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Developmental Botanic: A Case Study on Chromatographic Determination of Phytocompounds in the Roots of *Anthocleista nobilis* (G. Don.) as Pro-fertility

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Abstract

Medicinal plants have great importance in African medicine and are also used as precursors in drug discovery. The medicinal value of plants lies in their bioactive constituents which usually allow them to act as remedy in several ailments. Plant-based natural constituents can be derived from any part of the plant like roots, bark, leaves, flowers, fruits, seeds. The present study is designed determining phytocompounds present in *Anthocleista nobilis* root as pro-fertility, and its validation by gas chromatography mass spectrometry. GC-MS analysis of the methanol extract of *A. nobilis* root was performed using a Perkin Elmer GC Clarus 500 system comprising an Agilent technologies 5975 MSD model detector and a gas chromatograph interfaced to a mass spectrometer with the aid of the Turbo mass 5.0 software. The study results of the GC-MS analysis provided different phytochemical compounds possessing several biological activities such as dietary, antioxidant, anti-cardiac disorder, antimicrobial, antifungal, anticancer, anti-inflammatory activities etc. This study, therefore, showed that root of *Anthocleista nobilis* is source of biologically active metabolites. Furthermore, root extract revealed the presence of diverse chemical constituents. The experimental findings of this study justifies the use of *Anthocleista nobilis* in ethno-medicine, and suggest a recommendation of *A. nobilis* root as a plant of dietary and phytotherapeutic importance.

Keywords: *Anthocleista nobilis*, Phytochemicals, Chromatography, Herbal medicine

Introduction

The study of development has become essential for understanding any other area of biology. It is one of the fastest-growing and most exciting fields in biology, creating a frame-wok that integrates ecology, molecular biology, food science, regulatory hierarchies and many others in developing plant into different levels of importance (authors' unpublished data).

Furtherance to developing plant into different levels of therapeutic importance, botanical dietary supplements, sometimes called herbals or herbal dietary supplements, are products made from plants, plant parts, or plant extracts. They are meant to be consumed and contain one or more ingredients meant to supplement the diet for building cells, tissues, strengthening of organs, and system functioning; thereby enhancing healthy living. A recent study on botanical dietary supplement and fertility, analyzed the impact of diet in relation to fertility, specifically with how its role, when paired with assisted reproductive technology, can improve success; and that there is strong evidence that healthy preconception dietary patterns among both men and women of reproductive age have a beneficial effect on fertility (1).

The use of plants for medicinal purposes dates back to earlier recorded human history. Traditional medicines chiefly containing medicinal plants have always played a vital role as important alternatives to conventional medicines in developing countries. The use of medicinal plants or their products is more popular especially among the poor communities that inhabit rural areas and lack access to health. Alternatively, there has been an enormous increase in the demand of medicinal plants across the globe for their chemical diversity and for the production of newer therapeutic moieties to control various diseases. In spite of tremendous advancement made in the discovery of new synthetic drugs, medicinal plants have still retained their therapy in the literature. Therefore, research on medicinal plants always remained a potential area of investigation (2). Plant-based natural compounds can be derived from any part of the plant like roots, bark, leaves, flowers, fruits and seeds. Screening for active compounds from plants has led to the invention of new medicinal drugs which have different protection and treatment roles against various diseases or conditions (3). Modern method describing the identification and quantification of active constituents in plants material may be useful for proper standardization of herbal and its formulation. Use of plants as a source of medicine has been inherited and is an important component of the health care, and as well, becomes the source of many potent and powerful drugs (4). Many years ago, people around the globe have healed the sick with herbal derived remedies, and handed down through generation among the indigenous populations (5).

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The medicinal values of plants, *Anthocleista nobilis* for instance, lies in the bioactive phytochemical constituents that produces definite physiological effects on human body and protect them from various diseases. Plants defend themselves from pathogens and other herbivore enemies by elaborating a variety of bioactive secondary metabolites, and probably, minerals that may have multiple molecular sites of action. Accordingly, exploitation of these useful plants has spread rapidly to safeguard increasing population from various pathogens and ailments (6). Phytochemicals are protective and disease preventing particularly for some forms of cancer and heart disease (7). For thousands of years, people all over the world have used medicinal plants as base in making traditional medicines and had given great advantages to mankind to come up with new remedies (8). Medicinal plants contain bioactive compounds, for instance, saponins, tannins, flavonoids, essential oils etc. These phytochemicals are produced as a result of normal metabolic activities of plants and are also known as secondary metabolites (9). These are the originator of medicinal properties within plants, as exemplification, antimicrobial (10), antioxidant (11), and most importantly antidiabetic (12). The plant secondary metabolites are important for the human consumption as food and used in the pharmaceutical industry for required special attention (13). Many plants are good sources of antioxidants and other bioactive compounds containing phenolics, alkaloids, amino acids, ascorbic acid etc. Due to increasing demand, seeking therapeutic drugs from plants has grown tremendously. Such preparations contain various bioactive compounds of high therapeutic value and becoming popular in the area of medicine for their less expensive and less side effects etc., compared to modern allopathic drugs.

The traditional medicines in the last few decades emerged to have immense acknowledgements and it is estimated that 80% of community depend on traditional medicine for their primary healthcare (6). Traditional medicines are not only contributing to primary health care, but also in the development of modern drugs (14). Various types of traditional medicine and other medical practices referred to as complementary or alternative medicine are increasingly used in both developing and developed countries. Countries like India and China are popularly known when it comes to traditional medicines because they believe them to be safer, more effective and inexpensive (15).

Ayurveda stresses the use of plant-based medicine and treatments. But when compared, the Chinese medicine is more established than Ayurvedic medicine. This is due to even after Chinese people migrating to other countries they still follow their own culture. And also the Chinese people wherever in the world are actively participating in export and import of their medical system (16). It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life style, new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. Potentially valuable treasures in medicinal plants remain unexplored. By considering the scope of these medicinal plants we have to use more amounts of time and resources into developing medicines by medicinal plants. If we can come back to our nature, culture and tradition on use of medicinal plants, it can therefore, bring up a bright and healthy new generation (16). In Africa too, there is an abundance of natural resources such as plants and therefore, the indigenous society are inseparable from the natural environment. The ethnic groups are utilizing their traditional knowledge and experience in inheriting them to their younger generations in order to treat ailments; and their daily lives are depended on nature and this has influenced and helped them in forming traditional knowledge (17). Extraction is the main step for the recovery and isolation of bioactive components from plant parts. The analysis and extraction of plant matrices play an important role in the development, modernization and quality control of herbal formulation. Therefore, the extraction of bioactive compounds from plants for developmental/ therapeutic targets also requires identification of active principles (6).

Gas chromatography- mass spectrometry (GC-MS) is used as a technique that serves a broad range of applications aimed at sample identification, quantitative determination or both. The sample identification (qualitative analysis) needs a high degree of selectivity whereas quantitative analysis requires high accuracy (the precision and trueness) (18). GC-MS is one of the valuable tool for the identification of phytochemical compounds. It combines two analytical techniques to a single method of analyzing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of the components separately. It is the best technique to identify the bioactive compounds/constituents of long chain hydrocarbon, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc., (19, 20). The first step in investigating the presence of metabolites in any medicinal plants is by phytochemical screening that gives a broad idea on the nature of chemical constituents (21). To identify the compound, processing data from GC-MS must fulfil two criteria which includes; correct determination of mass spectrum of individual compounds; and accurate calculation of the abundance of chromatographic peaks corresponding to those compounds in each sample. Moreover, for sample introduction into GC-MS, there are three considerations. Firstly, the constituent of the sample must be volatile and secondly the analytes must be present at concentration which is appropriate to it. Thirdly, while injecting the sample, the sample must not degrade the separation (18).

The therapeutic effects of medicinal plants, which are used as a food-relish in folk medicine, are well documented. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modelled on plant substances. Aspirin, atropine, artimesinin, colchicine, digoxin, ephredrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine are a few important examples of what medicinal plants have given us in the past. Most of these plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people and some of these could not be substituted despite the enormous advancement in synthetic chemistry (22).

The Palma-Christi plant, an ecological ubiquitous, is scientifically known as *Anthocleista nobilis* (G. Don.) belonging to Loganiaceae family. It is a small to medium-sized tree growing to 30 m tall. It is commonly found in tropical African habitats such as the Mascarene Islands and Madagasccar as well as Southern, Western, and Eastern part of Nigeria. Also, it is a ubiquitous flora found densely grown everywhere in the forest, farmland, swampy area of land, roadsides among others in tropical rain forest. The bark is smooth and pale grey. The inner bark is cream-yellow and granular, whereas the twig has two spines above the leaf axis. The leaves are simple, broad and opposite, crowded at the end of branches, and petiole is 1-6 cm long. It is a photoautotroph. It exhibits tap root system, and the root can be erect, bend or curve. It is commonly called Candelabrum, Cabbage tree, Cabbage palm, or Palma christi in English language. It is also locally known as Uko nkirisi in Igbo language, Apa-Ora in Yoruba language, Kwari in Hausa language, Ogugu in Ilaje/Ikale language, and Duwa kuchi in Nupe language (23, 24). Conventionally, *A. nobilis* is used in the treatment of fever, stomach ache, diarrhea, and gonorrhea. It is also used as strong purgative, diuretic, and as poultice for treating sores in parts of West Africa. It is used as vapour bath for the treatment of leprosy, venereal diseases, and dysmenorrheal. In Mbano community in Imo State, Nigeria, the root bark decoctions are mostly used in the treatment of diabetes mellitus, gastrointestinal worms, malaria, and jaundice (25), while in Ilaje and Ikale communities in Ondo State, Nigeria, the root tinctures are mostly used as antioxidant, to regulate menstruation, for aiding conception, and in the treatment of rheumatism and arthritis. It is also reported to be used in local medicine in parts of West Africa for curing fever, arthritis, stomach ache, diarrhea, and gonorrhea, and as poultice for sores (26). The source material, *Anthocleista nobilis*, is a plant of strong medicinal properties; and this claim has been corroborated by the earlier report of Erhabor *et al*. (27), that *A. nobilis* root is consumed with other herbal agents to relieves sexual depression, stroke and various cardiovascular disorders, which are one out of the many causes of inadequate sexual functions in humans. And that the botanical is said to contain some minerals (Zn, Cu etc.,) as pro-fertility in human males (28). Nevertheless, there is no enough research performed on determination of the chemical composition of its root in literature upon the large geographic distribution. Hence, in continuation to the on-going project on the survey and bioactivity testing of pro-fertility plants, I herein report, the GC-MS based phytocompounds profiling of the crude extract from the root of *A. nobilis* with the aim of confirming the ethnomedicinal use of the plant towards harnessing its potentials in therapeutic/ developmental targets.

Material and Methods

Chemicals, reagents and instruments

Hexane, chloroform, ethyl acetate, methanol, syringe, Whatman's syringe filter and rotary evaporator were obtained from Sigma-Aldrich. All chemicals were of analytical grade and used without any further purification. The GC-MS analysis was conducted on a Perkin Elmer Clarus 500, GC-MS spectrometer equipped with Vf-5 MS fused silica capillary column of 30 m x 0.25 i.d and 0.25 µm film thickness.

Plant material and sample collection

The roots of *Anthocleista nobilis* were collected in a forest along Okitipupa-Ore road, Ondo State, Nigeria. Samples were identified and authenticated by a Botanist.

Preparation of plant material

The fresh roots of *Anthocleista nobilis* were detached from the whole uprooted plant, rinsed in water and spread on laboratory tables where they were dried under room temperature. The plant material were then transferred to an oven set at 40 ˚C for 5-10 minutes before been reduced to fine powder with the aid of a mechanical grinder.

Preparation of extract

200 g of the powdered plant material was macerated in 1 litre of methanol for 48 hours. The mixture was sieved using porcelain cloth and was further filtered using No.1 Whatman filter paper. The filtrate was concentrated using rotary evaporator and the crude concentrate was then stored at 4 ˚C until required for further experiment.

Gas chromatography (GC) - mass spectrometry (MS) analysis of Anthocleista nobilis roots

GC-MS was employed to detect the phytocomponents enhancing the bioactivity of the medicinal plant, *Anthocleista nobilis.* The sample of *Anthocleista nobilis* roots was prepared and diluted using methanol. 2 Ml of crude sample was suctioned using syringe and filtered by using Whatman's syringe filter (0.2 µm) and transferred into glass vials. Then, 1 µL of distilled sample was analysed by injecting into GC-MS with a split injector at 300˚C. The Vf – 5 MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm) was employed. The temperature programme was 50 ˚C, held for 10 minutes, increased at 3 ˚C/minutes for 250 ˚C and finally hold for 10 minutes. Inert helium gas was employed as a carrier gas at a constant flow rate of 1.0 Ml/minutes.

Identification of phytocomponents

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standards and Technology (NIST) having more than 62,000 patterns of the spectrum of the known components stored in the NIST library. The compound were identified by comparison of their retention indices (RI) with those provided in NIST library. Identification was assumed when a good match of RI was achieved (29).

Results and Discussion

It is believed that the use of chemical solvent in extraction, preservation and column chromatography procedure will affect the amount of the phytocomponents extracted. As a result, phytocompounds possessing diverse biological activities were assayed and found in the methanol root extract of *Anthocleista nobilis*. The total ion chromatogram (TIC) of methanol root extract of *Anthocleista nobilis* showing the GC-MS profile of the compounds identified is given in Figure 1. GC-MS chromatogram of the methanol extract of the plant material showed twenty two peaks which indicated the presence of twenty two phytocompounds. The mass spectra coupled with the chemical structure of the identified compounds are presented in Figure 2, while names of compounds, molecular formula, molecular weight, retention time, and bioactivity of the detected compounds are shown in Table 1.

The chromatographic determination of phytocomponuds in this study conforms/ relates with the work of Sharangouda *et al*. (22), where data of the antifertility characteristics and associated effects of chromatographic fractions of crude petroleum ether of *C. medica* seeds were presented to elucidate the active principle; and that further investigation on the plant extract, isolation of novel constituents were undertaken and carried out through thin layer chromatography. Also, the yet to be published preliminary phytochemical studies of the plant in question, *A. nobilis* roots, showed the presence of alkaloids, flavonoids, saponin, glycosides and tannins in its methanol extract. This result equally agrees with the findings of Sharangouda *et al*. (22), while studying the preliminary phytochemical of *C. medica* petroleum ether extract.

Furtherance to the findings of this study (Table 1), the plant has been implicated containing antioxidative compounds (30-32) that could play vital role enhancing fertility in humans; and according to Meena and Sreenivasula (33), successful fertility depends on various factors to include antioxidative status. As observed in this study, the phytocompounds identified are a group of naturally occurring compounds that are present in plants (33), and when consumed by mammals, they may have protective effects against certain forms of cancer, cardiovascular diseases (as observed in Table 1), osteoporosi and may also prevent undesirable menopausal symptoms. Dietary exposure to phytocompounds is common for both animals and humans. Exposures occur through regular dietary intake or through nutritional supplements of phytocompounds (33).

No doubt, the phytocompounds in the findings of this study, were identified based on the mass fragmentation pattern and comparing the peak area and retention time of the NIST database; in which the most abundant compound being 5-Hydroxymethylfurfural (13.64%: occurring thrice) used as antioxidant agent (31), followed by 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (9.09%: occurring twice) used as antimicrobial, anti-inflammatory, antioxidant agent (27), and Heptanoic acid (9.09%: occurring twice) of unknown medicinal property (Table 1). On comparison of the mass spectra of constituents with the main library, all the compound's data base gave more than 100% match as well as confirmatory compound structure match.

The compound, 2,4-Dihydroxy- 2,5- dimethyl-3 (2H)-furan-3-one (Table 1) had been implicated as dietary and food-grade flavour ingredient (30). This finding conforms to the report that "diet is one piece of the fertility puzzle, and it happens to be a piece that someone can control, along with alcohol consumption, smoking habits, and stress levels. But, "someone is not going to unblock a fallopian tube or cure a lack of sperm with just diet alone". Rather, your nutrition matters for your overall health, and there is good research that it can affect someone's fertility as well (authors' unpublished data).

Mass spectrometry becomes a vital tool in the hands of the organic chemists and biochemists, because of its potential to supply the definitive, qualitative and quantitative information on molecules based on their structural compositions. Gas chromatography attached to a Mass Spectrometer (GC-MS) enables mixture of small molecules mainly organic compounds of low molecular weight (<600) which can be analysed (6, 34).

Plants produce diverse phytochemicals known as secondary metabolites. It is well known that plants produce these metabolites to protect themselves from pathogenic attacks. Hitherto, from the available supportive literature, secondary metabolites of plant origin possesses several biological activities such as antimicrobial, antifungal, anticancer, antioxidant, antiviral, antiulcer, and anti-inflammatory activities (35), for relief, and as well as management of illnesses in mankind. Owing to the biological activities of the plant-derived metabolites, as observed in the methanol extract of *Anthocleista nobilis* root, many source material of plant origin have becomes field of great scientific interest, and further study of these phytoconstituents may prove the medicinal importance in future.

From the information provided so far; it can be inferred that natural bioactive phytocompounds have been suggested as alternative sources for dietary, antioxidant, antibacterial, antimicrobial, anti-inflammatory, anti-acne, antibiotics etc. The chemical features of these constituents differ considerably among different species. This approach is alluring, in part, because they constitute a potential source of bioactive compounds that have been professed by the general public as comparatively safe, and often act at multiple and novel target sites, thereby increasing the potential of addressing various human inadequacies, sexual health inclusive.

Conclusion

From the result obtained for GCMS analysis of the methanol root extract of *Anthocleista nobilis*, there contain phytocompounds that could be used to treat or control diverse ailments like depression, stroke, inflammation, acne, cardiovascular disorder, and skin infections. The correlation among the phytochemical constituents with their biological activities is now being the matter of innovative thought. Thus, this type of study may give information on nature of active principles present in the medicinal plant. These phytocompounds presumed to be responsible for eliciting the medicinal activity of this plant, *Anthocleista nobilis*, and its extract may be a good biochemical agent that could be added as a chemical basis in therapeutics.

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Ethical issue

Author is aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Author adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

Author declare that there is no conflict of interests.

Author's contribution

Author of this study have a complete contribution for data collection, data analysis and manuscript writing.

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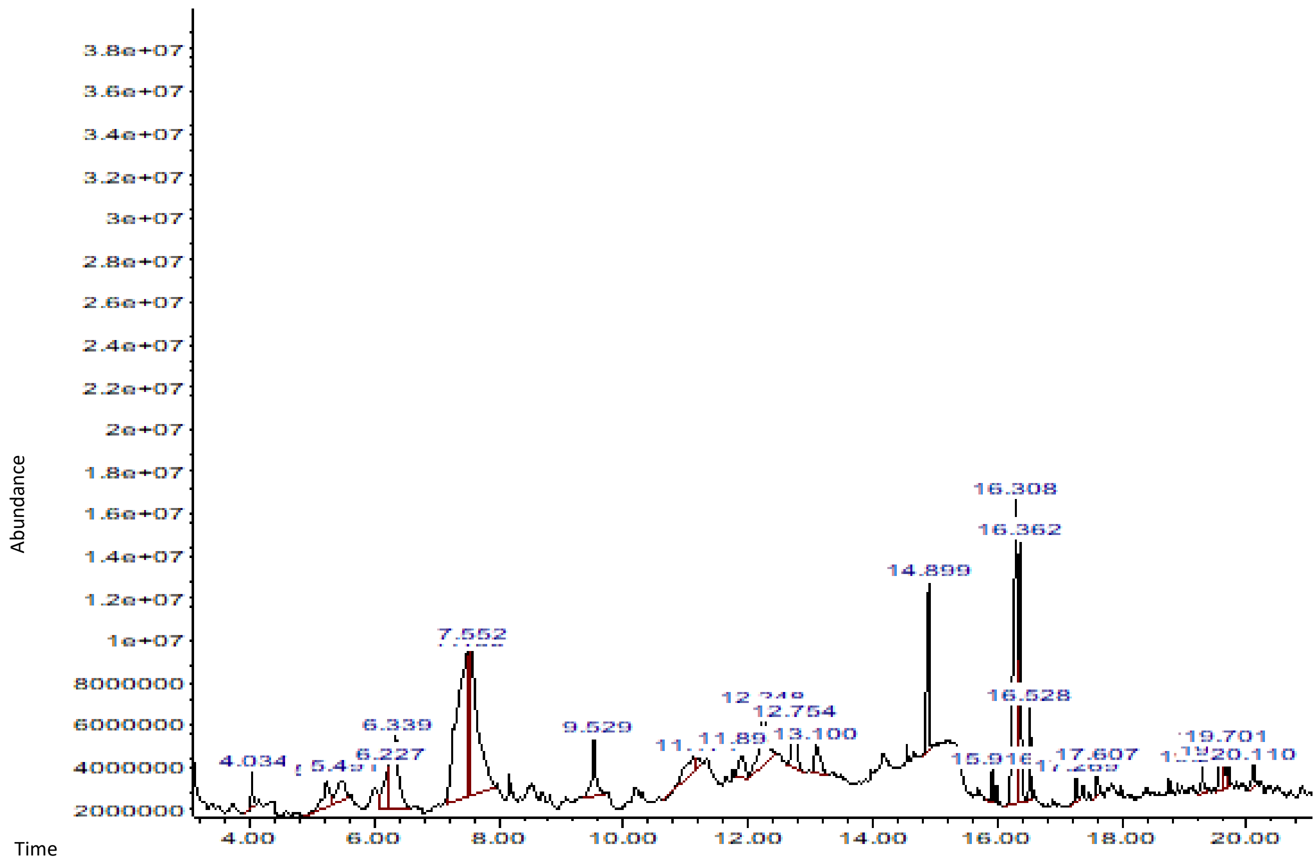
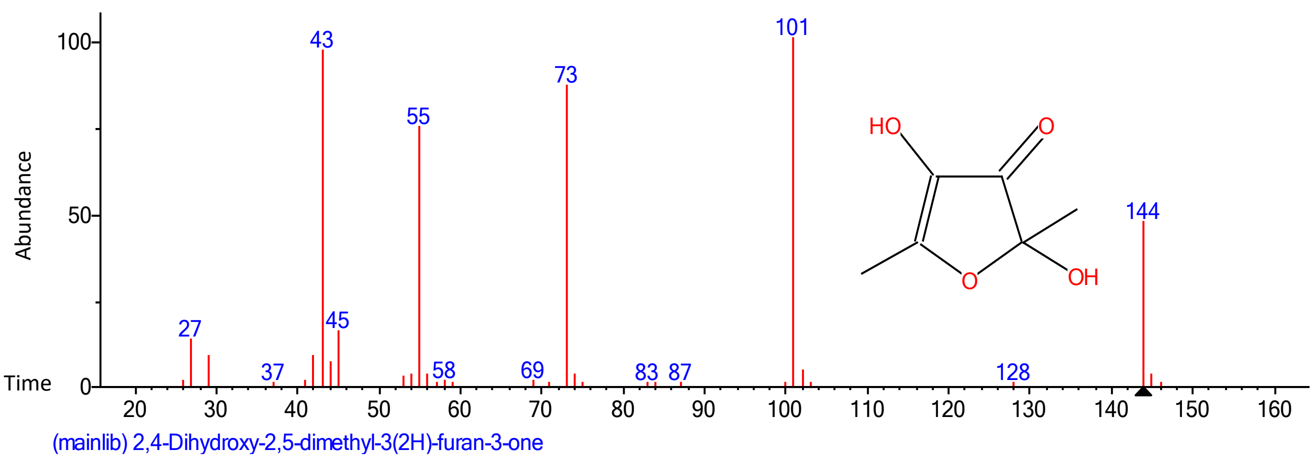
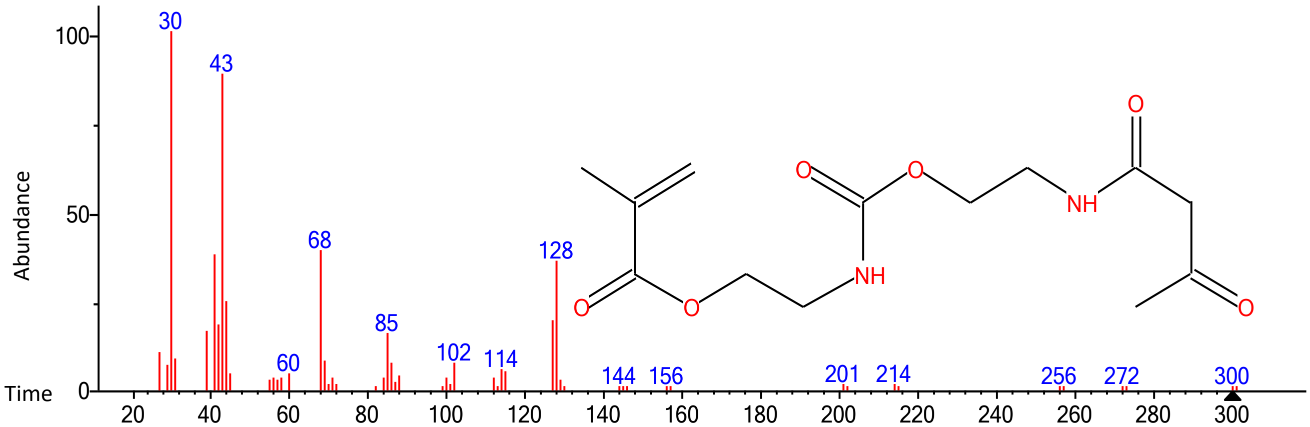
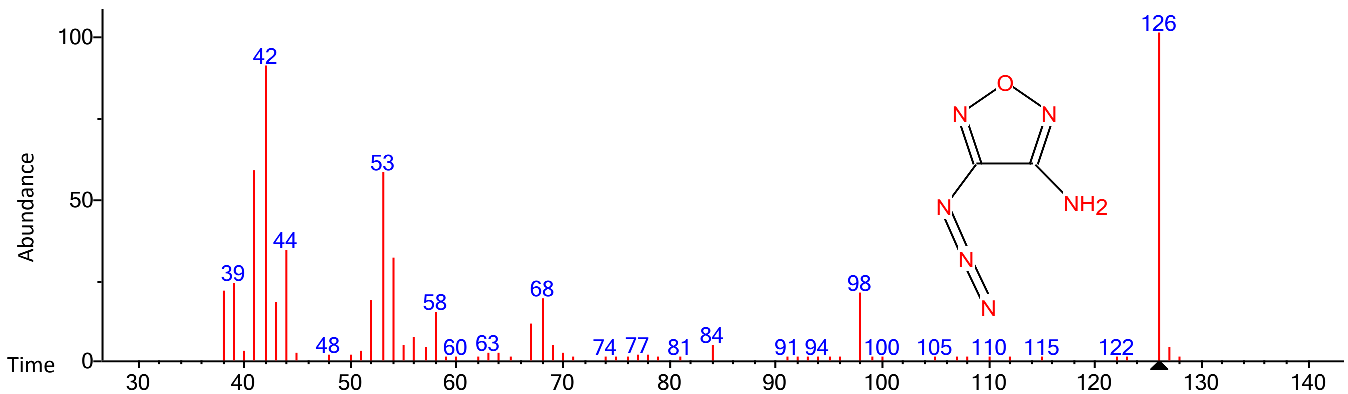


the Total Ion Chromatogram (TIC) of methanol root extract of *Anthocleista nobilis*

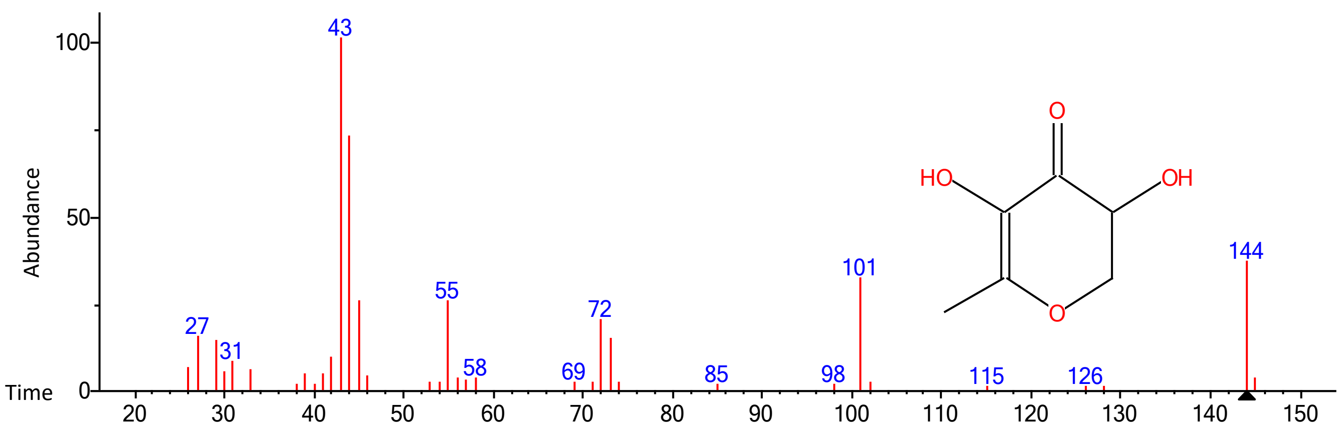




(mainlib) 2-propenoic acid, 2-methyl-, 2-[[[2-[(1,3-dioxobutyl)amino]ethoxy]carbonyl]amino]ethyl ester



(mainlib) Furazanamine, 4-azido-



(mainlib) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

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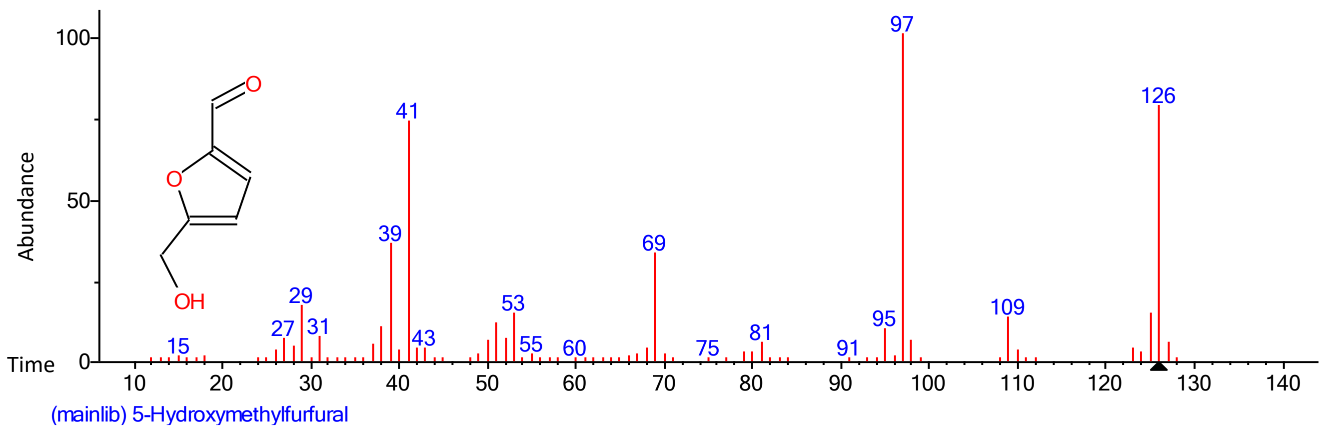

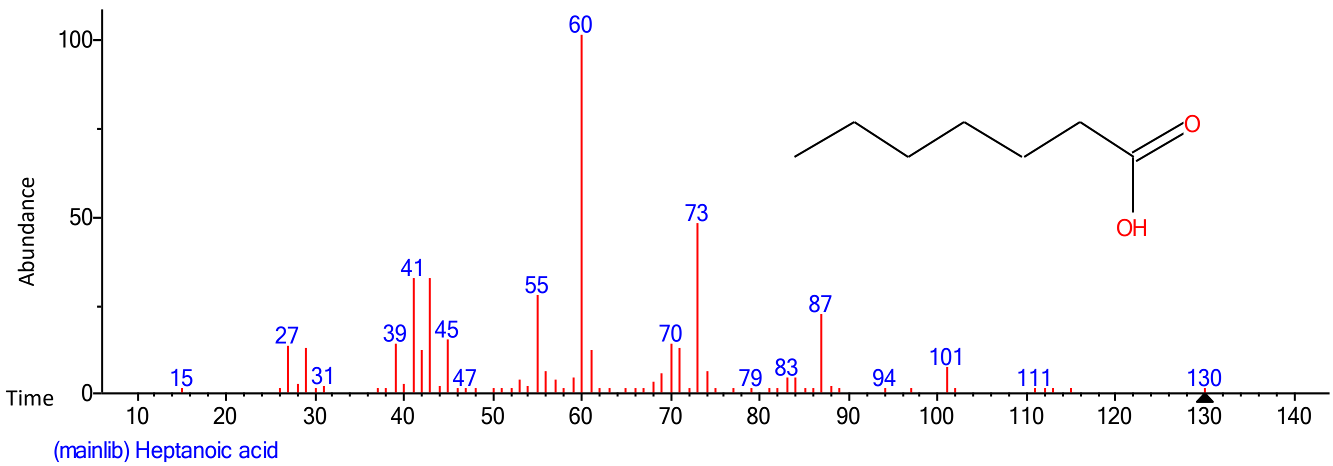

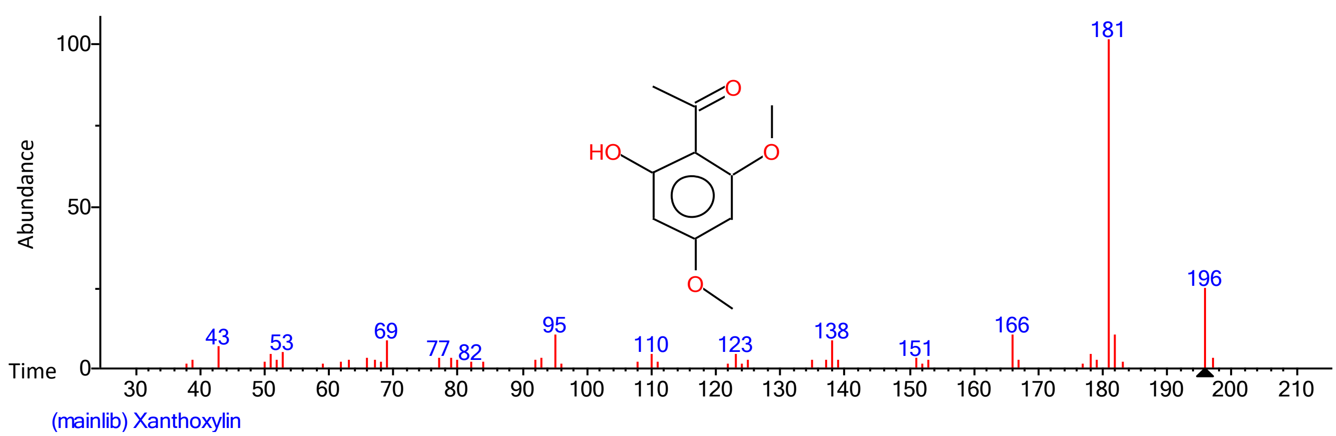

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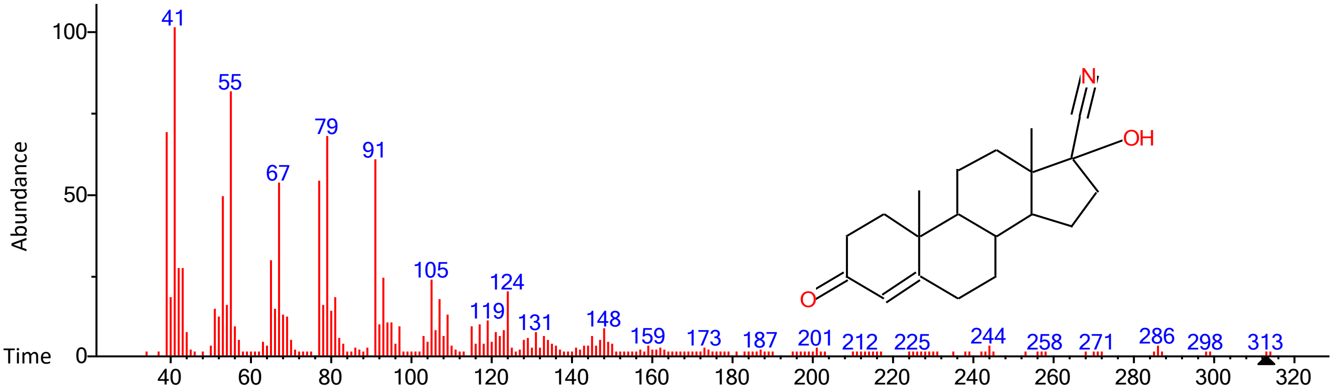
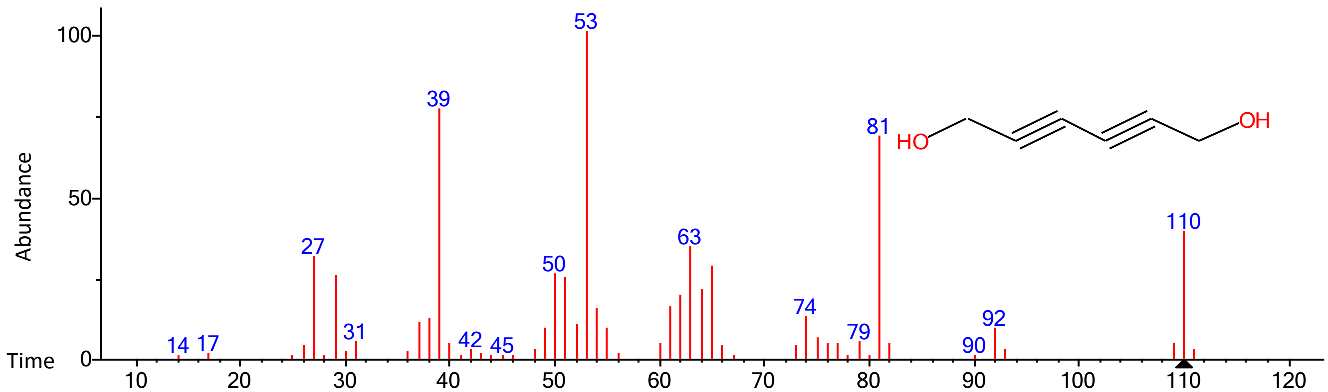
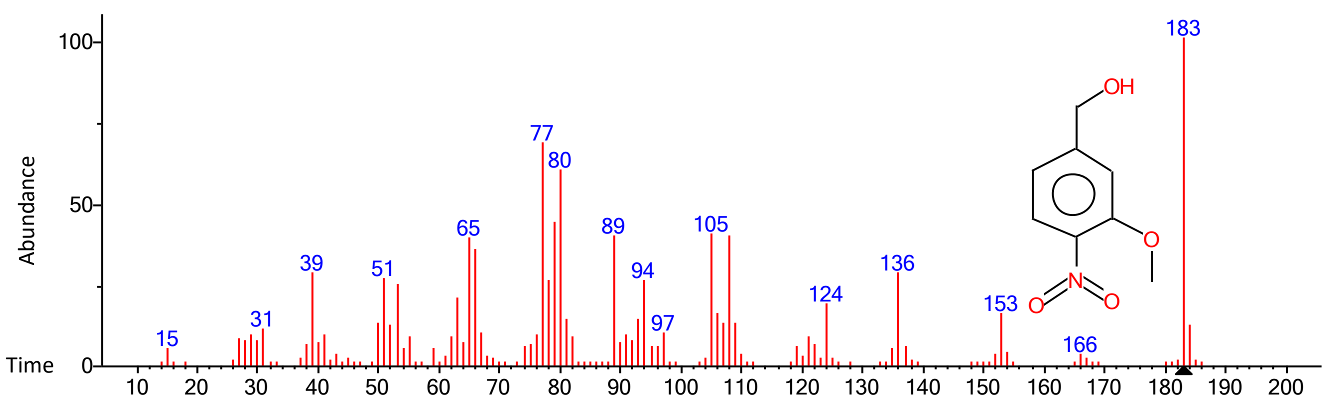


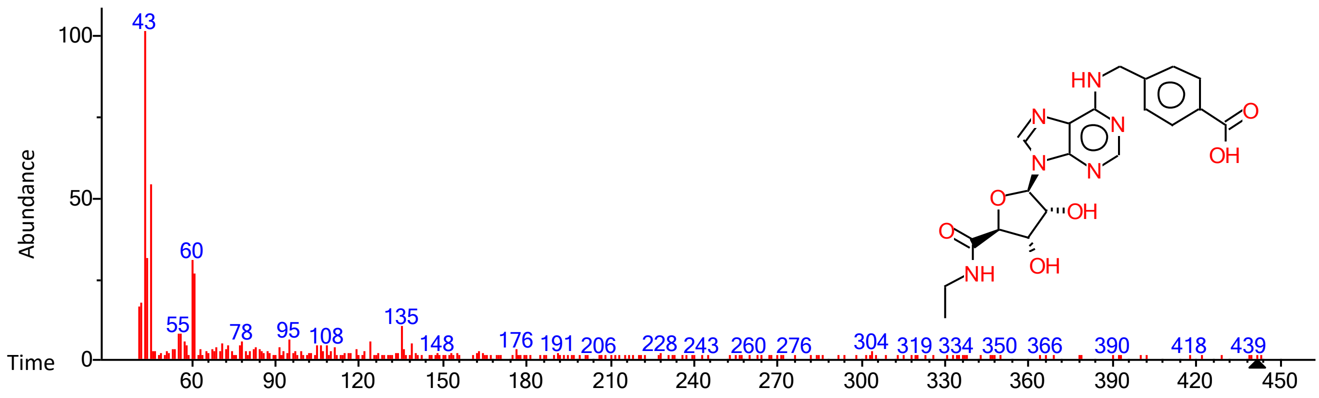
(mainlib) Preg-4-en-3-one, 17α-hydroxy-17β-cyano-



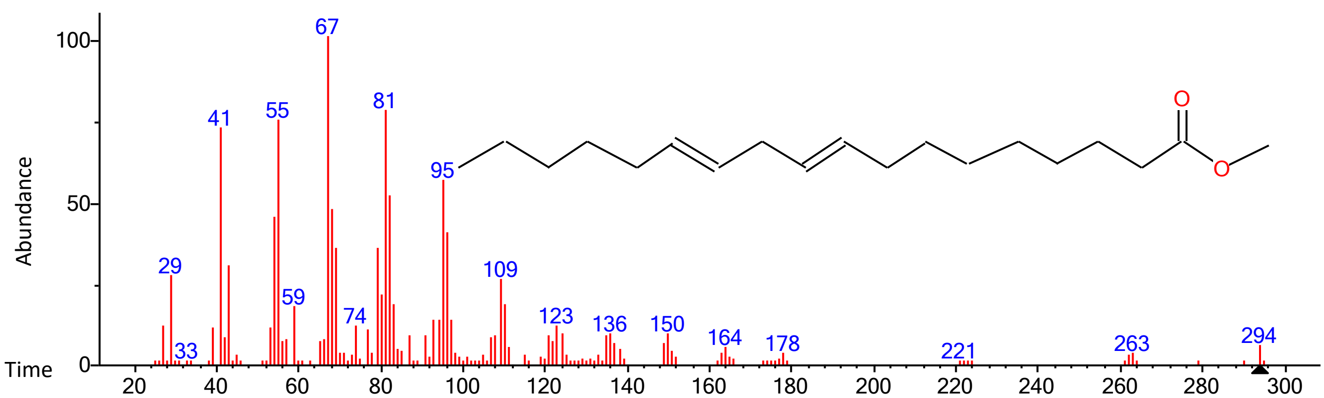
(mainlib) 2,4-Hexadiyne-1,6-diol



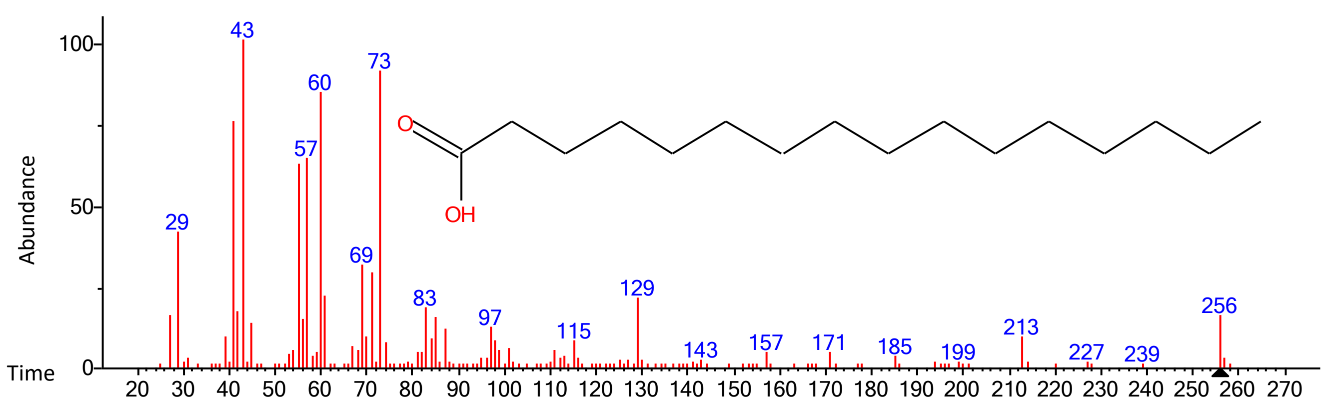
(mainlib) Benzenemethanol, 3-methoxy-4-nitro-



(mainlib) Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-



(mainlib) 9,12-Octadecadienoic acid, methyl ester



(mainlib) n-Hexadecanoic acid

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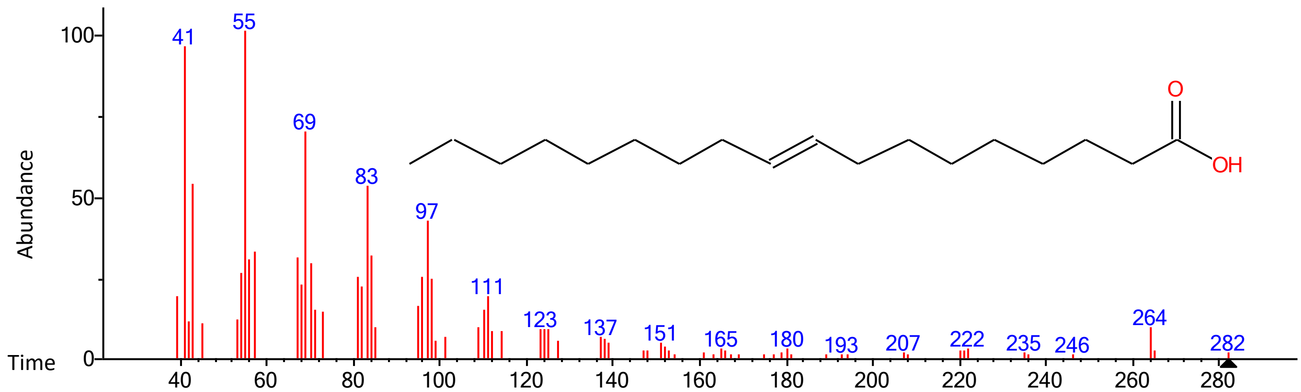

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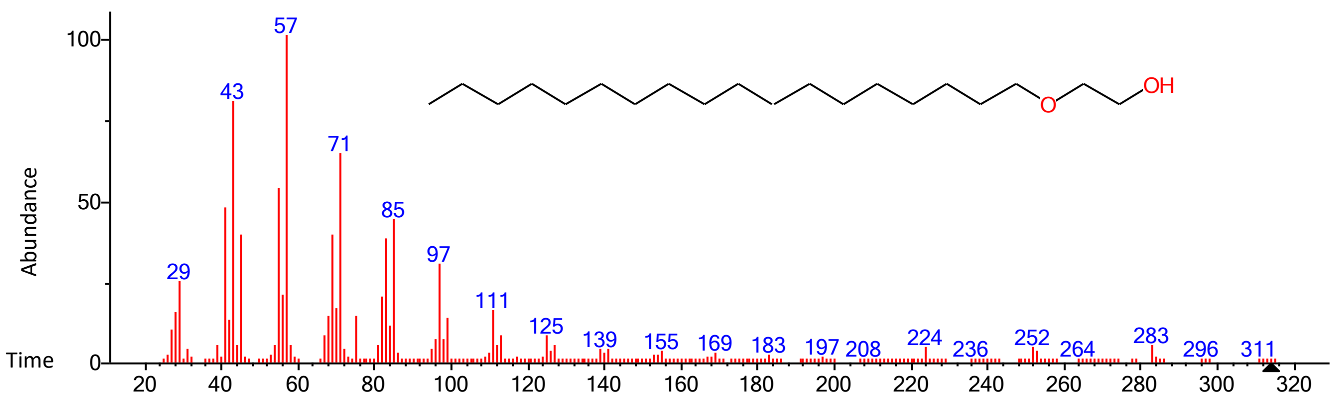
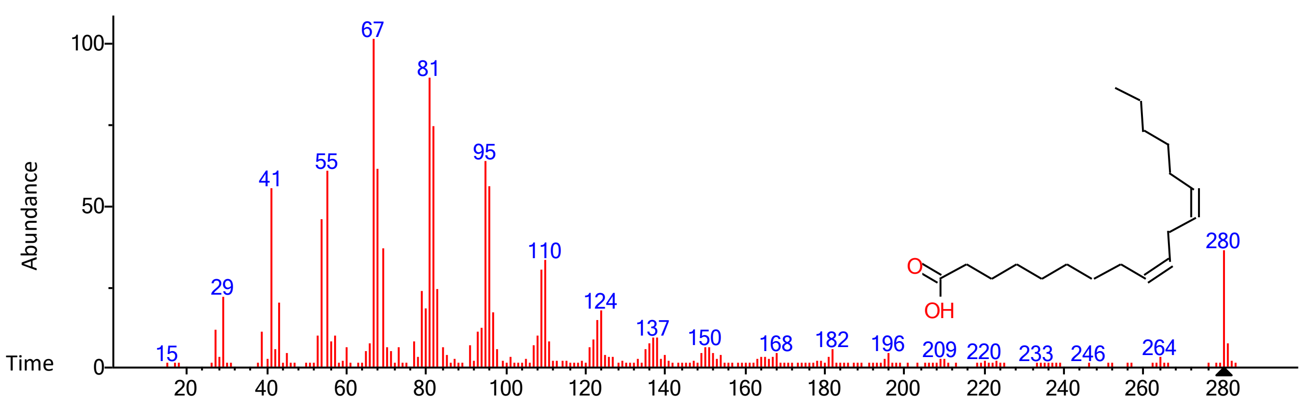
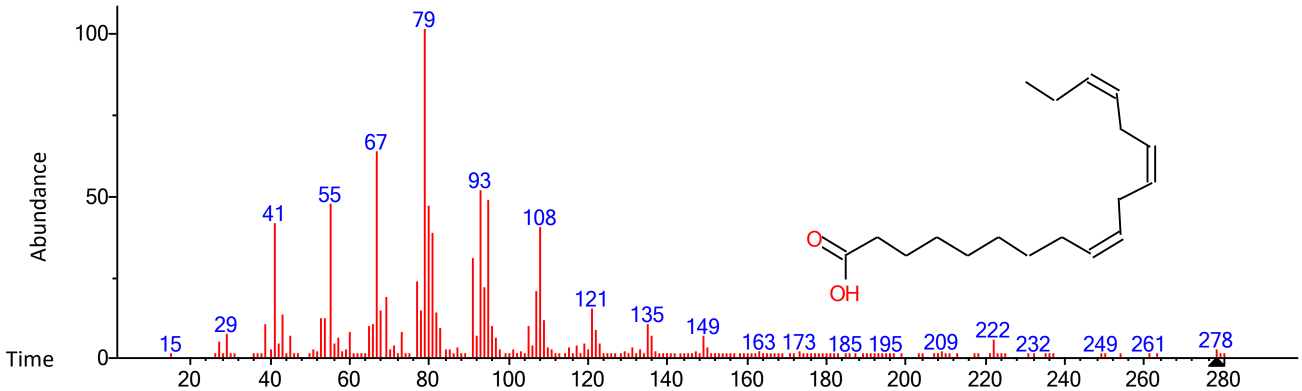


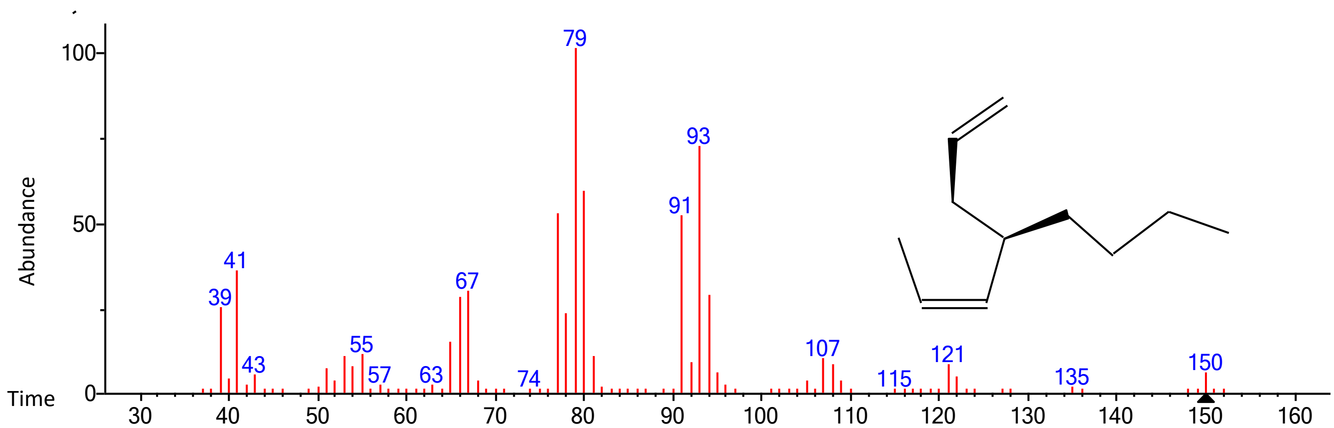
(mainlib) Ethanol, 2-(octadecyloxy)-



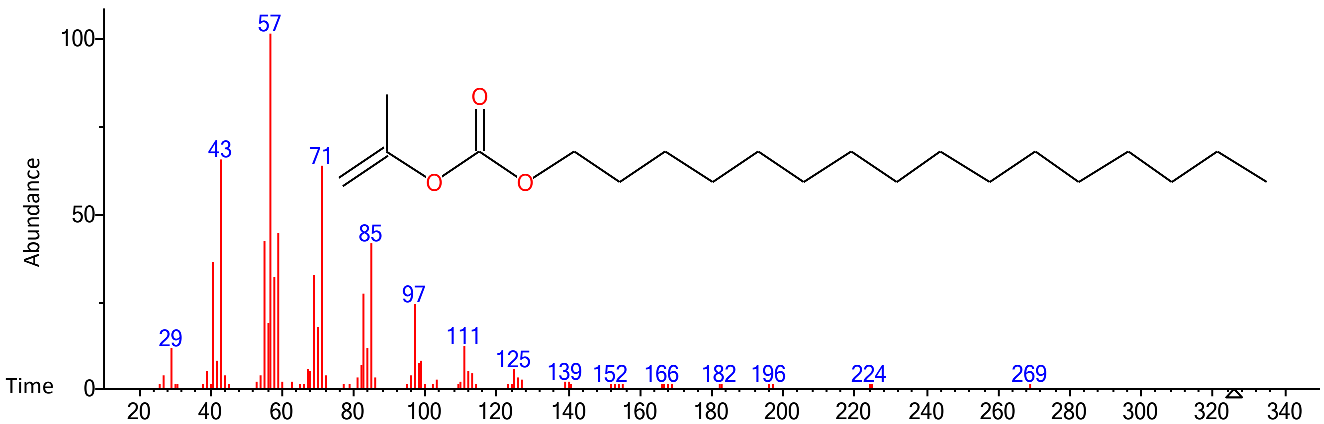
(mainlib) 9,12-Octadecadienoic acid (Z,Z)-



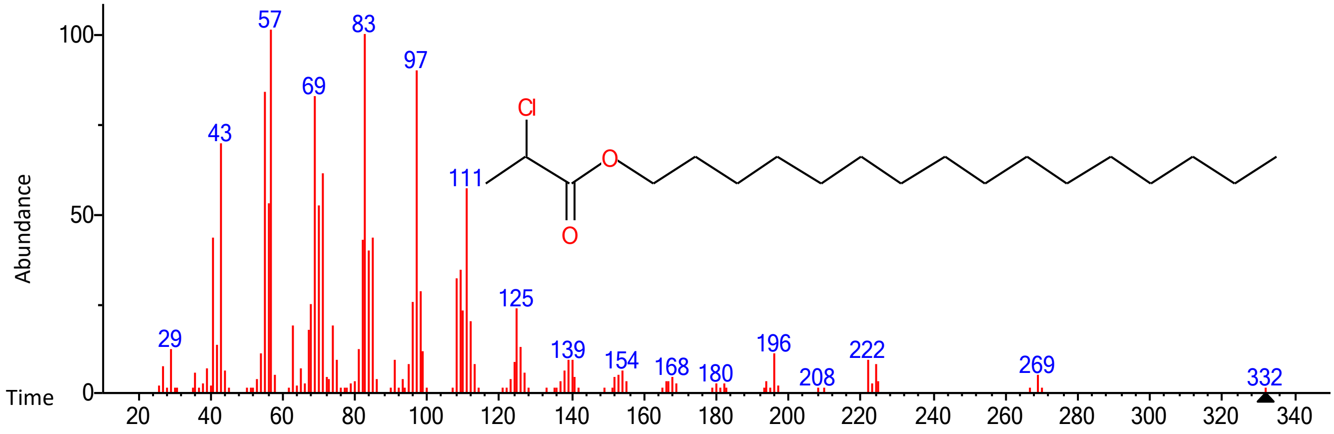
(mainlib) 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-

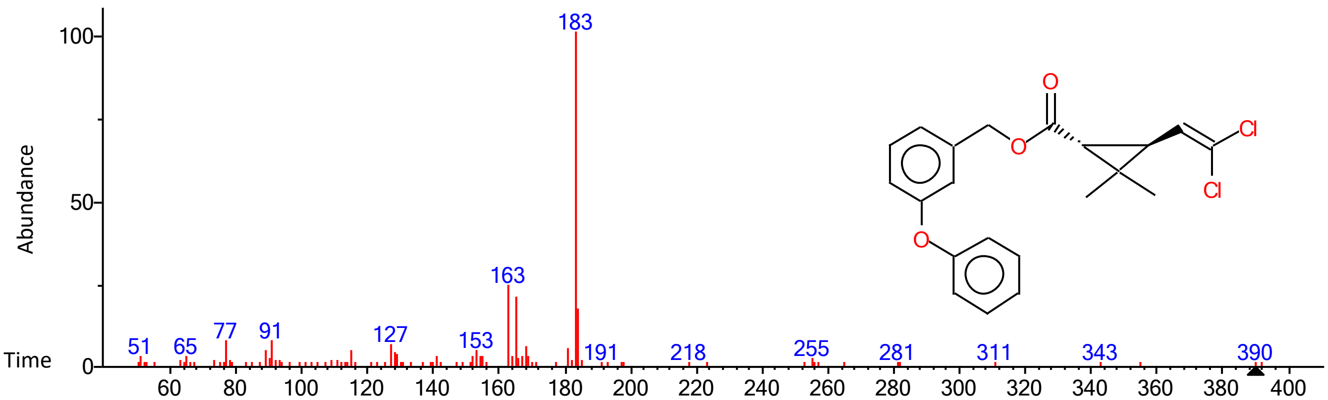


(mainlib) cis-3-Butyl-4-vinyl-cyclopentene



(mainlib) Carbonic acid, hexadecyl prop-1-en-2-yl ester





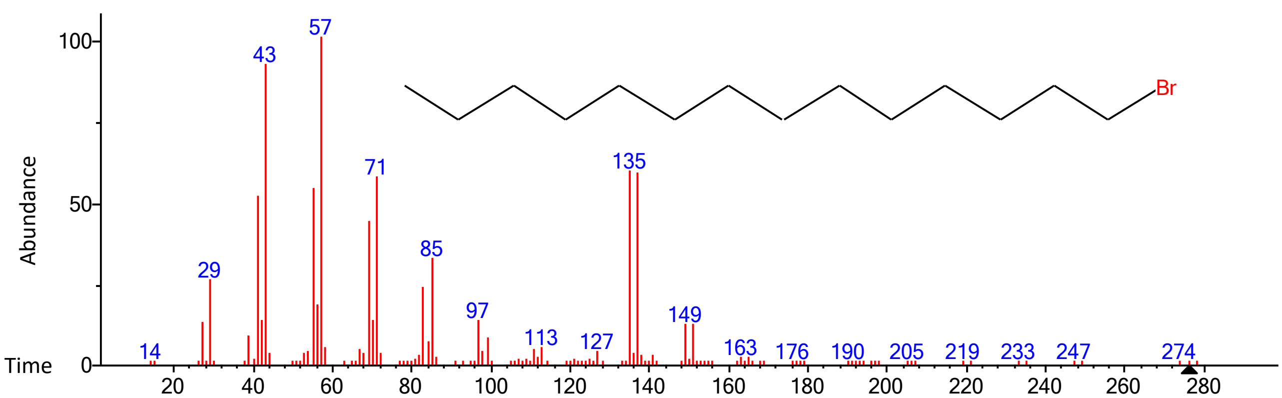


Figure 2. Shows the mass spectra of the twenty two phytocompounds identified by GC-MS analysis in *Anthocleista nobilis* root

Table 1. The names, molecular formula, molecular weight, retention time and bioactivity of the compounds identified in methanol root extract of *Anthocleista nobilis* by GC-MS

No.	Name of Compound	Molecular Formula	Molecular weight (g/mol)	Retention time (min.)	Bioactivity
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12	4.034	Dietary, Food-grade flavour ingredient (30)
2	2-propenoic acid, 2-methyl-,2[[2[(1,3 dioxobutyl)amino] ethoxy]carbonyl]amino]ethyl ester	$C_{13}H_{20}N_2O_6$	300.31	5.236	Flavouring agent, antibacterial (30)
3	Furazanamine,4-azido-	$C_2H_3N_6O$	127.09	5.491	Unknown
4	4H-Pyran-4-one,2,3-dihydro-3,5 dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	6.227	Antimicrobial, anti-inflammatory and antioxidant capacity (30)
5	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	7.485	Antioxidant (31)
6	Xanthoxylin(2-hydroxy-4 methoxyphenyl)-	$C_9H_{10}O_3$	166.17	9.529	Unknown
7	Heptanoic acid	$C_7H_{14}O_2$	130.18	11.118	Unknown
8	Preg-4-en-3-one,17.alpha.-hydroxy -17.beta.-cyano-	$C_{21}H_{32}O_2$	316.5	11.893	Unknown
9	2,4-Hexadiyne-1,6-diol	$C_6H_6O_2$	110.11	12.248	Antibiotic (30)
10	Benzenemethanol,3 methoxy-4-nitro	$C_8H_9NO_4$	183.16	12.754	Unknown
11	Adenosine,4-'de(hydroxymethyl)-4'-[N-ethylaminoformyl]-	$C_{20}H_{22}N_6O_6$	442.4	13.100	Unknown
12	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.899	Antioxidant, anti-inflammatory (32)
13	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	15.916	Anti-inflammatory (21)
14	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	16.308	Antimicrobial, anti-acne (30)
15	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	16.362	Anti-inflammatory (32)
16	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	$C_{18}H_{30}O_2$	278.4	17.269	Preventive against cardiovascular diseases (7)
17	cis-3-Butyl-4-vinyl-cyclopentene	$C_{11}H_{18}$	150.26	17.607	Unknown
18	Carbonic acid, hexadecyl prop-1-en-2-yl ester	$C_{20}H_{38}O_3$	326.5	19.289	Flavouring agent (32)
19	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.5	19.578	Unknown
20	2-Chloropropionic acid, hexadecyl ester	$C_{19}H_{37}ClO_2$	332.9	19.628	Unknown
21	Cyclopropanecarboxylic acid, 3-(2, 2-dichloroethenyl)-2,2-dimethyl,(3phenoxyphenyl)methyl ester,trans-	$C_{21}H_{20}Cl_2O_3$	391.3	19.701	Unknown
22	Tetradecane, 1-bromo-	$C_{14}H_{29}Br$	277.28	20.110	Antioxidant (32)

J Infertil Reprod Biol, 2021, Volume 9, Issue 1, Pages: 7-21. https://doi.org/10.47277/JIRB/9(1)/7

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