

Full Length Research Paper

Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS)

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Received 29 January, 2014; Accepted 20 March, 2015

In this study, the alkaloid compounds of *Solanum nigrum* have been evaluated. The chemical compositions of the leaf methanol extract of *S. nigrum* were investigated using Gas chromatography-mass spectroscopy (GC-MS). GC-MS analysis of *S. nigrum* alkaloid leaf methanol extract revealed the existence of the cyclopentasiloxane-decamethyl, L-proline, ethylester, 2-ethyl-1-butanol, methyl ether, cyclopentasiloxane-ocamethyl, betanedioic acid, hydroxyl, diethyl, ester, 1.1.3.3.5.5.7.7-octamethyl-1-7-(2methyl-propoxy) tetrasiloxane-1, dodecanoic acid, 3-hydroxy-, ethyl ester, cyclopentasiloxane-ocamethyl, dodecanedioic acid, bistert-butyl dimethylsilyl ester, 2-pyrrolidinecarboxylic acid-5-oxo, ethyl ester, 1-dodecanamine, N.N-dimethyl, cyclooctasiloxane, hexadecamethyl, 5-keto-2, 2-dimethylheptanoic acid, ethyl ester, cyclodecasiloxane, eicosamethyl, 9.12.15-octadecatrienoic acid, octadecanoic acid, octadecenal, 9-octadecenamamide, octadecane, 3-ethyl-5-(ethylbutyl), N-acetyl-L-tryptophan ethyl ester, ethyl iso-allocholate, phthalic acid, di(2-propylpentyl)ester and 17-(1.5-Dimethylhexyl)-10. 13-dimethyl-2.3.4.7.8.9.10.11. 12.13.14.15.16.17-tetradecahydro-1H. Alkaloids extract from leaf of *S. nigrum* were assayed for *in vitro* antibacterial activity against *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aerogenosa* and *Klebsiella pneumonia* using the diffusion method in agar. The zone of inhibition was compared with different standard antibiotics. The diameters of inhibition zones ranged from 0.8 to 2.01 mm for all treatments.

Key words: Alkaloids, antibacterial activity, gas chromatography-mass spectroscopy (GC-MS) analysis, *Solanum nigrum*.

INTRODUCTION

Plants are rich source of secondary metabolites with interesting biological activities (Koduru et al., 2006). Several plant products have been shown to exert a protective role against the formation of free radicals and

playing a beneficial role in maintaining disease condition (Ajitha et al., 2001). *Solanum nigrum* is a common weed in gardens, fields and waste-land throughout the country (up to 1500 m altitude) (Figure 1). It is found in Baghdad,

Basrah, Kut, Tal-Kaif, and Sulaimaniya. Leaves of *S. nigrum* contain solanin; solanidin are poisonous to cattle, sheep, horses and goat. The effects of poison are necrosis, paralysis, salivation, vomiting and diarrhoea. The medicinal value of drug plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body. These chemicals include alkaloids, flavanoids, glucosides, tannins, gums, resins, essential oils, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals, etc. Very few of these chemicals are toxic also (Haraguchi et al., 1999; Sashikumar et al., 2003).

The photochemicals with adequate antibacterial activity will be used for the treatment of bacterial infections (Iwu et al., 1999; World Health Organization, 2002; Purohit and Vyas, 2004; Krishnaraju et al., 2005). Successful extraction is largely dependent on the type of solvent used in the extraction procedure. The most often tested extracts are water extract as a sample of extract that is primarily used in traditional medicine and extracts from organic solvents, such as methanol, as well as ethyl acetate, acetone, chloroform, and dichloromethane. Diffusion and dilution method are two types of susceptibility test used to determine the antibacterial efficacy of plant extracts. Diffusion method is a qualitative test which allows classification of bacteria as susceptible or resistant to the tested plant extract according to the size of diameter of the zone of inhibition (Alves et al., 2000; Palombo and Semple, 2001; Uzun et al., 2004; Cos et al., 2006; Ncube et al., 2008; Stanojević et al., 2010). Considering the high economical and pharmacological importance of secondary plant metabolites, industries are deeply interested in utilizing plant tissue culture technique for large scale production of these substances (Misawa, 1994). The aim of this study is to assess the antibacterial activity of alkaloids extracts from the leaves of *S. nigrum*, which can be the basis for the synthesis of new antibiotics. This is because of increase in the emergence of bacterial strains resistant to multiple clinical disease.

MATERIALS AND METHODS

Collection and preparation of plant

In this research, the leaves were dried at room temperature for 13 days and when properly dried the leaves were powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Extraction and identification of alkaloids

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made

alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test.

Mayer's test

To the filtrate in test tube I, 1 ml of Mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendoff's test

To the filtrate in test tube II, 1 ml of Dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Wagner's test

To the filtrate in tube III, 1 ml of Wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis of the methanol extract of *S. nigrum* was carried out using a Clarus 500 Perkin- elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold-Perkin Elmer Turbomass 5.1 spectrometer with an Elite-1 (100% Dimethyl poly siloxane), 30 m × 0.25 mm ID × 1 µm of capillary column. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system operated in electron impact mode with ionization energy of 70 eV. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed at 110°C (isothermal for 2 min), with an increase of 100°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45 to 450 (m/z). The mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver 5.2.

Measurement of antibacterial activity

The antibacterial activity of alkaloids was determined using agar well diffusion method. Wells of 5 mm diameter were punched in the agar medium with sterile cork borer and filled with plant alkaloid extract. Standard antibiotics, penicillin, kanamycin, cefotaxime, streptomycin and rifampin (1 mg/ml) were also tested for their antibacterial activity. The plates were incubated at 37°C for

Table 1. Compounds present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

Serial No.	Phytochemical compound	Formula	Molecular weight	Exact mass	Structure
1	Cyclopentasiloxane. decamethyl	C ₁₀ H ₃₀ O ₅ Si ₅	370	370.093956	Figure 3
2	L-Proline, ethylester	C ₇ H ₁₃ NO ₂	143	143.094628	Figure 4
3	2-Ethyl-1-butanol, methyl ether	C ₇ H ₁₆ O	116	116.1201153	Figure 5
4	Cyclopentasiloxane. ocamethyl	C ₈ H ₂₄ O ₄ Si ₄	296	296.075165	Figure 6
5	Betanedioic acid , hydroxyl,diethyl, ester	C ₈ H ₁₄ O ₅	190	190.084124	Figure 7
6	1.1.3.3.5.5.7.7-Octamethy-l-7-(2methyl-propoxy) tetrasiloxane-1	C ₁₂ H ₃₄ O ₅ Si ₄	370	370.14833	Figure 8
7	Dodecanoic acid, 3-hydroxy-, ethyl ester	C ₁₄ H ₂₈ O ₃	244	244.203845	Figure 9
9	Dodecanedioic acid. Bistert-butyl dimethylsilyl ester	C ₂₄ H ₅₀ O ₄ Si ₂	458	458.324762	Figure 10
10	2-Pyrrolidinecarboxylic acid-5-oxo, ethyl ester	C ₇ H ₁₁ NO ₃	157	157.073894	Figure 11
11	1-Dodecanamine, N.N-dimethyl	C ₁₄ H ₃₁ N	213	213.24565	Figure 12
12	Cyclooctasiloxane, hexadecamethyl	C ₁₆ H ₄₈ O ₈ Si ₈	592	592.15033	Figure 13
13	5-keto-2, 2-dimethylheptanoic acid, ethyl ester	C ₁₁ H ₂₀ O ₃	200	200.141245	Figure 14
14	Cyclodecasiloxane, eicosamethyl	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	740	740.187912	Figure 15
15	9.12.15-Octadecatrienoic acid	C ₁₈ H ₃₀ O ₂	278	278.22458	Figure 16
16	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	284.27153	Figure 17
17	Octadecenal	C ₁₈ H ₃₄ O	266	266.260965	Figure 18
18	9-Octadecenamide	C ₁₈ H ₃₅ NO	281	281.271864	Figure 19
19	Octadecane, 3-ethyl-5-(ethylbutyl)	C ₂₆ H ₅₄	366	366.422552	Figure 20
20	N-Acetyl-L-tryptophan ethyl ester	C ₁₅ H ₁₈ N ₂ O ₃	274	274.131742	Figure 21
21	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	436.318874	Figure 22
22	Phthalic acid, di(2-propylpentyl)ester	C ₂₄ H ₃₈ O ₄	390	390.27701	Figure 23
23	17-(1.5-Dimethylhexyl)-10. 13-dimethyl-2.3.4.7.8.9.10.11. 12.13.14.15.16.17-tetradecahydro-1H	C ₂₇ H ₄₆ O	386	386.354866	Figure 24

The negative control was added without adding the cultures to know the sterile conditions. Then Petri dishes were placed in the refrigerator at 4°C or at room temperature for 1 h for diffusion, then incubate at 37°C for 24 h. Observation was done on zone of inhibition which produced different antibiotics. Measurement was done using a scale and the average of two diameters of each zone of inhibition was recorded.

RESULTS AND DISCUSSION

GC-MS analysis of alkaloid compound clearly

showed the presence of twenty three compounds. The alkaloid compound, formula, molecular weight and exact mass are as shown in Table 1. The GC-MS chromatogram of the 23 peak of the compounds detected are as shown in Figure 2. Chromatogram GC-MS analysis of the methanol extract of *S. nigrum* showed the presence of twenty three major peaks and the components corresponding to the peaks were determined as follows. The first setup peaks were determined to be cyclopentasiloxane-decamethyl (Figure 3). The second peaks were indicated to be L-proline,

ethylester (Figure 4). The next peaks was considered to be 2-ethyl-1-butanol, methyl ether, cyclopentasiloxane-ocamethyl, betanedioic acid, hydroxyl, diethyl, ester, 1.1.3.3.5.5.7.7-Octamethy-l-7-(2methyl-propoxy) tetrasiloxane-1, dodecanoic acid, 3-hydroxy-, ethyl ester, cyclopentasiloxane-ocamethyl, dodecanedioic acid, bistert-butyl dimethylsilyl ester, 2-pyrrolidinecarboxylic acid-5-oxo, ethyl ester, 1-dodecanamine, N.N-dimethyl, cyclooctasiloxane, hexadecamethyl, 5-keto-2, 2-dimethylheptanoic acid, ethyl ester, cyclodecasiloxane,

Table 2. Zone of inhibition (mm) of test bacterial strains to alkaloid leaf extracts of *Solanum nigrum* (L.) and standard antibiotics.

Alkaloid antibiotic	<i>K. pneumonia</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>E. coli</i>
Alkaloid	1.8±0.42	1.4±0.59	1.9±0.61	2.01±0.51	1.7±0.62
Kanamycin	0.8±0.3	0.5±0.4	0.6±0.2	0.4±0.1	0.9±0.1
Cefotaxime	1.3±0.5	1.5±0.1	1.2±0.1	1.2±0.6	1.2±0.3
Penicillin	1.1±0.2	1±0.5	1±0.4	1±0.2	1.6±0.1
Streptomycin	1.2±0.3	1.1±0.3	1.3±0.5	1.7±0.2	1.3±0.6
Rifampin	1.1±0.1	1.1±0.1	1.2±0.5	0.6±0.1	0.8±0.2



Figure 1. Leaves of *Solanum nigrum*.

eicosamethyl, 9.12.15-octadecatrienoic acid, octadecanoic acid, octadecenal, 9-octadecenamide, octadecane, 3-ethyl-5-(ethylbutyl), N-Acetyl-L-tryptophan ethyl ester, ethyl iso-allocholate, phthalic acid, di(2-propylpentyl)ester and 17-(1.5-Dimethylhexyl)-10. 13-dimethyl-2.3.4.7.8.9.10.11.12.13.14.15.16.17-tetradecahydro-1H (Figures 5 to 23). Among the

identified phyto-compounds are the property of anti-oxidant and antimicrobial activities (Stainer et al., 1986; Singh et al., 1998; Prescott et al., 1999; Kumar et al., 2001; Purohit and Vyas, 2004; John and Senthilkumar, 2005; Venkatesan et al., 2005; Santh et al., 2006; Sazada et al., 2009). Plant based antimicrobials have enormous therapeutic potential as they can serve the

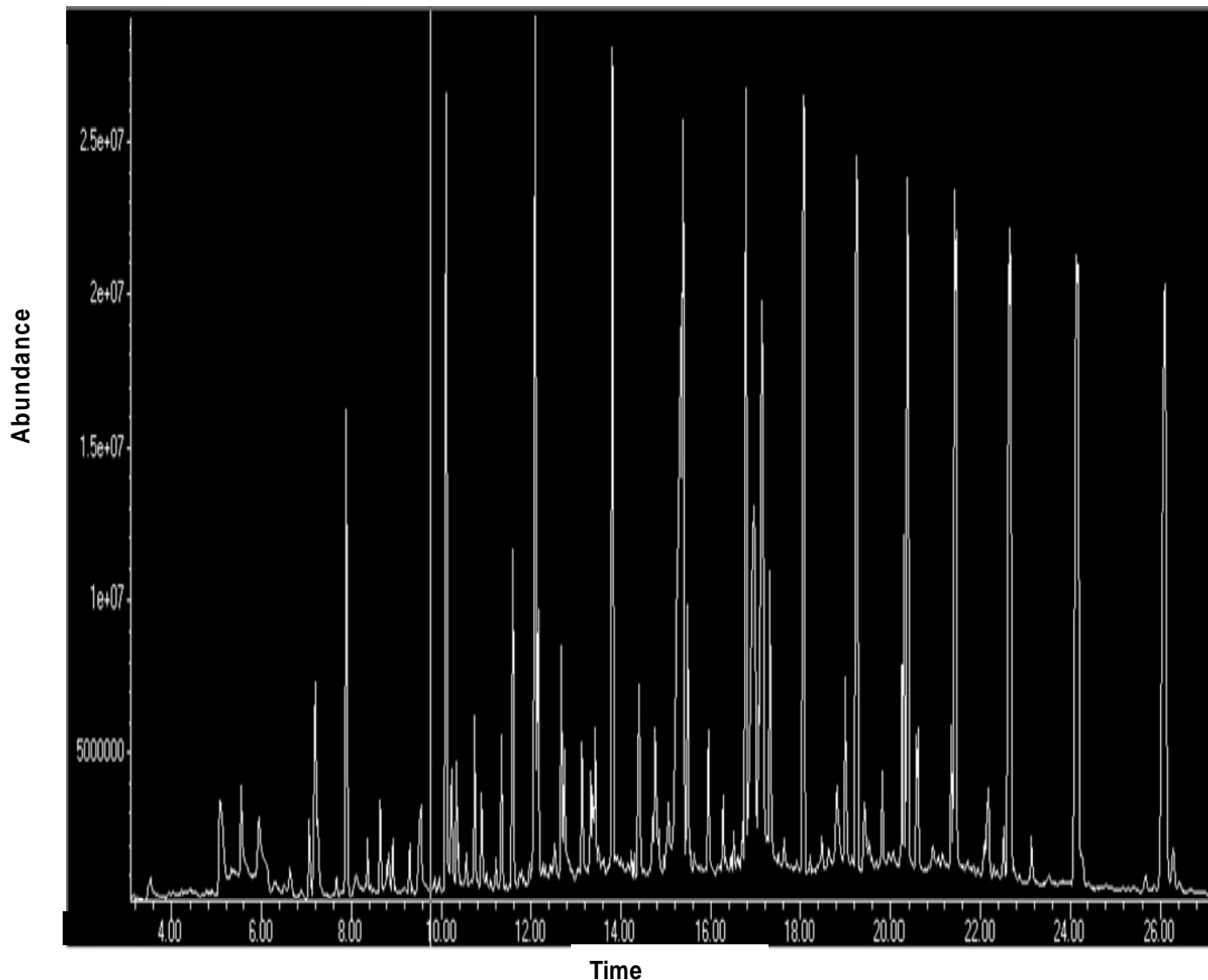


Figure 2. GC-MS Profile of leaves extract of *Solanum nigrum*.

purpose with lesser side effects. Continued further exploration of plant derived antimicrobials is needed today.

The results of the antimicrobial activity of the extracts of leaves of *S. nigrum* are as shown in Table 2. It was observed that the sensitivity tests show the effect of crude extracted alkaloids from seeds and roots of different bacterial strains, giving varying diameters depending on the tested strains.

The clear zone of growth inhibition was noted around the well due to diffusion of alkaloid compound. The diameter of the zone denotes the relative susceptibility of the test microorganism to a particular antimicrobial. The obtained results of the crude extracts were compared with the standard antibiotics such as penicillin, kanamycin, cefotaxime, streptomycin and rifampin. All

the tested organisms are highly sensitive to the methanol leaf extract (1.4 to 2 mm) than the standard antibiotics which showed more or less activity (0.4 to 1.7 mm).

The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health (Walton and Brown, 1999). Further works on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes. *S. nigrum* is the most potent plant against pathogenic microorganisms. However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *S. nigrum* extracts looking toward a pharmaceutical use.

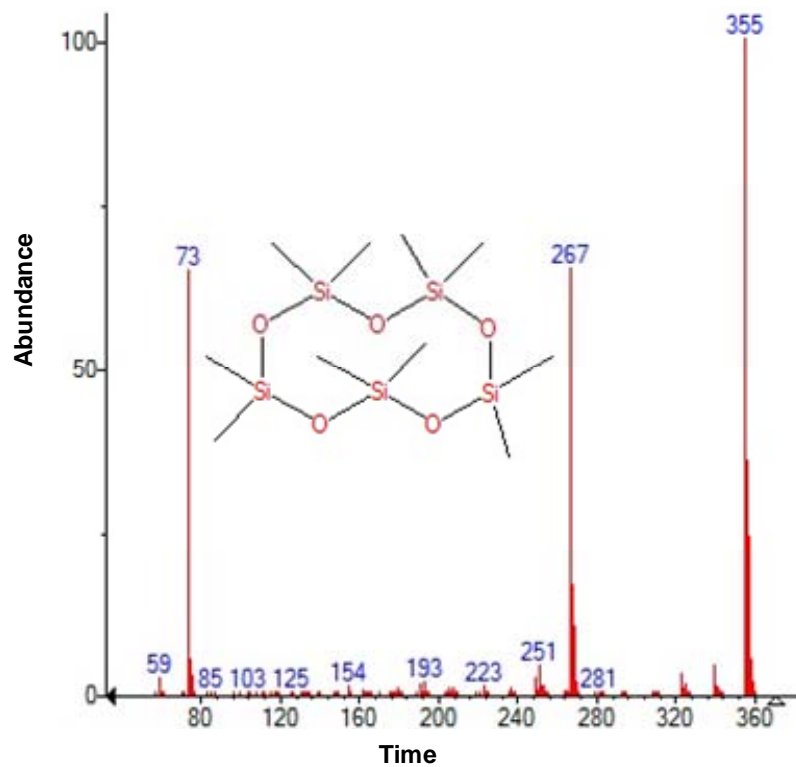


Figure 3. Structure of cyclopentasiloxane-decamethyl present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

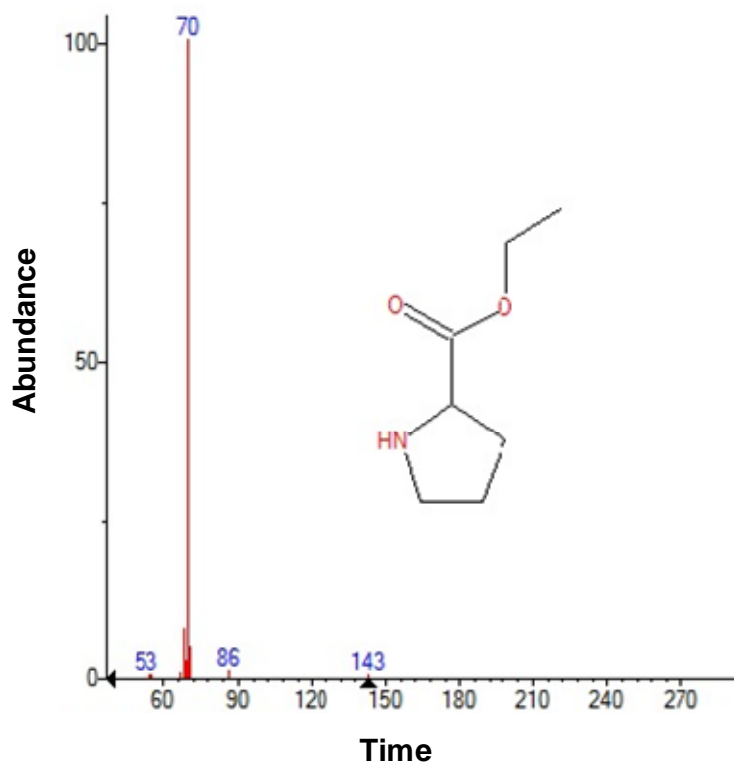


Figure 4. Structure of L-Proline, ethylester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

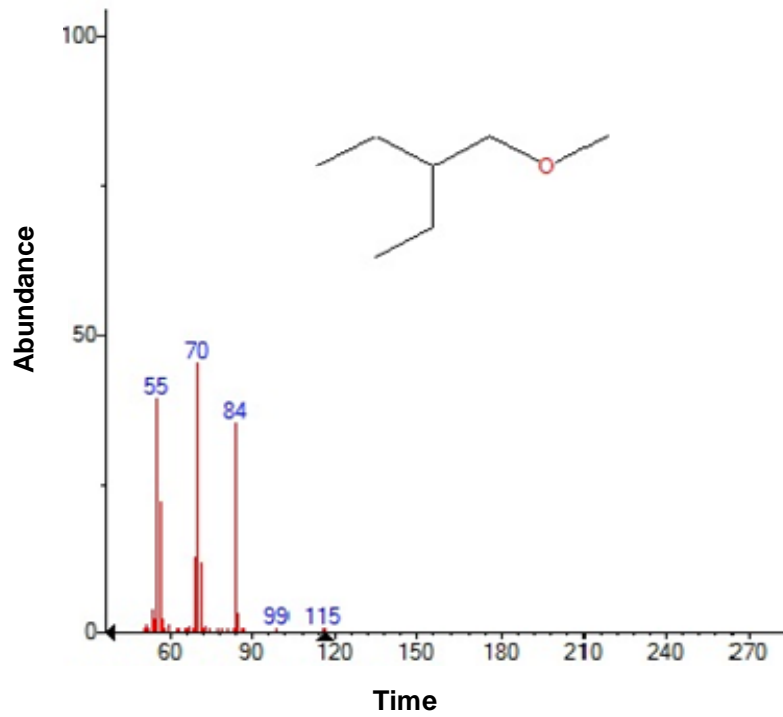


Figure 5. Structure of 2-ethyl-1-butanol, methyl ether present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

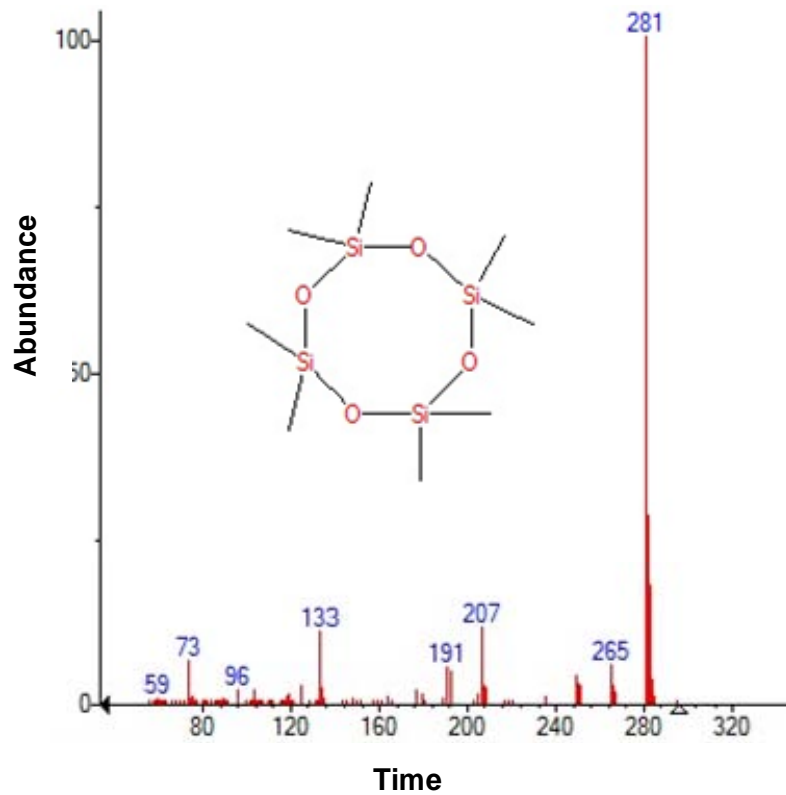


Figure 6. Structure of cyclopentasiloxane-ocamethyl present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

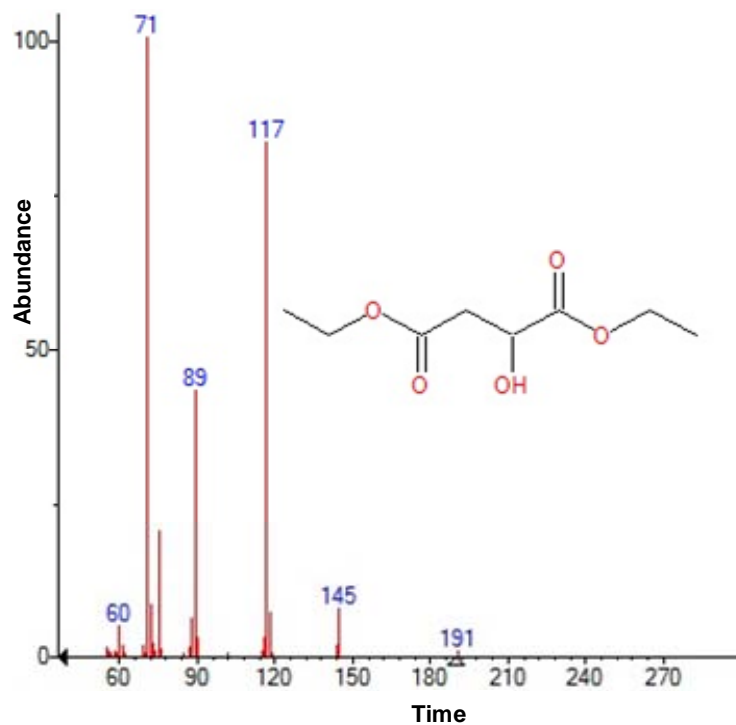


Figure 7. Structure of betanedioic acid, hydroxyl, diethyl, ester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

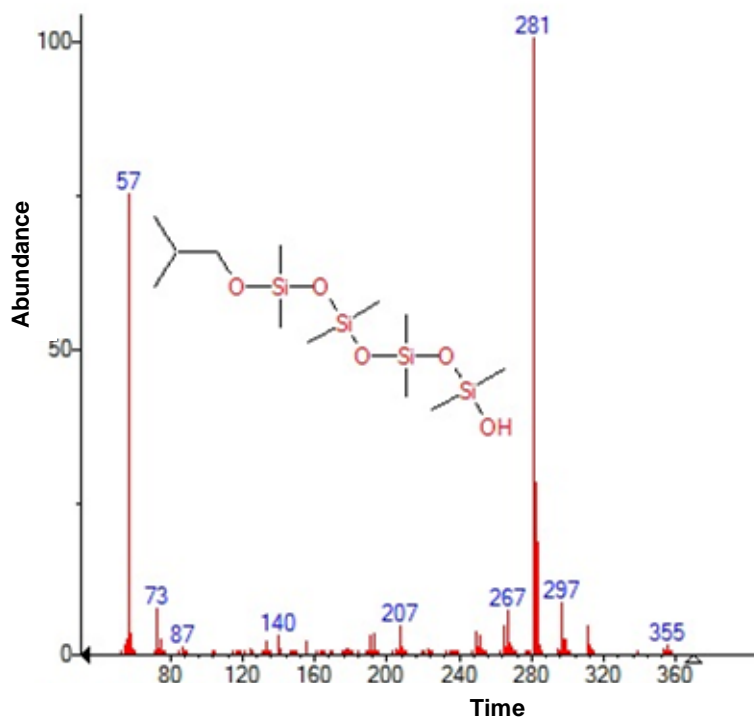


Figure 8. Structure of 1.1.3.3.5.5.7.7-Octamethyl-1-(2methyl-propoxy) tetrasiloxane-1 present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

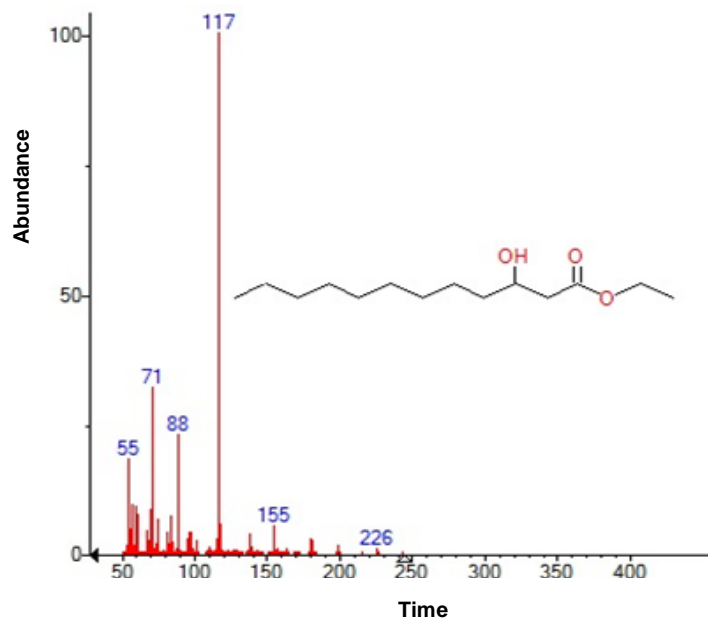


Figure 9. Structure of dodecanoic acid, 3-hydroxy-, ethyl ester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

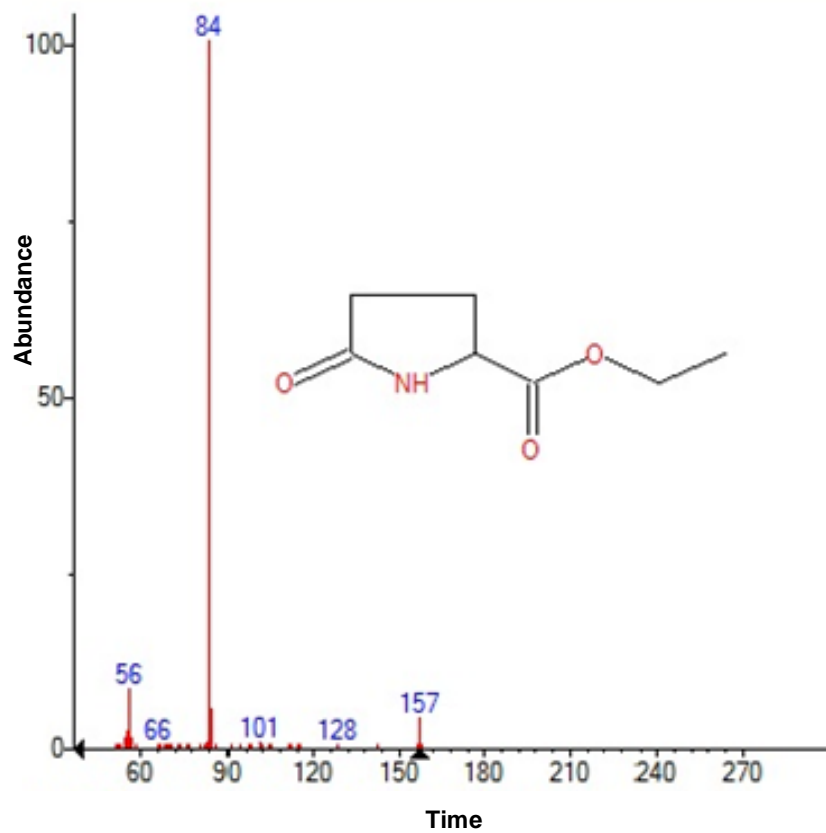


Figure 10. Structure of 2-pyrrolidinecarboxylic acid-5-oxo, ethyl ester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

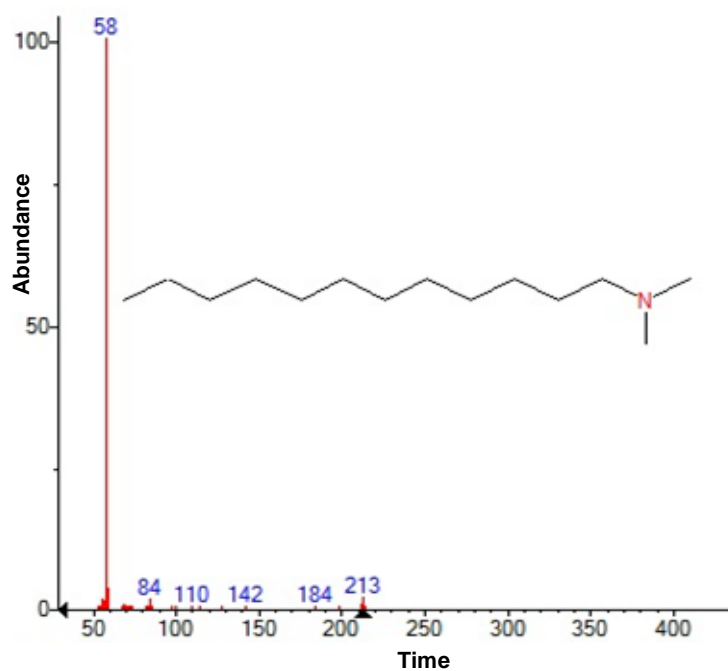


Figure 11. Structure of 1-dodecanamine, N,N-dimethyl present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

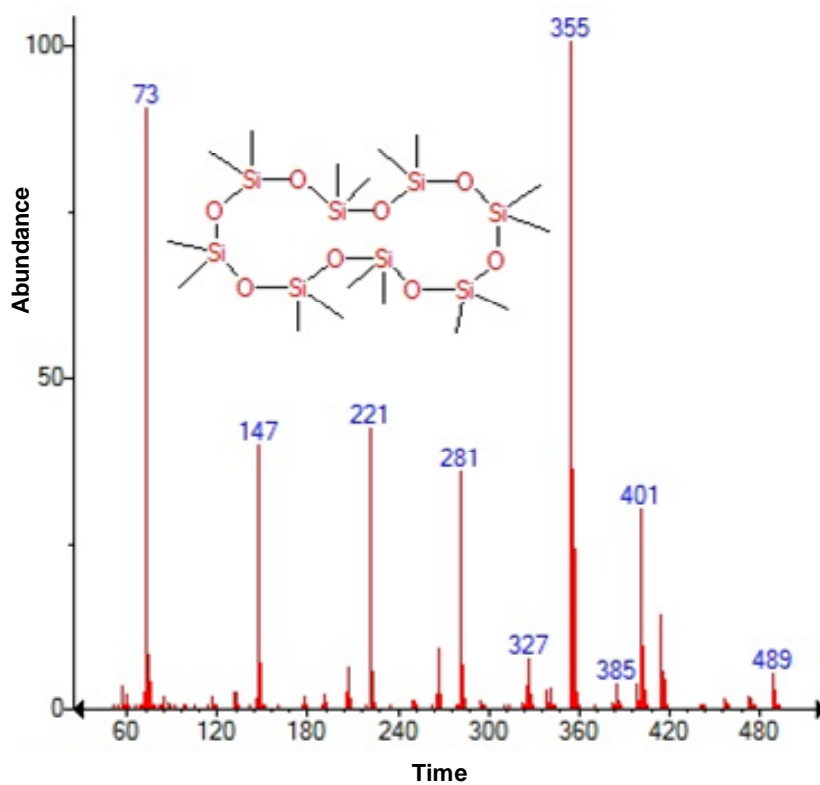


Figure 12. Structure of cyclooctasiloxane, hexadecamethyl present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

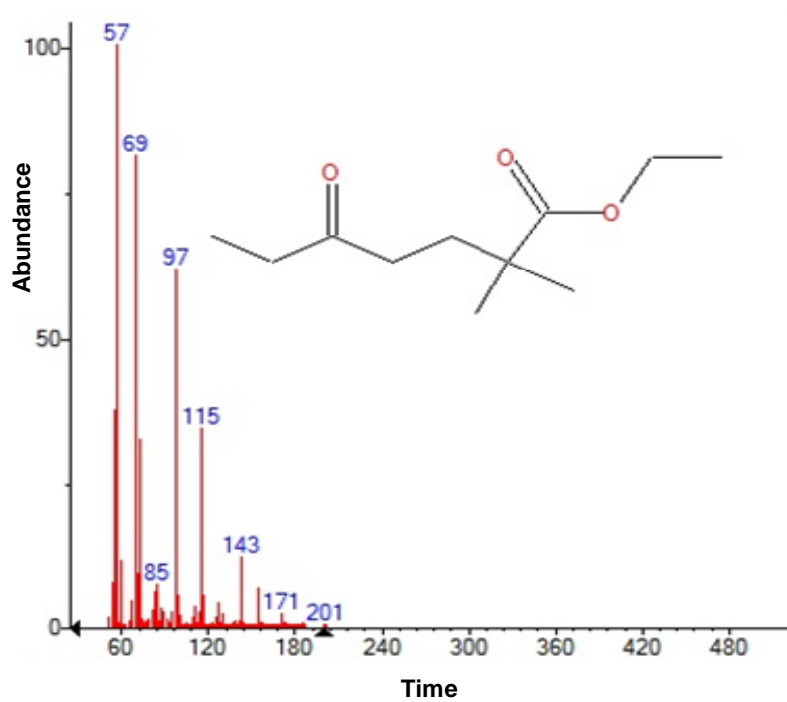


Figure 13. Structure of 5-keto-2,2-dimethylheptanoic acid, ethyl ester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

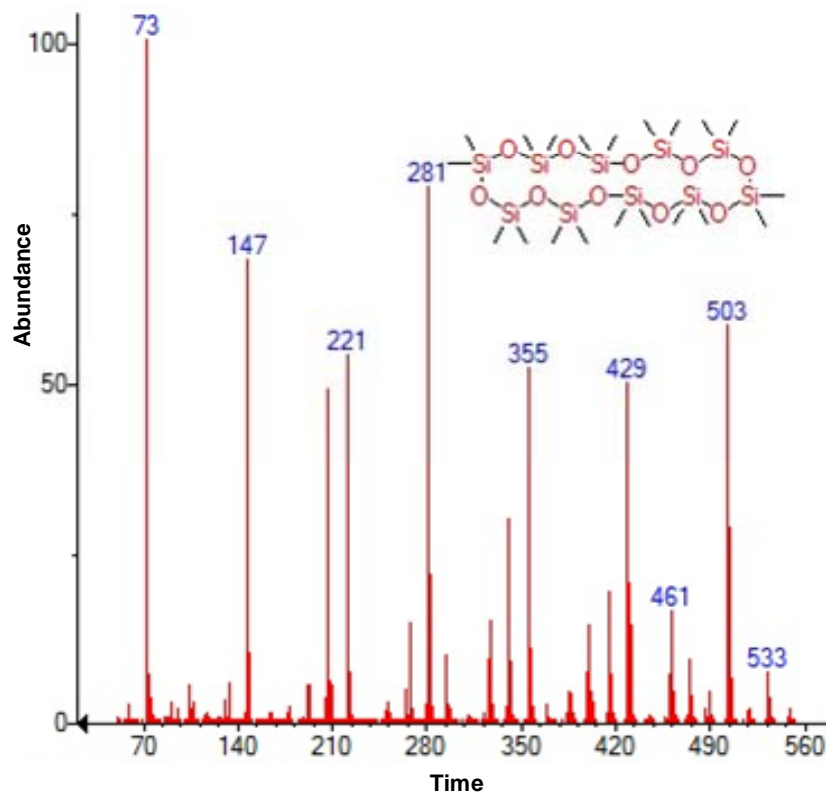


Figure 14. Structure of cyclodecasiloxane, eicosamethyl present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

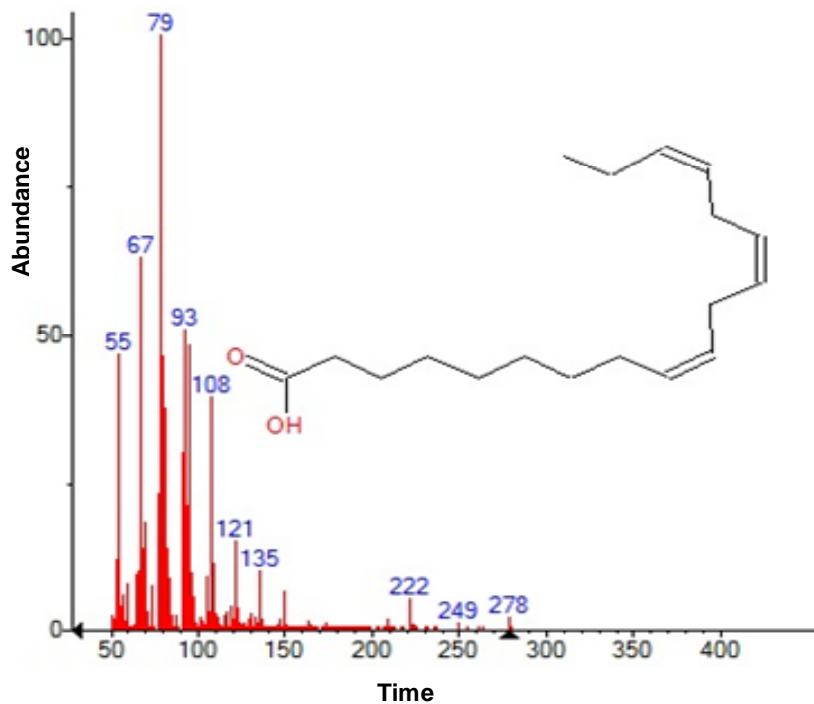


Figure 15. Structure of 9,12,15-octadecatrienoic acid present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

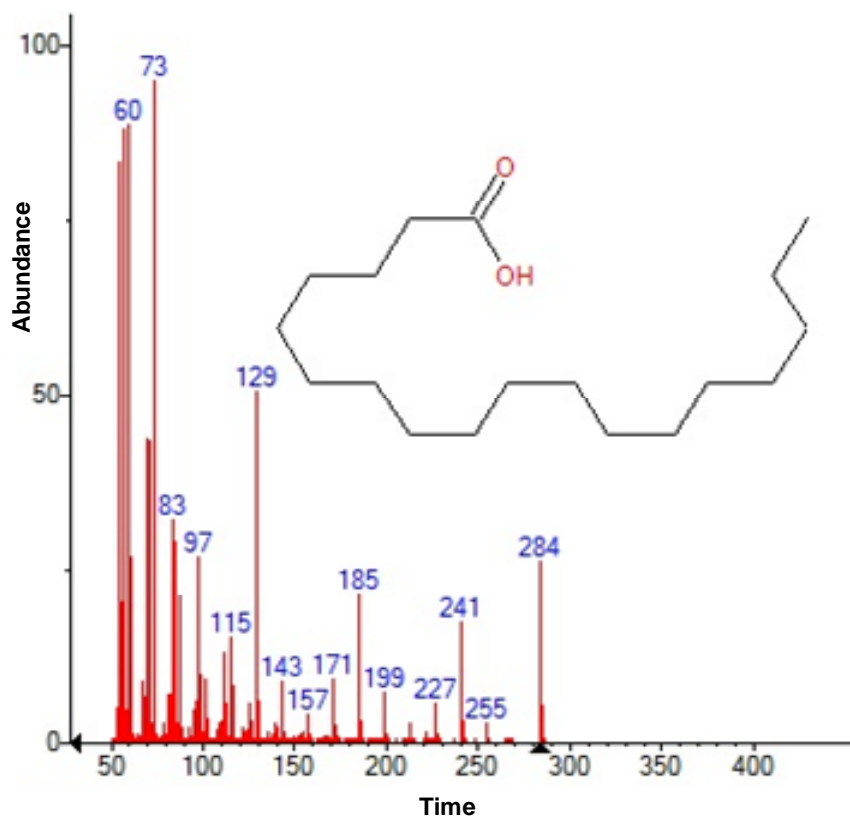


Figure 16. Structure of octadecanoic acid present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

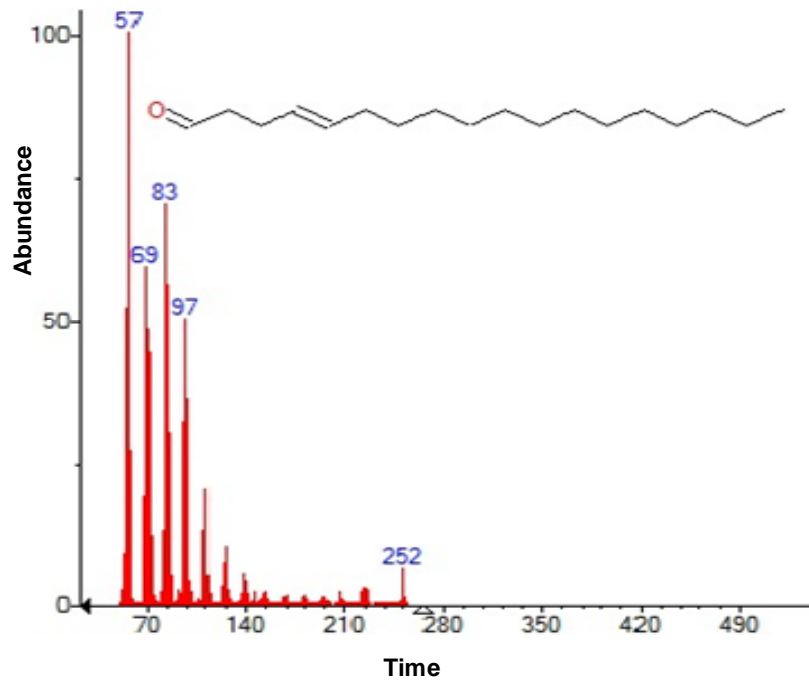


Figure 17. Structure of octadecenal present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

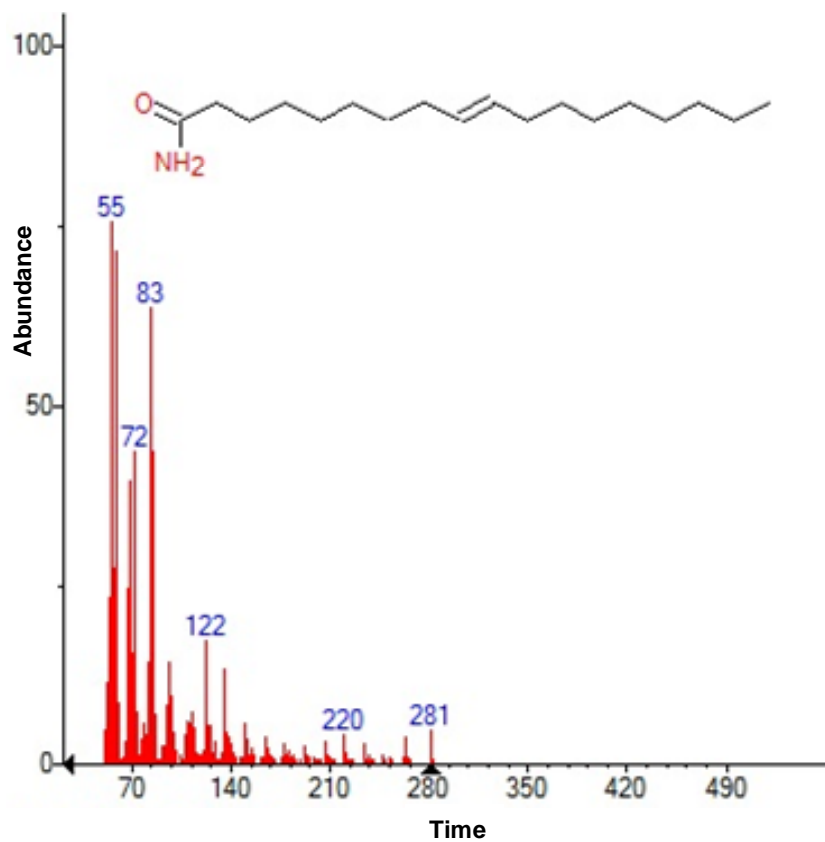


Figure 18. Structure of 9-octadecenamide present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

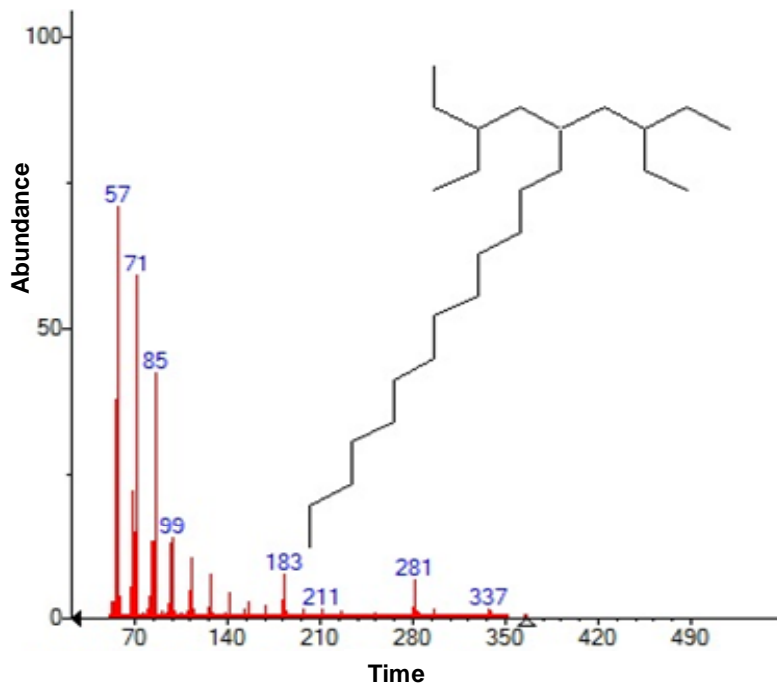


Figure 19. Structure of octadecane, 3-ethyl-5-(ethylbutyl) present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

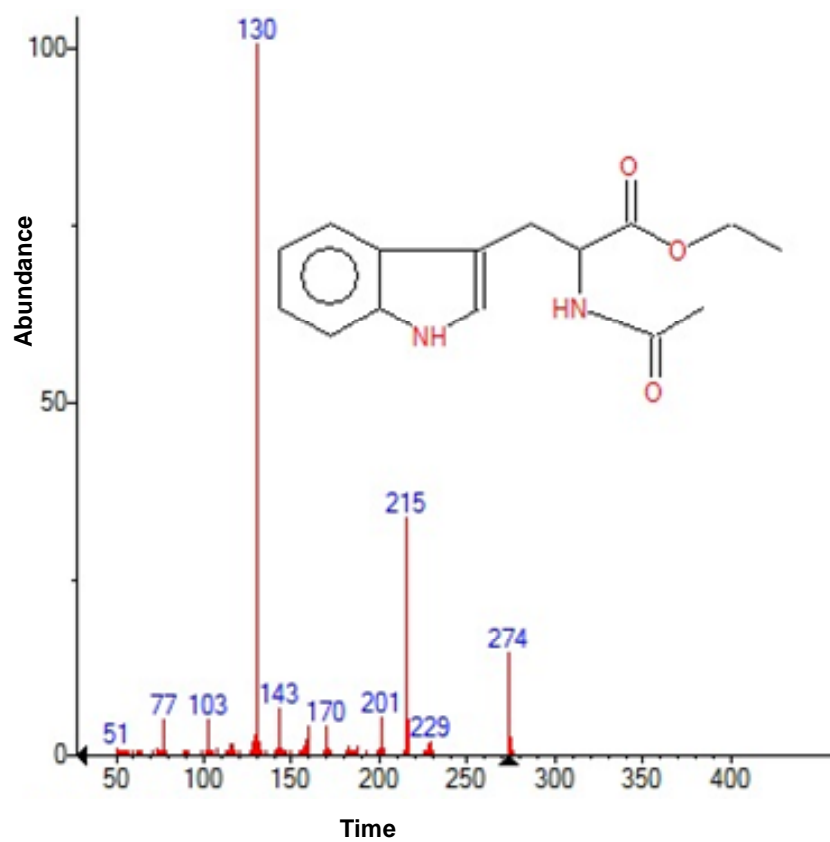


Figure 20. Structure of N-acetyl-L-tryptophan ethyl ester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

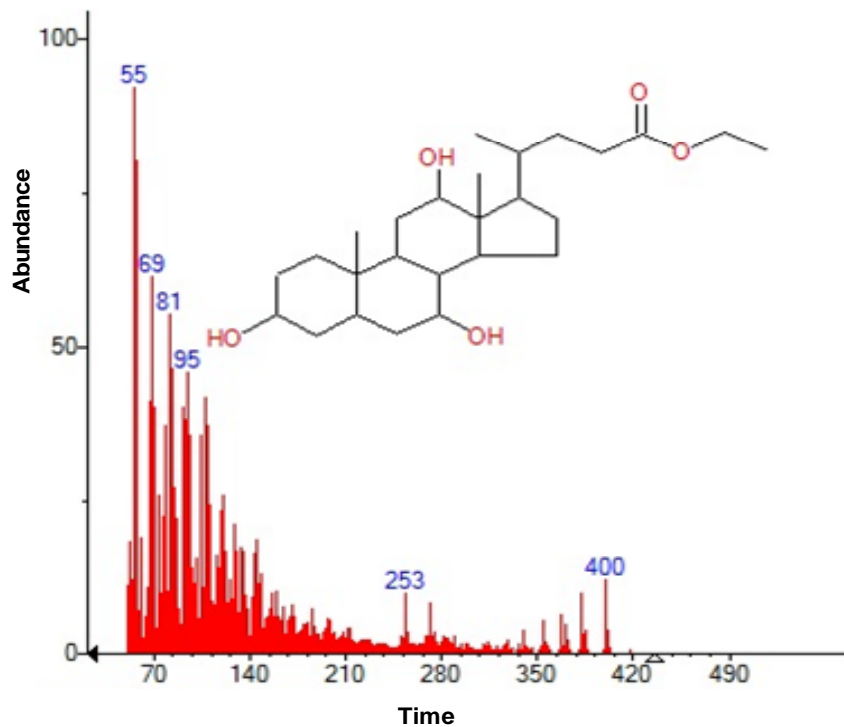


Figure 21. Structure of ethyl iso-allocholate present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

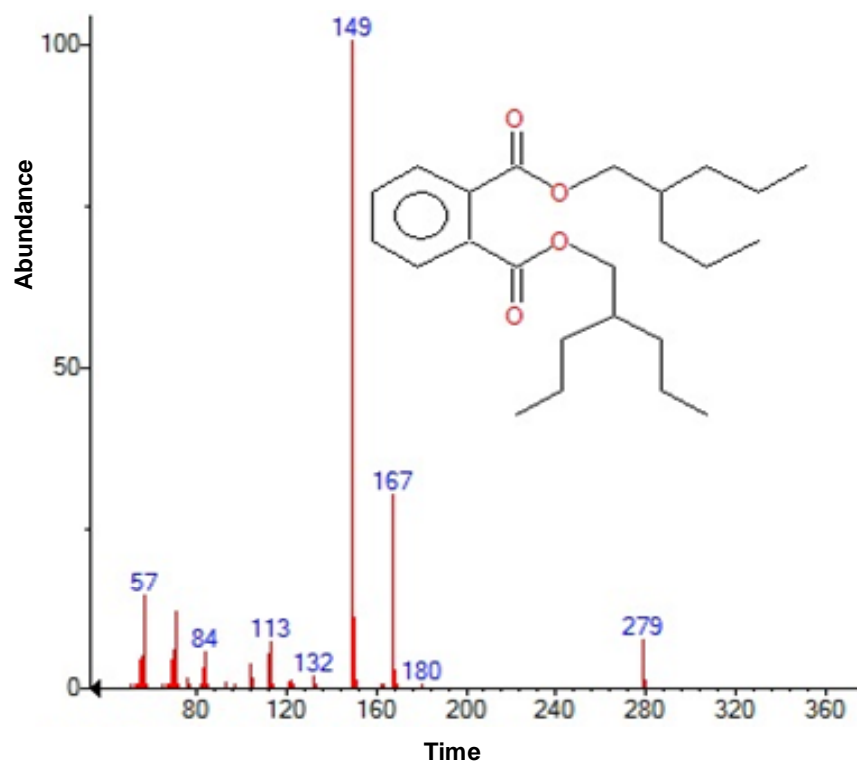


Figure 22. Structure of phthalic acid, di(2-propylpentyl)ester present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

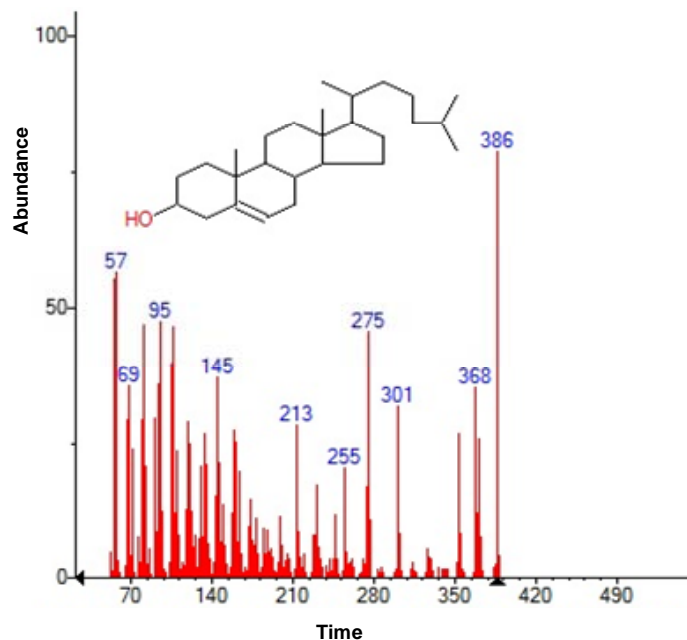


Figure 23. Structure of 17-(1.5-Dimethylhexyl)-10, 13-dimethyl-2,3,4,7,8,9,10,11, 12,13,14,15,16,17-tetradecahydro-1H present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

Conclusion

Twenty three chemical alkaloids constituents have been identified from ethanolic extract of the *S. nigrum* by GC-MS. *In vitro* antibacterial evaluation of *S. nigrum* forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds.

ACKNOWLEDGEMENT

The authors thank Dr. Abdul-Kareem Al-Bermani, Lecturer, Department of Biology, for valuable suggestions and encouragement.

Conflict of interest

Authors declare that there are no conflicts of interests

REFERENCES

Ajitha M, Rajanarayana K (2001). Role of oxygen free radicals in human disease. *Indian Drugs*.38:545-554.
 Alves TM, Silva AF, Brandão M, Grandi TS, Smânia E, Smânia Júnior A, Zani C (2000). Biological Screening of Brazilian Medicinal Plants. *Memórias do Instituto. Oswaldo Cruz* 95:(3)367-373.
 Cos P, Vlietinck AJ, Berghe DV, Maes L (2006). Anti-infective potential of natural products: How to develop a stronger in vitro proof-of-concept. *J. Ethnopharmacol.* 106(3) 290-302.
 Evans WC (2002). *Trease and Evans Pharmacognosy*, 15th edition.

WB Saunders Company Ltd, London. Pp. 137-139,230-240.
 Haraguchi H, Kataoka S, Okamoto S, Hanafi M, Shibata K (1999). Antimicrobial triterpenes from *Ilex integra* and the mechanism of antifungal action. *Phytother. Residence* 13:151-156.
 Iwu MW, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin. In: Janick J (ed.), *Perspectives on New Crops and New Uses*. Alexandria, VA: ASHS Press. pp. 457-462.
 John Britto S, Senthilkumar S (2005). Antibacterial activity of *Solanum incanum* L. leaf extracts. *Asian J. Microbiol. Biotech. Environ. Sci.* 3:65-66.
 Koduru S, Grierson DS, Afolayan AJ (2006). Antimicrobial Activity of *Solanum aculeastrum*. *Pharm. Biol.* 44:266-283.
 Krishnaraju AV, Rao TV, Sundararaju D (2005). Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int. J. Appl. Sci. Eng.* 2:125-134.
 Kumar VP, Shashidhara S, Kumar MM, Sridhara BY (2001). Cytoprotective role of *Datura Stramonium* against gentamicin-induced kidney cell (vero cells) damage in vitro. *Fitoterapia* 72:481-486.
 Misawa M (1994). Plant tissue culture. An alternative for production of useful metabolites. *FAO Agriculture Services Bulletin*, Rome. No. 108
 Ncube NS, Afolayan AJ, Okoh AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol.* 7(12):1797-1806.
 Palombo EA, Semple SJ (2001). Antibacterial activity of traditional Australian medicinal plants. *J. Ethnopharmacol.* 77(2-3):151-15.
 Prescott LM, Harley JP, Klein DN (1999). *Microbiology* 4th ed. Bistrit: The McGraw-Hill Companies Inc. pp. 685.
 Purohit SS, Vyas SP (2004). *Medicinal plants cultivation a scientific approach including processing and financial guidelines*. 1st edition. Publishers Agrobios, Jodhpur, India. pp. 1-3.
 Santh RT (2006). Antibacterial activity of *Adhatoda vasica* leaf extract. *Asian J. Microbiol. Biotech. Environ. Sci.* 8(2):287-289.
 Sashikumar JM, Remya M, Janardhanan K (2003). Antimicrobial activity of ethno medicinal plants of Nilgiri biosphere reserve and Western Ghats. *Asian J. Microbiol. Biotechnol. Environ. Sci.* 5:183-185.
 Sazada S, Arti V, Ayaz AR, Fraha J, Mukesh K (2009). Preliminary

- phytochemical analysis of some important medicinal and aromatic plants. 3(5-6):188-195.
- Singh SK, Saroj K, Tirupathi UJ, Singh AK, Singh RH (1998). An antimicrobial principle from *Speranhtus indicus*. Int. J. Crude Drug 26:235-239.
- Stainer RY, Ingraham JL, Wheelis ML (1986). General Microbiology, 5th ed. London: The MacMillan Press Ltd.
- Venkatesan M, Vishwanathan MB, Ramesh N, Lakshmanaperumalsamy P (2005). Antibacterial potential from Indian *Suregada angustifolia*. J. Ethnopharmacol. 99(3):349-52.
- Stanojević D, Čomić L, Stefanović O (2010). In vitro synergy between *Salvia officinalis* L. and some preservatives. Cent. Eur. J. Biol. 5 (4) 491-495.
- Uzun E, Sariyar G, Adsersen A, Karakoc B, Otük G, Oktayoglu E, Pirildar S (2004). Traditional medicine in Sakarya Province (Turkey) and antimicrobial activities of selected species. J. Ethnopharmacol. 95(2-3):96-287.
- Walton J, Brown DE (1999). Chemical from plants, perspectives on plant secondary products. Imperial College Press London UK. 2-5
- World Health Organization (2002). WHO Traditional medicine strategy 2002-2005, World Health Organization.