dihydrodeoxystreptomycin | 9.01 | 567.2868 | | Dihydrodeoxystreptomycin | $C_{21} H_{41} N_7 O_{11}$ | $C_{21} H_{41} N_7 O_{11}$ | $C_{21} H_{41} N_7 O_{11}$ | –0.7 | 4 |
| C16 Sphinganine | 9.863 | 273.2654 | 656625 | C16 Sphinganine | $C_{16} H_{35} N O_2$ | $C_{16} H_{35} N O_2$ | $C_{16} H_{35} N O_2$ | 4.97 | 1 |
| Desmethyl pirenzepine | 10.072 | 337.1589 | 107611 | Desmethyl pirenzepine | $C_{18} H_{19} N_5 O_2$ | $C_{18} H_{19} N_5 O_2$ | $C_{18} H_{19} N_5 O_2$ | –14.88 | 10 |
| Droperidol | 10.121 | 379.169 | | Droperidol | $C_{22} H_{22} F N_3 O_2$ | $C_{22} H_{22} F N_3 O_2$ | $C_{22} H_{22} F N_3 O_2$ | 1.68 | 10 |
| Gln Ala Tyr | 10.143 | 380.1705 | 7832424 | Gln Ala Tyr | $C_{17} H_{24} N_4 O_6$ | $C_{17} H_{24} N_4 O_6$ | $C_{17} H_{24} N_4 O_6$ | –2.46 | 10 |
| Droperidol | 10.447 | 379.1668 | | Droperidol | $C_{22} H_{22} F N_3 O_2$ | $C_{22} H_{22} F N_3 O_2$ | $C_{22} H_{22} F N_3 O_2$ | 7.52 | 10 |
| Droperidol | 10.811 | 379.1672 | 334484 | Droperidol | $C_{22} H_{22} F N_3 O_2$ | $C_{22} H_{22} F N_3 O_2$ | $C_{22} H_{22} F N_3 O_2$ | 6.32 | 10 |
| Colforsin | 12.266 | 410.2308 | 1559726 | Colforsin | $C_{22} H_{34} O_7$ | $C_{22} H_{34} O_7$ | $C_{22} H_{34} O_7$ | –0.94 | 10 |
| 12-oxo-9-octadecynoic acid | 12.37 | 294.2205 | | 12-oxo-9-octadecynoic acid | $C_{18} H_{30} O_3$ | $C_{18} H_{30} O_3$ | $C_{18} H_{30} O_3$ | –3.26 | 10 |
| Dihydrojasmonic acid, methyl ester | 12.909 | 226.1557 | 619762 | Dihydrojasmonic acid, methyl ester | $C_{13} H_{22} O_3$ | $C_{13} H_{22} O_3$ | $C_{13} H_{22} O_3$ | 5.27 | 10 |
| 12-oxo-9-octadecynoic acid | 12.37 | 294.2205 | 406966 | 12-oxo-9-octadecynoic acid | $C_{18} H_{30} O_3$ | $C_{18} H_{30} O_3$ | $C_{18} H_{30} O_3$ | –3.26 | 10 |
| Granisetron metabolite 4 | 13.173 | 314.1796 | 135159 | Granisetron metabolite 4 | $C_{17} H_{22} N_4 O_2$ | $C_{17} H_{22} N_4 O_2$ | $C_{17} H_{22} N_4 O_2$ | –17.02 | 10 |
| Hydrocortisone caproate | 13.318 | 460.2826 | | Hydrocortisone caproate | $C_{27} H_{40} O_6$ | $C_{27} H_{40} O_6$ | $C_{27} H_{40} O_6$ | –0.27 | 4 |
| 15d-PGA2 | 13.39 | 316.2027 | 1729506 | 15d-PGA2 | $C_{20} H_{28} O_3$ | $C_{20} H_{28} O_3$ | $C_{20} H_{28} O_3$ | 3.69 | 10 |
| 8Z,11Z,14Z-heptadecatrienal | 13.488 | 248.2154 | 2535271 | 8Z,11Z,14Z-heptadecatrienal | $C_{17} H_{28} O$ | $C_{17} H_{28} O$ | $C_{17} H_{28} O$ | –5.52 | 2 |
| 5,4'-Dimethoxy-7-hydroxyflavone | 13.489 | 298.0828 | 637712 | 5,4'-Dimethoxy-7-hydroxyflavone | $C_{17} H_{14} O_5$ | $C_{17} H_{14} O_5$ | $C_{17} H_{14} O_5$ | 4.43 | 10 |
| Carbetapentane | 13.492 | 333.2289 | 2335442 | Carbetapentane | $C_{20} H_{31} N O_3$ | $C_{20} H_{31} N O_3$ | $C_{20} H_{31} N O_3$ | 4.46 | 1 |
| 19-Oxotestosterone | 13.582 | 288.2078 | | 19-Oxotestosterone | $C_{19} H_{28} O_2$ | $C_{19} H_{28} O_2$ | $C_{19} H_{28} O_2$ | 4 | 10 |
| Estradiol methyl ether | 13.645 | 286.1923 | | Estradiol methyl ether | $C_{19} H_{26} O_2$ | $C_{19} H_{26} O_2$ | $C_{19} H_{26} O_2$ | 3.48 | 8 |
| 19-Oxo-all-trans-retinoic acid | 13.983 | 314.187 | 188984 | 19-Oxo-all-trans-retinoic acid | $C_{20} H_{26} O_3$ | $C_{20} H_{26} O_3$ | $C_{20} H_{26} O_3$ | 3.76 | 10 |
| GPGro(8:0/8:0)[U] | 14.02 | 498.2598 | 493247 | GPGro(8:0/8:0)[U] | $C_{22} H_{43} O_{10} P$ | $C_{22} H_{43} O_{10} P$ | $C_{22} H_{43} O_{10} P$ | –0.74 | 10 |
| Atenolol | 14.068 | 266.1636 | 1412268 | Atenolol | $C_{14} H_{22} N_2 O_3$ | $C_{14} H_{22} N_2 O_3$ | $C_{14} H_{22} N_2 O_3$ | –2.11 | 10 |
| 12-oxo-9-octadecynoic acid | 14.116 | 294.2206 | 946469 | 12-oxo-9-octadecynoic acid | $C_{18} H_{30} O_3$ | $C_{18} H_{30} O_3$ | $C_{18} H_{30} O_3$ | –3.64 | 10 |
| Trp Lys Val | 14.116 | 431.2534 | 902068 | Trp Lys Val | $C_{22} H_{33} N_5 O_4$ | $C_{22} H_{33} N_5 O_4$ | $C_{22} H_{33} N_5 O_4$ | –0.44 | 10 |
| Artelinic acid | 14.164 | 418.1978 | 734247 | Artelinic acid | $C_{23} H_{30} O_7$ | $C_{23} H_{30} O_7$ | $C_{23} H_{30} O_7$ | 3.3 | 10 |
| Osthol | 14.389 | 244.1089 | | Osthol | $C_{15} H_{16} O_3$ | $C_{15} H_{16} O_3$ | $C_{15} H_{16} O_3$ | 4.17 | 8 |
| 15-deoxy-delta-12,14-PGD2 | 14.393 | 334.2133 | 294544 | 15-deoxy-delta-12,14-PGD2 | $C_{20} H_{30} O_4$ | $C_{20} H_{30} O_4$ | $C_{20} H_{30} O_4$ | 3.35 | 10 |
| Lys Pro Trp | 14.442 | 429.2372 | | Lys Pro Trp | $C_{22} H_{31} N_5 O_4$ | $C_{22} H_{31} N_5 O_4$ | $C_{22} H_{31} N_5 O_4$ | 0.88 | 8 |
| Arg Phe Asn | 14.485 | 435.2237 | 971069 | Arg Phe Asn | $C_{19} H_{29} N_7 O_5$ | $C_{19} H_{29} N_7 O_5$ | $C_{19} H_{29} N_7 O_5$ | –1.51 | 10 |
| 6-ethyl-tetradecanoic acid | 14.526 | 256.2413 | 1026313 | 6-ethyl-tetradecanoic acid | $C_{16} H_{32} O_2$ | $C_{16} H_{32} O_2$ | $C_{16} H_{32} O_2$ | –4.23 | 10 |
| Leu Gln Arg | 14.526 | 415.2574 | 1344677 | Leu Gln Arg | $C_{17} H_{33} N_7 O_5$ | $C_{17} H_{33} N_7 O_5$ | $C_{17} H_{33} N_7 O_5$ | –7.46 | 10 |
| Dihydrotetrabenazine glucuronide | 14.529 | 495.2456 | | Dihydrotetrabenazine glucuronide | $C_{25} H_{37} N O_9$ | $C_{25} H_{37} N O_9$ | $C_{25} H_{37} N O_9$ | 2.51 | 8 |

(*Contd...*)

**Table S1:** (*Continued*)

| Compound label | RT | Mass | Abund | Name | Formula | MFG Formula | DB Formula | DB Diff (pp) | Hits (DB) |
|---|---|---|---|---|---|---|---|---|---|
| 4-hydroxy palmitic acid | 14.754 | 272.2362 | 671477 | 4-hydroxy palmitic acid | $C_{16}H_{32}O_3$ | $C_{16}H_{32}O_3$ | $C_{16}H_{32}O_3$ | –3.7 | 10 |
| 1-Pyrrolidinepropanol, a-(4-hydroxycyclohexyl)-a-phenyl-,cis-glucuronide | 14.844 | 479.2498 | 349875 | 1-Pyrrolidinepropanol, a-(4-hydroxycyclohexyl)-a-phenyl-,cis-glucuronide | $C_{25}H_{37}NO_8$ | $C_{25}H_{37}NO_8$ | $C_{25}H_{37}NO_8$ | 4.51 | 10 |
| Trp Ile Lys | 14.844 | 445.2689 | 575188 | Trp Ile Lys | $C_{23}H_{35}N_5O_4$ | $C_{23}H_{35}N_5O_4$ | $C_{23}H_{35}N_5O_4$ | –0.03 | 10 |
| Lys Tyr Trp | 14.921 | 495.2508 | | Lys Tyr Trp | $C_{26}H_{33}N_5O_5$ | $C_{26}H_{33}N_5O_5$ | $C_{26}H_{33}N_5O_5$ | –5.39 | 10 |
| Mifepristone (RU 486 | 15.078 | 429.2734 | 540376 | Mifepristone (RU 486 | $C_{29}H_{35}NO_2$ | $C_{29}H_{35}NO_2$ | $C_{29}H_{35}NO_2$ | –15.41 | 5 |
| 9S,10-epoxy-10,12Zoctadecadienoic acid | 15.126 | 294.2184 | 719238 | 9S,10-epoxy-10,12Zoctadecadienoic acid | $C_{18}H_{30}O_3$ | $C_{18}H_{30}O_3$ | $C_{18}H_{30}O_3$ | 3.74 | 10 |
| 8,13-dihydroxy-9,11-octadecadienoic acid | 15.175 | 312.2289 | 648056 | 8,13-dihydroxy-9,11-octadecadienoic acid | $C_{18}H_{32}O_4$ | $C_{18}H_{32}O_4$ | $C_{18}H_{32}O_4$ | 3.62 | 10 |
| Trp Ile Lys | 15.227 | 445.2681 | | Trp Ile Lys | $C_{23}H_{35}N_5O_4$ | $C_{23}H_{35}N_5O_4$ | $C_{23}H_{35}N_5O_4$ | 1.91 | 10 |
| Benzoquinoneacetic acid | 15.329 | 166.0257 | 1529767 | Benzoquinoneacetic acid | $C_8H_6O_4$ | $C_8H_6O_4$ | $C_8H_6O_4$ | 5.75 | 8 |
| Lactone of PGF-MUM | 15.367 | 296.1612 | 2444385 | Lactone of PGF-MUM | $C_{16}H_{24}O_5$ | $C_{16}H_{24}O_5$ | $C_{16}H_{24}O_5$ | 3.84 | 4 |
| Mifepristone (RU 486) | 15.461 | 429.273 | 1844522 | Mifepristone (RU 486) | $C_{29}H_{35}NO_2$ | $C_{29}H_{35}NO_2$ | $C_{29}H_{35}NO_2$ | –14.48 | 5 |
| 4-Nonylphenol | 15.602 | 220.1818 | | 4-NONYLPHENOL | $C_{15}H_{24}O$ | $C_{15}H_{24}O$ | $C_{15}H_{24}O$ | 4.14 | 5 |
| Lactone of PGF-MUM | 15.647 | 296.1612 | 1012282 | Lactone of PGF-MUM | $C_{16}H_{24}O_5$ | $C_{16}H_{24}O_5$ | $C_{16}H_{24}O_5$ | 4.11 | 4 |
| Mibefradil | 15.933 | 495.2873 | 327819 | Mibefradil | $C_{29}H_{38}FN_3O_3$ | $C_{29}H_{38}FN_3O_3$ | $C_{29}H_{38}FN_3O_3$ | 4.94 | 1 |
| Benzoquinoneacetic acid | 16.075 | 166.0257 | 1036299 | Benzoquinoneacetic acid | $C_8H_6O_4$ | $C_8H_6O_4$ | $C_8H_6O_4$ | 5.7 | 8 |
| Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxocyclopentyl]-octanoate | 16.117 | 310.1769 | 1570376 | Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate | $C_{17}H_{26}O_5$ | $C_{17}H_{26}O_5$ | $C_{17}H_{26}O_5$ | 3.49 | 1 |
| 5a,7a-Dihydroxy-11-ketotetranorprostanoic acid | 16.263 | 300.1948 | | 5a,7a-Dihydroxy-11-ketotetranorprostanoic acid | $C_{16}H_{28}O_5$ | $C_{16}H_{28}O_5$ | $C_{16}H_{28}O_5$ | –3.67 | 4 |
| Benzoquinoneacetic acid | 16.438 | 166.0257 | 1022176 | Benzoquinoneacetic acid | $C_8H_6O_4$ | $C_8H_6O_4$ | $C_8H_6O_4$ | 5.48 | 8 |
| GPEtnNMe(18:1(9Z)/18:1(9Z)) | 16.482 | 757.559 | 958354 | GPEtnNMe(18:1(9Z)/18:1(9Z)) | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | 4.11 | 6 |
| CRUSTECDYSONE | 16.484 | 480.3093 | | CRUSTECDYSONE | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | –1.2 | 3 |
| methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate | 16.489 | 310.1769 | 497571 | methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate | $C_{17}H_{26}O_5$ | $C_{17}H_{26}O_5$ | $C_{17}H_{26}O_5$ | 3.63 | 1 |
| Ser Tyr Arg | 16.617 | 424.2059 | 811359 | Ser Tyr Arg | $C_{18}H_{28}N_6O_6$ | $C_{18}H_{28}N_6O_6$ | $C_{18}H_{28}N_6O_6$ | 2.7 | 10 |
| Asp Arg Ile | 16.661 | 402.2241 | | Asp Arg Ile | $C_{16}H_{30}N_6O_6$ | $C_{16}H_{30}N_6O_6$ | $C_{16}H_{30}N_6O_6$ | –3.62 | 10 |
| GPGro(18:0/18:1(9Z))[U] | 16.661 | 776.5736 | 466792 | GPGro(18:0/18:1(9Z))[U] | $C_{42}H_{81}O_{10}P$ | $C_{42}H_{81}O_{10}P$ | $C_{42}H_{81}O_{10}P$ | –21.68 | 3 |
| GPEtn(17:0/18:0)[U] | 16.704 | 733.5616 | | GPEtn(17:0/18:0)[U] | $C_{40}H_{80}NO_8P$ | $C_{40}H_{80}NO_8P$ | $C_{40}H_{80}NO_8P$ | 0.73 | 10 |
| GPEtnNMe(18:1(9Z)/18:1(9Z)) | 16.842 | 757.5591 | 638279 | GPEtnNMe(18:1(9Z)/18:1(9Z)) | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | 3.99 | 6 |
| CRUSTECDYSONE | 16.847 | 480.3091 | | CRUSTECDYSONE | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | –0.92 | 3 |
| Emedastine | 16.891 | 302.2106 | | Emedastine | $C_{17}H_{26}N_4O$ | $C_{17}H_{26}N_4O$ | $C_{17}H_{26}N_4O$ | 0.23 | 3 |
| 26,26,26,27,27,27-hexafluoro-1alpha,24-dihydroxyvitaminD3 / 26,26,26,27,27,27-hexafluoro-1alpha,24 | 17.078 | 524.2725 | | 26,26,26,27,27,27-hexafluoro-1alpha,24-dihydroxyvitaminD3 / 26,26,26,27,27,27-hexafluoro-1alpha,24 | $C_{27}H_{38}F_6O_3$ | $C_{27}H_{38}F_6O_3$ | $C_{27}H_{38}F_6O_3$ | 0.07 | 4 |

(*Contd...*)

**Table S1:** (*Continued*)

| Compound label | RT | Mass | Abund | Name | Formula | MFG Formula | DB Formula | DB Diff (pp) | Hits (DB) |
|---|---|---|---|---|---|---|---|---|---|
| (Z)-N-(2-hydroxyethyl)icos-11-enamide | 17.122 | 353.328 | | (Z)-N-(2-hydroxyethyl)icos-11-enamide | $C_{22}H_{43}NO_2$ | $C_{22}H_{43}NO_2$ | $C_{22}H_{43}NO_2$ | 3.84 | 1 |
| Crustecdysone | 17.21 | 480.3091 | | Crustecdysone | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | –0.74 | 1 |
| Emedastine | 17.255 | 302.2107 | | Emedastine | $C_{17}H_{26}N_4O$ | $C_{17}H_{26}N_4O$ | $C_{17}H_{26}N_4O$ | –0.25 | 8 |
| (Z)-N-(2-hydroxyethyl)icos-11-enamide | 17.499 | 353.3279 | 1205775 | (Z)-N-(2-hydroxyethyl)icos-11-enamide | $C_{22}H_{43}NO_2$ | $C_{22}H_{43}NO_2$ | $C_{22}H_{43}NO_2$ | 4.31 | 1 |
| Anandamide (20:2, n-6) | 17.564 | 351.3124 | | Anandamide (20:2, n-6) | $C_{22}H_{41}NO_2$ | $C_{22}H_{41}NO_2$ | $C_{22}H_{41}NO_2$ | 3.68 | 1 |
| Idebenone | 17.565 | 338.208 | 1728222 | Idebenone | $C_{19}H_{30}O_5$ | $C_{19}H_{30}O_5$ | $C_{19}H_{30}O_5$ | 3.8 | 4 |
| Crustecdysone | 17.565 | 480.3089 | | CRUSTECDYSONE | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | $C_{27}H_{44}O_7$ | –0.46 | 3 |
| Khivorin | 17.832 | 586.2789 | | Khivorin | $C_{32}H_{42}O_{10}$ | $C_{32}H_{42}O_{10}$ | $C_{32}H_{42}O_{10}$ | –1.84 | 4 |
| GPEtn(17:0/18:0)[U] | 17.836 | 733.5613 | | GPEtn(17:0/18:0)[U] | $C_{40}H_{80}NO_8P$ | $C_{40}H_{80}NO_8P$ | $C_{40}H_{80}NO_8P$ | 1.17 | 10 |
| (23R,25R)-1alpha,25-dihydroxyvitamin D3 26,23-lactone / (23R,25R)-1alpha,25-dihydroxycholecalciferol | 17.875 | 444.2883 | 493447 | (23R,25R)-1alpha,25-dihydroxyvitamin D3 26,23-lactone / (23R,25R)-1alpha,25-dihydroxycholecalciferol | $C_{27}H_{40}O_5$ | $C_{27}H_{40}O_5$ | $C_{27}H_{40}O_5$ | –1.55 | 7 |
| (+/-)-12-HEPE | 18.01 | 318.2185 | | (+/-)-12-HEPE | $C_{20}H_{30}O_3$ | $C_{20}H_{30}O_3$ | $C_{20}H_{30}O_3$ | 3.06 | 10 |
| Met Phe Asn | 18.013 | 410.1648 | 476522 | Met Phe Asn | $C_{18}H_{26}N_4O_5S$ | $C_{18}H_{26}N_4O_5S$ | $C_{18}H_{26}N_4O_5S$ | –5.79 | 10 |
| GPEtnNMe(18:1(9Z)/18:1(9Z)) | 18.193 | 757.5596 | 740512 | GPEtnNMe(18:1(9Z)/18:1(9Z)) | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | 3.35 | 6 |
| KHIVORIN | 18.197 | 586.279 | 566438 | KHIVORIN | $C_{32}H_{42}O_{10}$ | $C_{32}H_{42}O_{10}$ | $C_{32}H_{42}O_{10}$ | –1.99 | 4 |
| L-Phosphatidic acid | 18.243 | 596.3728 | | L-Phosphatidic acid | $C_{29}H_{57}O_{10}P$ | $C_{29}H_{57}O_{10}P$ | $C_{29}H_{57}O_{10}P$ | –6.45 | 9 |
| 5,14,15-trihydroxy-6,8,10,12-Eicosatetraenoic acid | 18.332 | 352.2241 | | 5,14,15-trihydroxy-6,8,10,12-Eicosatetraenoic acid | $C_{20}H_{32}O_5$ | $C_{20}H_{32}O_5$ | $C_{20}H_{32}O_5$ | 2.52 | 2 |
| Met Phe Asn | 18.38 | 410.164 | 588484 | Met Phe Asn | $C_{18}H_{26}N_4O_5S$ | $C_{18}H_{26}N_4O_5S$ | $C_{18}H_{26}N_4O_5S$ | –4.04 | 10 |
| GPEtnNMe(18:1(9Z)/18:1(9Z)) | 18.559 | 757.5596 | 761669 | GPEtnNMe(18:1(9Z)/18:1(9Z)) | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | $C_{42}H_{80}NO_8P$ | 3.35 | 6 |
| Khivorin | 18.559 | 586.2792 | 801629 | Khivorin | $C_{32}H_{42}O_{10}$ | $C_{32}H_{42}O_{10}$ | $C_{32}H_{42}O_{10}$ | –2.36 | 4 |
| Khayanthone | 18.654 | 570.2841 | 1424582 | Khayanthone | $C_{32}H_{42}O_9$ | $C_{32}H_{42}O_9$ | $C_{32}H_{42}O_9$ | –2.17 | 3 |
| Allotetrahydrocortiso | 18.746 | 366.2394 | 1685758 | Allotetrahydrocortiso | $C_{21}H_{34}O_5$ | $C_{21}H_{34}O_5$ | $C_{21}H_{34}O_5$ | 3.31 | 10 |
| Khayanthone | 19.047 | 570.2844 | | Khayanthone | $C_{32}H_{42}O_9$ | $C_{32}H_{42}O_9$ | $C_{32}H_{42}O_9$ | –2.63 | 3 |
| Allotetrahydrocortisol | 19.129 | 366.2394 | | Allotetrahydrocortisol | $C_{21}H_{34}O_5$ | $C_{21}H_{34}O_5$ | $C_{21}H_{34}O_5$ | 3.45 | 10 |
| Lagochilin | 19.256 | 356.2571 | | Lagochilin | $C_{20}H_{36}O_5$ | $C_{20}H_{36}O_5$ | $C_{20}H_{36}O_5$ | –2.25 | 10 |
| 9,12-dimethoxy-13-hydroxy-10-octadecenoic acid | 19.332 | 358.2728 | 2978444 | 9,12-dimethoxy-13-hydroxy-10-octadecenoic acid | $C_{20}H_{38}O_5$ | $C_{20}H_{38}O_5$ | $C_{20}H_{38}O_5$ | –2.49 | 10 |
| Iloprost | 19.518 | 360.2288 | | Iloprost | $C_{22}H_{32}O_4$ | $C_{22}H_{32}O_4$ | $C_{22}H_{32}O_4$ | 3.42 | 10 |
| 9,12-dimethoxy-13-hydroxy-10-octadecenoic acid | 19.659 | 358.2727 | | 9,12-dimethoxy-13-hydroxy-10-octadecenoic acid | $C_{20}H_{38}O_5$ | $C_{20}H_{38}O_5$ | $C_{20}H_{38}O_5$ | –2.14 | 10 |
| PGF2alpha methyl ether | 19.781 | 354.2777 | 504527 | PGF2alpha methyl ether | $C_{21}H_{38}O_4$ | $C_{21}H_{38}O_4$ | $C_{21}H_{38}O_4$ | –2.03 | 3 |
| PGF2alpha methyl ether | 20.126 | 354.2776 | | PGF2alpha methyl ether | $C_{21}H_{38}O_4$ | $C_{21}H_{38}O_4$ | $C_{21}H_{38}O_4$ | –1.68 | 3 |
| 3beta, 6alpha, 7beta-Trihydroxy-5beta-cholan-24-oic Acid | 20.334 | 408.2855 | | 3beta, 6alpha, 7beta-Trihydroxy-5beta-cholan-24-oic Acid | $C_{24}H_{40}O_5$ | $C_{24}H_{40}O_5$ | $C_{24}H_{40}O_5$ | 4.97 | 10 |
| 3beta, 6alpha, 7beta-Trihydroxy-5beta-cholan-24-oic Acid | 20.638 | 408.2856 | | 3beta, 6alpha, 7beta-Trihydroxy-5beta-cholan-24-oic Acid | $C_{24}H_{40}O_5$ | $C_{24}H_{40}O_5$ | $C_{24}H_{40}O_5$ | 4.83 | 10 |

**Table S2:** List of compounds obtained from negative ionization of HRLCMS- MSMS- QTOF analysis.

| Compound label | RT | Mass | Abund | Name | Formula | MFG Formula | DB Formula | DB Diff (ppm) | Hits (DB) |
|---|---|---|---|---|---|---|---|---|---|
| Hydrocortisone-17-butyrate | 11.083 | 432.2505 | 430912 | Hydrocortisone-17- butyrate | $C_{25}H_{36}O_6$ | $C_{25}H_{36}O_6$ | $C_{25}H_{36}O_6$ | 1.7 | 10 |
| Phe His Lys | 11.091 | 430.2341 | | Phe His Lys | $C_{21}H_{30}N_6O_4$ | $C_{21}H_{30}N_6O_4$ | $C_{21}H_{30}N_6O_4$ | –2.8 | 10 |
| His His Lys | 12.363 | 420.2277 | 1517429 | His His Lys | $C_{18}H_{28}N_8O_4$ | $C_{18}H_{28}N_8O_4$ | $C_{18}H_{28}N_8O_4$ | –10.25 | 3 |
| Trp Thr Ile | 12.436 | 418.2125 | | Trp Thr Ile | $C_{21}H_{30}N_4O_5$ | $C_{21}H_{30}N_4O_5$ | $C_{21}H_{30}N_4O_5$ | 21.84 | 10 |
| Manoalide | 12.678 | 416.2552 | | Manoalide | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | 2.64 | 10 |
| Phe His Lys | 13.53 | 430.2332 | | Phe His Lys | $C_{21}H_{30}N_6O_4$ | $C_{21}H_{30}N_6O_4$ | $C_{21}H_{30}N_6O_4$ | –0.81 | 10 |
| Ramipril | 13.862 | 416.2327 | | Ramipril | $C_{23}H_{32}N_2O_5$ | $C_{23}H_{32}N_2O_5$ | $C_{23}H_{32}N_2O_5$ | –3.84 | 10 |
| Phe Lys Pro | 13.972 | 390.2184 | | Phe Lys Pro | $C_{20}H_{30}N_4O_4$ | $C_{20}H_{30}N_4O_4$ | $C_{20}H_{30}N_4O_4$ | 21.16 | 6 |
| 3-Oxochola-1,4,6-trien-24-oic Acid | 14.077 | 368.2341 | | 3-Oxochola-1,4,6-trien-24-oic acid | $C_{24}H_{32}O_3$ | $C_{24}H_{32}O_3$ | $C_{24}H_{32}O_3$ | 2.91 | 5 |
| Manoalide | 14.251 | 416.2552 | 639473 | Manoalide | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | 2.52 | 10 |
| His His Lys | 14.487 | 420.2297 | 2218246 | His His Lys | $C_{18}H_{28}N_8O_4$ | $C_{18}H_{28}N_8O_4$ | $C_{18}H_{28}N_8O_4$ | –15.06 | 3 |
| Tylosin | 14.63 | 915.4984 | 192031 | Tylosin | $C_{46}H_{77}NO_{17}$ | $C_{46}H_{77}NO_{17}$ | $C_{46}H_{77}NO_{17}$ | 22.7 | 1 |
| Trp Thr Ile | 14.659 | 418.2137 | | Trp Thr Ile | $C_{21}H_{30}N_4O_5$ | $C_{21}H_{30}N_4O_5$ | $C_{21}H_{30}N_4O_5$ | 18.98 | 10 |
| His His Lys | 14.8 | 420.2292 | 1005931 | His His Lys | $C_{18}H_{28}N_8O_4$ | $C_{18}H_{28}N_8O_4$ | $C_{18}H_{28}N_8O_4$ | –13.91 | 3 |
| Ile Glu Arg | 15.045 | 416.238 | | Ile Glu Arg | $C_{17}H_{32}N_6O_6$ | $C_{17}H_{32}N_6O_6$ | $C_{17}H_{32}N_6O_6$ | 0.87 | 10 |
| Ramipril | 15.396 | 416.2342 | | Ramipril | $C_{23}H_{32}N_2O_5$ | $C_{23}H_{32}N_2O_5$ | $C_{23}H_{32}N_2O_5$ | –7.31 | 10 |
| 12-oxo-ETE | 15.408 | 318.219 | | 12-oxo-ETE | $C_{20}H_{30}O_3$ | $C_{20}H_{30}O_3$ | $C_{20}H_{30}O_3$ | 1.65 | 10 |
| 13R-hydroxy-9E,11Z- octadecadienoic acid | 16.081 | 296.2341 | | 13R-hydroxy-9E,11Z-octadecadienoic acid | $C_{18}H_{32}O_3$ | $C_{18}H_{32}O_3$ | $C_{18}H_{32}O_3$ | 3.42 | 10 |
| Manoalide | 16.112 | 416.256 | | Manoalide | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | 0.77 | 10 |
| Manoalide | 16.387 | 416.2561 | | Manoalide | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | $C_{25}H_{36}O_5$ | 0.5 | 10 |
| Hydrocortisone-17-butyrate | 16.483 | 432.2509 | | Hydrocortisone-17- butyrate | $C_{25}H_{36}O_6$ | $C_{25}H_{36}O_6$ | $C_{25}H_{36}O_6$ | 0.63 | 10 |
| 12-oxo-ETE | 16.621 | 318.2194 | | 12-oxo-ETE | $C_{20}H_{30}O_3$ | $C_{20}H_{30}O_3$ | $C_{20}H_{30}O_3$ | 0.39 | 10 |
| 5,8,11-Eicosatriynoic acid | 17.49 | 300.2093 | 2279283 | 5,8,11-Eicosatriynoic acid | $C_{20}H_{28}O_2$ | $C_{20}H_{28}O_2$ | $C_{20}H_{28}O_2$ | –1.38 | 10 |
| Ophiobolin A | 17.93 | 400.262 | | Ophiobolin A | $C_{25}H_{36}O_4$ | $C_{25}H_{36}O_4$ | $C_{25}H_{36}O_4$ | –1.72 | 2 |
| Oleandolide | 18.241 | 386.2255 | 369549 | Oleandolide | $C_{20}H_{34}O_7$ | $C_{20}H_{34}O_7$ | $C_{20}H_{34}O_7$ | 12.74 | 2 |
| Ophiobolin A | 18.32 | 400.2623 | | Ophiobolin A | $C_{25}H_{36}O_4$ | $C_{25}H_{36}O_4$ | $C_{25}H_{36}O_4$ | –2.34 | 2 |
| 2E,5Z,8Z,11Z,14Z-eicosapentaenoic acid | 18.334 | 302.2248 | | 2E,5Z,8Z,11Z,14Z-eicosapentaenoic acid | $C_{20}H_{30}O_2$ | $C_{20}H_{30}O_2$ | $C_{20}H_{30}O_2$ | –0.86 | 10 |
| Gln Lys Lys | 18.62 | 402.2574 | 2389381 | Gln Lys Lys | $C_{17}H_{34}N_6O_5$ | $C_{17}H_{34}N_6O_5$ | $C_{17}H_{34}N_6O_5$ | 4.19 | 10 |
| Roxithromycin | 18.62 | 836.5041 | | Roxithromycin | $C_{41}H_{76}N_2O_{15}$ | $C_{41}H_{76}N_2O_{15}$ | $C_{41}H_{76}N_2O_{15}$ | 24.53 | 1 |
| Gln Lys Lys | 18.975 | 402.2569 | 1622676 | Gln Lys Lys | $C_{17}H_{34}N_6O_5$ | $C_{17}H_{34}N_6O_5$ | $C_{17}H_{34}N_6O_5$ | 5.47 | 10 |

(*Contd...*)

**Table S2:** (*Continued*)

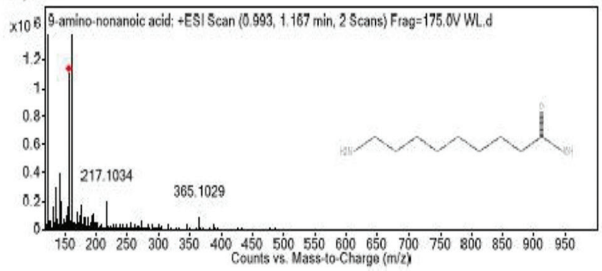
| Compound label | RT | Mass | Abund | Name | Formula | MFG Formula | DB Formula | DB Diff (ppm) | Hits (DB) |
|---|---|---|---|---|---|---|---|---|---|
| (22R)-1alpha,22,25-trihydroxy 24a,24b-dihomo-20-epivitamin D3 / (22R)-1alpha,22,25-trihydroxy-24a,24 | 21.44 | 460.357 | | (22R)-1alpha,22,25-trihydroxy-24a,24b-dihomo-20-epivitamin D3 / (22R)-1alpha,22,25-trihydroxy-24a,24 | $C_{29}H_{48}O_4$ | $C_{29}H_{48}O_4$ | $C_{29}H_{48}O_4$ | –3.84 | 10 |
| 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | 24.015 | 774.5322 | | 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | –5.1 | 5 |
| 5-[2-(hydroxymethyl)-5- methylphenoxy]-2,2-dimethyl- Pentanoic acid (GemfibrozilM4) | 24.047 | 266.155 | | 5-[2-(hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-Pentanoic acid (Gemfibrozil M4) | $C_{15}H_{22}O_4$ | $C_{15}H_{22}O_4$ | $C_{15}H_{22}O_4$ | –12.09 | 3 |
| 5-[2-(hydroxymethyl)-5- methylphenoxy]-2,2-dimethyl- Pentanoic acid (GemfibrozilM4) | 24.404 | 266.1549 | | 5-[2-(hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-Pentanoic acid (Gemfibrozil M4) | $C_{15}H_{22}O_4$ | $C_{15}H_{22}O_4$ | $C_{15}H_{22}O_4$ | -11.71 | 3 |
| 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | 24.727 | 774.5319 | | 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | –4.75 | 5 |
| 5-[2-(hydroxymethyl)-5- methylphenoxy]-2,2-dimethyl- Pentanoic acid (GemfibrozilM4) | 24.727 | 266.1547 | | 5-[2-(hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-Pentanoic acid (Gemfibrozil M4) | $C_{15}H_{22}O_4$ | $C_{15}H_{22}O_4$ | $C_{15}H_{22}O_4$ | -11.01 | 3 |
| 1,2 di-(9Z,12Z,15Z- )-3-O-Beta-D- galactosyl-sn-glycerol | 25.04 | 774.5319 | | 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | –4.77 | 5 |
| 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | 25.688 | 774.5312 | | 1,2 di-(9Z,12Z,15Z-octadecatrienoyl)-3-O-Beta-D-galactosyl-sn-glycerol | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | $C_{45}H_{74}O_{10}$ | –3.87 | 5 |

**Spectroscopic data of allelopathic compounds obtained by HRLCMS- MSMS- QTOF analysis**

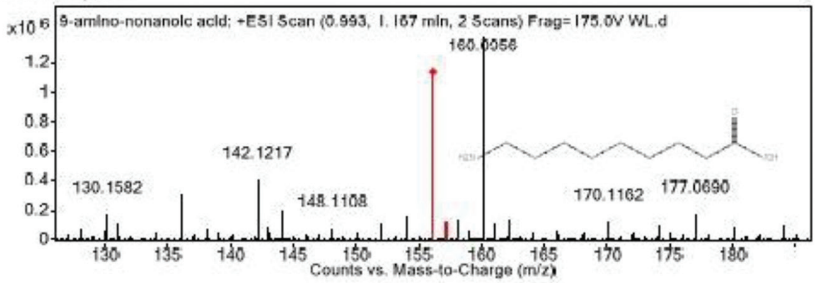
1. 9-amino-nonanoic acid

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| 9-amino-nonanoic acid | 9-amino-nonanoic acid | 156.137 | 1.097 | Auto MS/MS | 173.1404 |

MS Spectrum



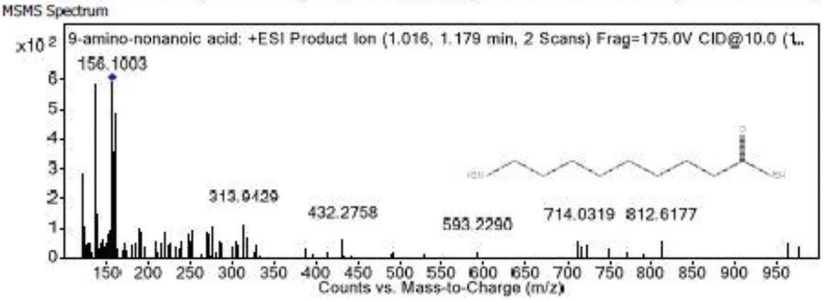

MS Zoomed Spectrum




**MS Spectrum Peak List**

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 122.0954 | | | 1 | 1374728.63 | | |
| 136.1112 | | | 1 | 296522.03 | | |
| 142.1217 | | | 1 | 397338.38 | | |
| 144.1007 | | | | 191334.19 | | |
| 156.137 | 156.1383 | 8.25 | 1 | 1099110.38 | C9 H19 N O2 | (M+H)+[-H2O] |
| 157.1406 | 157.1415 | 5.83 | 1 | 103340.01 | C9 H19 N O2 | (M+H)+[-H2O] |
| 158.1495 | 158.144 | -34.78 | 1 | 18695.27 | C9 H19 N O2 | (M+H)+[-H2O] |
| 160.0956 | | | 1 | 1371763.38 | | |
| 160.1306 | | | | 173236.14 | | |
| 217.1034 | | | | 194682.09 | | |

MSMS Spectrum




**MS/MS Spectrum Peak List**

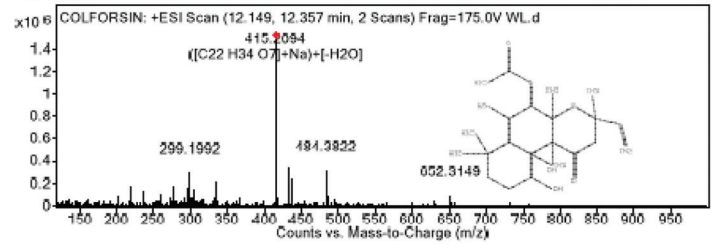
| *m/z* | z | Abund |
|---|---|---|
| 122.0956 | 1 | 277.14 |
| 123.0662 | | 100.53 |
| 137.0376 | | 577.67 |
| 138.0879 | | 144.73 |
| 156.1003 | 1 | 587.06 |
| 156.1356 | 1 | 532.07 |
| 158.095 | 1 | 350.75 |
| 159.0969 | | 105.44 |
| 160.0943 | | 482.1 |
| 313.9429 | | 109.23 |

**Compound Structure**

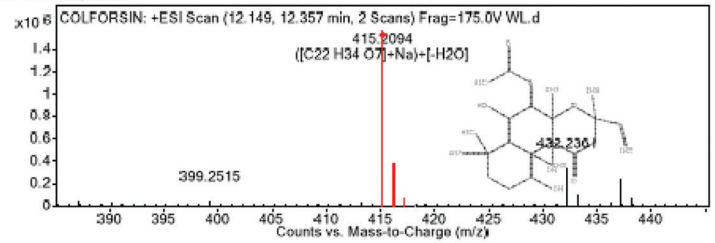
2. Colforsin

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| COLFORSIN | COLFORSIN | 415.2094 | 12.266 | Auto MS/MS | 410.2308 |

MS Spectrum



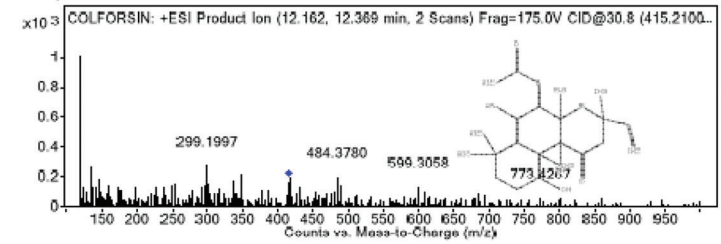

MS Zoomed Spectrum




**MS Spectrum Peak List**

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 219.1732 | | | 1 | 178159.56 | | |
| 277.2148 | | | 1 | 173578.34 | | |
| 299.1992 | | | 1 | 299222.31 | | |
| 335.218 | | | 1 | 206138.97 | | |
| 415.2094 | 415.2091 | -0.75 | 1 | 1559726.38 | C22 H34 O7 | (M+Na)+[-H2O] |
| 416.213 | 416.2125 | -1.24 | 1 | 376081.75 | C22 H34 O7 | (M+Na)+[-H2O] |
| 417.2168 | 417.2152 | -3.84 | 1 | 64053.52 | C22 H34 O7 | (M+Na)+[-H2O] |
| 432.2361 | | | 1 | 341178.41 | | |
| 437.1918 | | | 1 | 233730.05 | | |
| 484.3822 | | | 1 | 315402.66 | | |

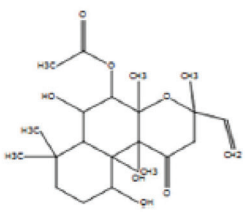
MSMS Spectrum




**MS/MS Spectrum Peak List**

| *m/z* | Calc m/z | Diff (ppm) | Abund | Formula | Ion |
|---|---|---|---|---|---|
| 120.0878 | | | 1011.69 | | |
| 133.0636 | | | 259.14 | | |
| 135.1159 | | | 153.92 | | |
| 145.1003 | | | 178.56 | | |
| 299.1997 | | | 272.11 | | |
| 336.2578 | | | 168 | | |
| 349.2001 | | | 202.72 | | |
| 415.214 | | | 159.45 | | |
| 416.213 | 416.2125 | -1.06 | 190.51 | C22 H34 O7 | (M+Na)+[-H2O] |
| 484.378 | | | 195.63 | | |

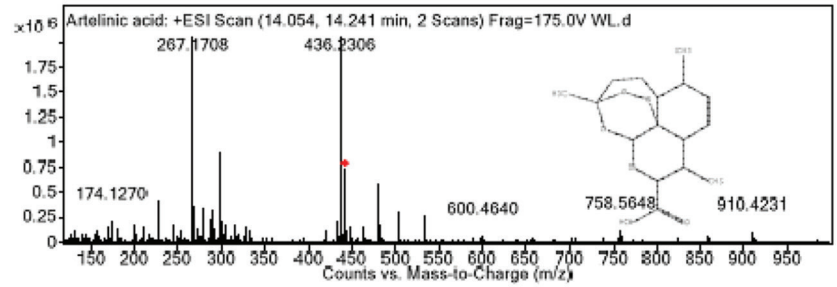
**Compound Structure**



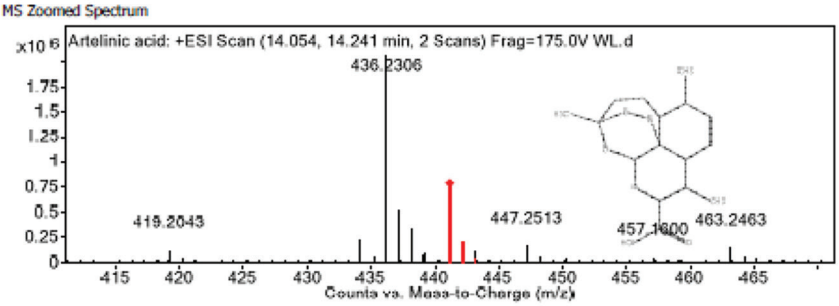
3. Artelinic acid

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| Artelinic acid | Artelinic acid | 441.186 | 14.164 | Auto MS/MS | 418.1978 |

MS Spectrum



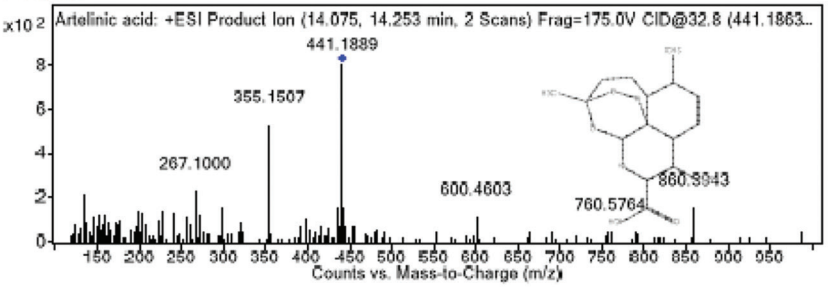

MS Zoomed Spectrum




MS Spectrum Peak List

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 227.1054 | | | 1 | 408567.13 | | |
| 267.1708 | | | 1 | 2555037 | | |
| 299.1991 | | | 1 | 895134.5 | | |
| 436.2306 | | | 1 | 2052762.5 | | |
| 437.2344 | | | 1 | 517373.44 | | |
| 441.186 | 441.1884 | 5.48 | 1 | 734247.06 | C23 H30 O7 | (M+Na)+ |
| 442.1897 | 442.1918 | 4.72 | 1 | 178828.27 | C23 H30 O7 | (M+Na)+ |
| 443.2 | 443.1944 | -12.66 | 1 | 101134.7 | C23 H30 O7 | (M+Na)+ |
| 444.2039 | 444.197 | -15.36 | 1 | 22996.32 | C23 H30 O7 | (M+Na)+ |
| 481.2562 | | | 1 | 579277.44 | | |

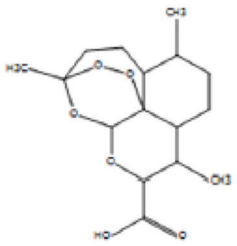
MSMS Spectrum




MS/MS Spectrum Peak List

| *m/z* | z | Abund |
|---|---|---|
| 133.0988 | | 206.17 |
| 267.1 | | 226.46 |
| 299.2017 | | 151.57 |
| 353.1351 | | 310.12 |
| 354.1373 | | 180.71 |
| 355.1507 | 1 | 526.03 |
| 436.2316 | 1 | 152.86 |
| 441.1889 | 1 | 803.7 |
| 442.1954 | 1 | 153.09 |
| 860.3943 | | 152.25 |

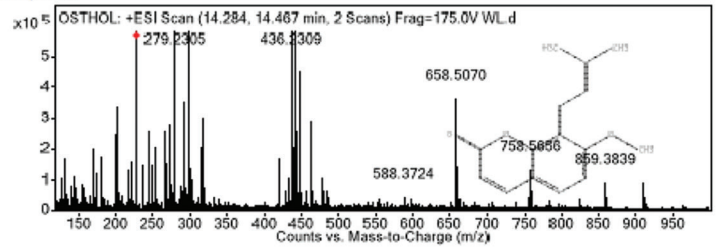
Compound Structure



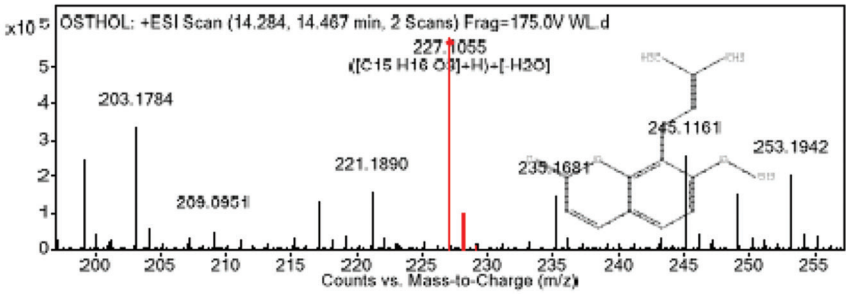
4. Osthol

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| OSTHOL | OSTHOL | 227.1055 | 14.389 | Auto MS/MS | 244.1089 |

MS Spectrum



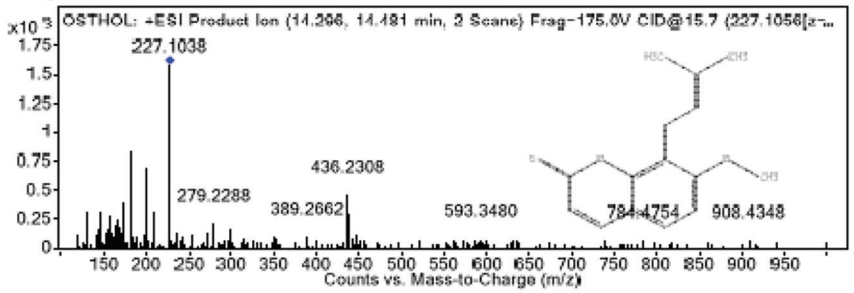

MS Zoomed Spectrum




**MS Spectrum Peak List**

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 227.1055 | 227.1067 | 5.12 | 1 | 579322.88 | C15 H16 O3 | (M+H)+[-H2O] |
| 228.1088 | 228.11 | 5.51 | 1 | 92507.27 | C15 H16 O3 | (M+H)+[-H2O] |
| 229.1185 | 229.1128 | -24.91 | 1 | 15041.33 | C15 H16 O3 | (M+H)+[-H2O] |
| 279.2305 | | | 1 | 928874.63 | | |
| 299.1992 | | | 1 | 592827.13 | | |
| 436.2309 | | | 1 | 2701717.75 | | |
| 437.2341 | | | 1 | 697284.88 | | |
| 438.2459 | | | 1 | 856833.63 | | |
| 441.1861 | | | 1 | 1007912.5 | | |
| 447.2511 | | | 1 | 447847.75 | | |

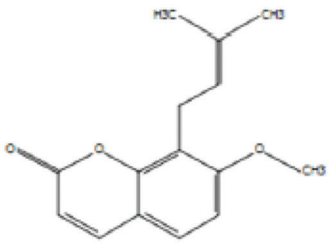
MSMS Spectrum




**MS/MS Spectrum Peak List**

| *m/z* | z | Abund |
|---|---|---|
| 131.0462 | | 303.36 |
| 145.101 | | 309.6 |
| 171.114 | | 381.12 |
| 181.0998 | 1 | 825.8 |
| 199.1089 | | 676.33 |
| 209.0915 | | 313.55 |
| 227.1038 | 1 | 1565.73 |
| 228.1082 | 1 | 314.81 |
| 436.2308 | | 446.13 |
| 437.2358 | | 293.83 |

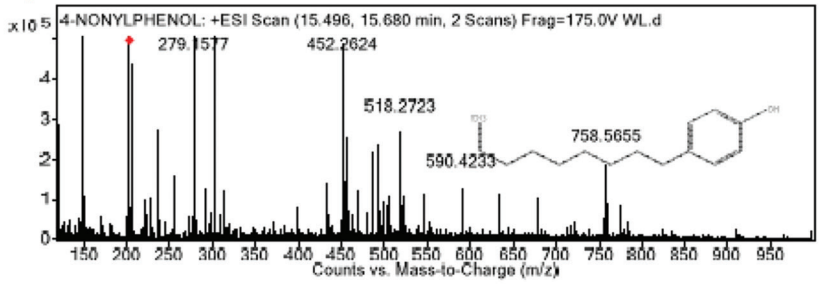
**Compound Structure**



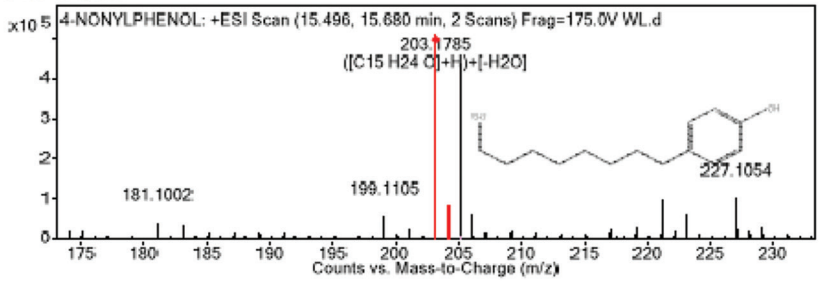
5. 4-Nonylphenol

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| 4-NONYLPHENOL | 4-NONYLPHENOL | 203.1785 | 15.602 | Auto MS/MS | 220.1818 |

MS Spectrum



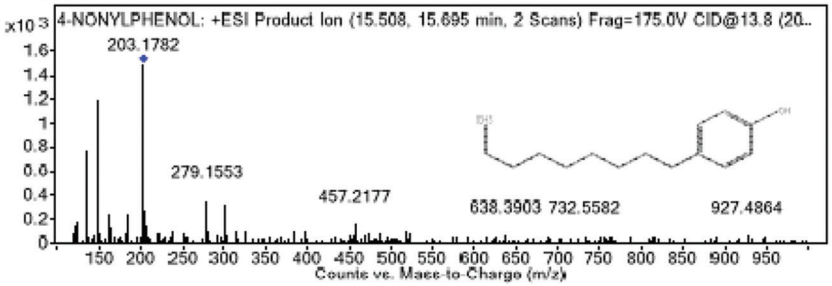

MS Zoomed Spectrum




**MS Spectrum Peak List**

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 121.0279 | | | | 287094 | | |
| 149.0224 | | | 1 | 1310015.38 | | |
| 203.1785 | 203.1794 | 4.43 | 1 | 508850.75 | C15 H24 O | (M+H)+[-H2O] |
| 204.1818 | 204.1828 | 4.82 | 1 | 77120.12 | C15 H24 O | (M+H)+[-H2O] |
| 205.0848 | | | 1 | 434300.34 | | |
| 237.1838 | | | 1 | 269290.88 | | |
| 279.1577 | | | 1 | 1832256 | | |
| 280.1614 | | | 1 | 330125.53 | | |
| 301.1395 | | | 1 | 983859.81 | | |
| 452.2624 | | | 1 | 487062.19 | | |

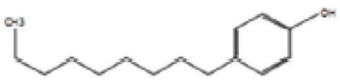
MSMS Spectrum




**MS/MS Spectrum Peak List**

| *m/z* | z | Abund |
|---|---|---|
| 133.0997 | | 753.21 |
| 135.1134 | | 187.92 |
| 147.1155 | 1 | 355.78 |
| 149.022 | | 1185.94 |
| 161.1329 | | 233.46 |
| 183.1153 | 1 | 229.52 |
| 203.1782 | 1 | 1490.24 |
| 204.1786 | 1 | 262.06 |
| 279.1553 | 1 | 349.21 |
| 301.1377 | 1 | 306.81 |

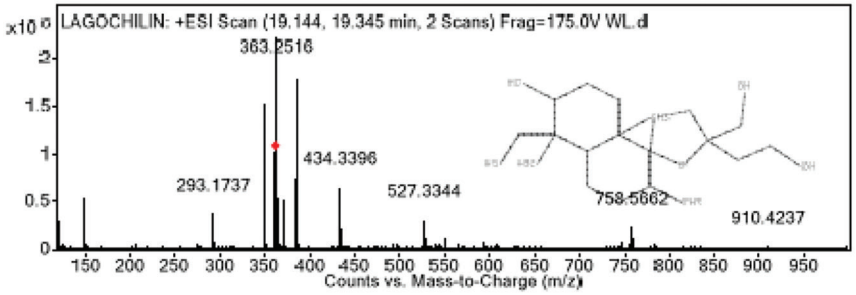
**Compound Structure**



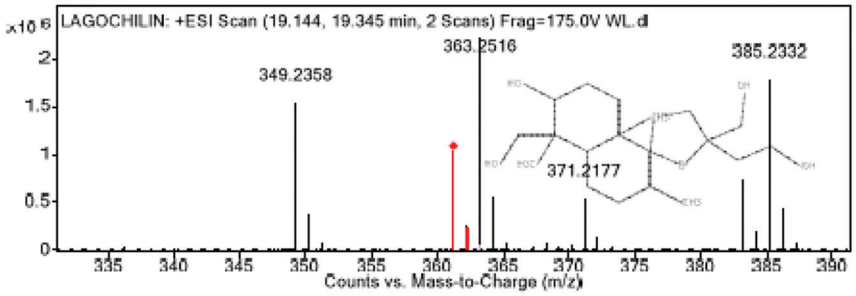
6. Lagochilin

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| LAGOCHILIN | LAGOCHILIN | 361.2357 | 19.256 | Auto MS/MS | 356.2571 |

MS Spectrum



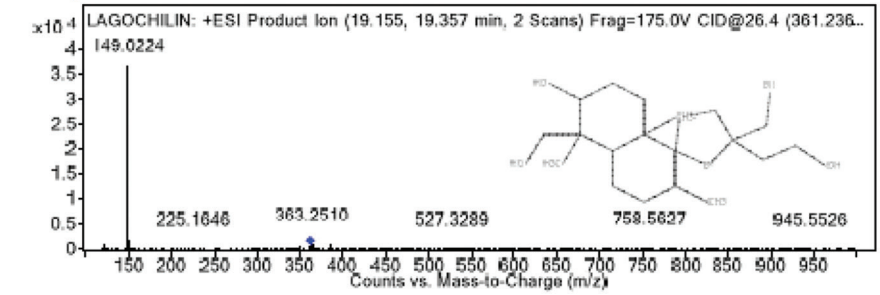

MS Zoomed Spectrum




MS Spectrum Peak List

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 149.0226 | | | 1 | 531516.38 | | |
| 349.2358 | | | 1 | 1517979.25 | | |
| 361.2357 | 361.2349 | -2.04 | 1 | 1012787.69 | C20 H36 O5 | (M+Na)+[-H2O] |
| 362.2394 | 362.2383 | -3.01 | 1 | 250942.03 | C20 H36 O5 | (M+Na)+[-H2O] |
| 363.2516 | | | 1 | 2217978.25 | | |
| 364.2551 | | | 1 | 536658.31 | | |
| 371.2177 | | | 1 | 514172.75 | | |
| 383.2176 | | | 1 | 737135.63 | | |
| 385.2332 | | | 1 | 1768841.13 | | |
| 434.3396 | | | 1 | 644861.63 | | |

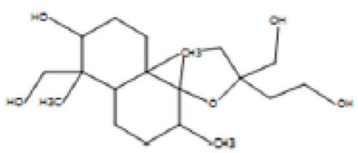
MSMS Spectrum




MS/MS Spectrum Peak List

| *m/z* | z | Abund |
|---|---|---|
| 121.0309 | | 612.54 |
| 149.0224 | 1 | 36511.01 |
| 149.0695 | 2 | 234.36 |
| 150.0265 | 1 | 1503.41 |
| 293.1716 | | 327.53 |
| 349.2354 | 1 | 342.35 |
| 361.2352 | 1 | 516.03 |
| 363.251 | | 984.62 |
| 364.2547 | | 657.05 |
| 385.2314 | 1 | 833 |

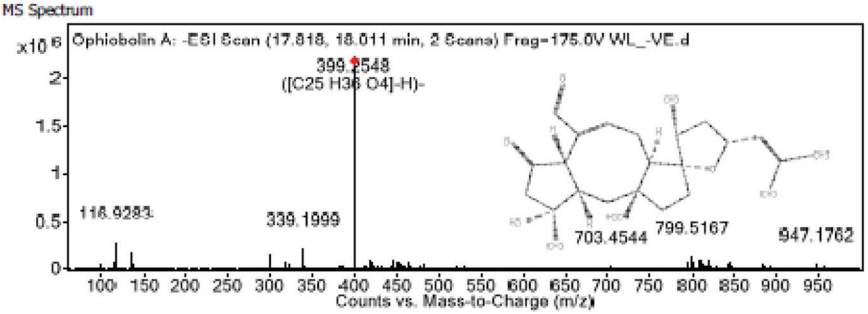
Compound Structure



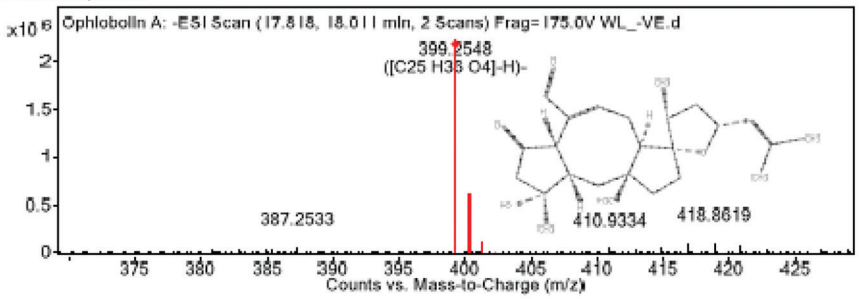
7. Ophiobolin A

| Compound Label | Name | *m/z* | RT | Algorithm | Mass |
|---|---|---|---|---|---|
| Ophiobolin A | **Ophiobolin A** | 399.2548 | 17.93 | Auto MS/MS | 400.262 |

MS Spectrum



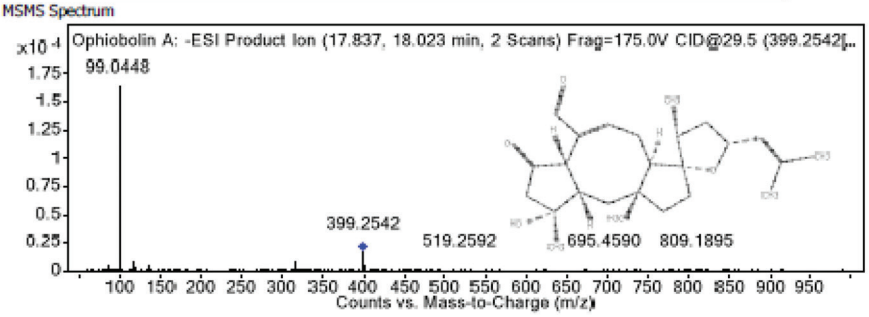

MS Zoomed Spectrum




**MS Spectrum Peak List**

| *m/z* | Calc m/z | Diff(ppm) | z | Abund | Formula | Ion |
|---|---|---|---|---|---|---|
| 116.9283 | | | 1 | 275570.81 | | |
| 134.8942 | | | 1 | 171447.81 | | |
| 299.2016 | | | 1 | 144303.25 | | |
| 339.1999 | | | 1 | 207582.75 | | |
| 399.2548 | 399.2541 | -1.86 | 1 | 2234338.25 | C25 H36 O4 | (M-H)- |
| 400.258 | 400.2575 | -1.33 | 1 | 603771 | C25 H36 O4 | (M-H)- |
| 401.2608 | 401.2604 | -1.06 | 1 | 85282.78 | C25 H36 O4 | (M-H)- |
| 446.8942 | | | | 94096.88 | | |
| 799.5167 | | | 1 | 127223.9 | | |
| 809.1836 | | | 1 | 95244.16 | | |

MSMS Spectrum




**MS/MS Spectrum Peak List**

| *m/z* | z | Abund |
|---|---|---|
| 87.0448 | 1 | 527.62 |
| 99.0448 | 1 | 16310.47 |
| 99.0827 | | 776.43 |
| 100.0481 | 1 | 727.23 |
| 101.0592 | 1 | 712.87 |
| 116.9283 | 1 | 657.64 |
| 134.8939 | 1 | 480.81 |
| 317.2113 | 1 | 662.97 |
| 399.2542 | 1 | 1563.35 |
| 400.2568 | 1 | 393.73 |

**Compound Structure**