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

Huda Jasim¹, Ameera Omran Hussein², Imad Hadi Hameed¹* and Muhanned Abdulhasan Kareem²

¹Department of Molecular Biology, Babylon University, Hilla City, Iraq.
²Babylon University, Centre of Environmental Research, Iraq.

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 spectrometry (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, hydroxy, diethyl, ester, 1.1.1.3.3.5.5.7.7-octamethyl-1-7-(2methyl-proproxy) tetrasiloxane-1, dodecanedioic acid, 3-hydroxy-, ethyl ester, cyclopentasiloxane-ocamethyl, dodecanedioic acid, bistert-butyldimethylsiyl 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-octadecanamide, 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 specroscopy (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 dichlormethane. 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 specroscopy (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 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, ceftoxime, streptomycin and refampin (1 mg/ml) were also tested for their antibacterial activity. The plates were incubated at 370°C for 24 h.
Table 1. Compounds present in the leaves extract of *Solanum nigrum* using GC-MS analysis.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Phytochemical compound</th>
<th>Formula</th>
<th>Molecular weight</th>
<th>Exact mass</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyclopentasiloxane. decamethyl</td>
<td>C_{10}H_{30}O_{5}Si_{5}</td>
<td>370</td>
<td>370.093956</td>
<td>Figure 3</td>
</tr>
<tr>
<td>2</td>
<td>L-Proline, ethylester</td>
<td>C_{7}H_{13}NO_{2}</td>
<td>143</td>
<td>143.094628</td>
<td>Figure 4</td>
</tr>
<tr>
<td>3</td>
<td>2-Ethyl-1-butanol, methyl ether</td>
<td>C_{16}H_{30}O</td>
<td>116</td>
<td>116.120115</td>
<td>Figure 5</td>
</tr>
<tr>
<td>4</td>
<td>Cyclopentasiloxane. ocamethyl</td>
<td>C_{8}H_{24}O_{4}Si_{4}</td>
<td>296</td>
<td>296.075165</td>
<td>Figure 6</td>
</tr>
<tr>
<td>5</td>
<td>Betanedioic acid, hydroxyl, diethyl, ethyl ester</td>
<td>C_{8}H_{14}O_{5}</td>
<td>190</td>
<td>190.084124</td>
<td>Figure 7</td>
</tr>
<tr>
<td>6</td>
<td>1.1.3.3.5.5.7.7-Octamethy-1-7-(2methyl-propoxy) tetrasiloxane-1</td>
<td>C_{12}H_{30}O_{5}Si_{4}</td>
<td>370</td>
<td>370.14833</td>
<td>Figure 8</td>
</tr>
<tr>
<td>7</td>
<td>Dodecanoic acid, 3-hydroxy-, ethyl ester</td>
<td>C_{14}H_{26}O_{3}</td>
<td>244</td>
<td>244.203845</td>
<td>Figure 9</td>
</tr>
<tr>
<td>8</td>
<td>Dodecanedioic acid, Bistert-butyldimethylsilyl ester</td>
<td>C_{24}H_{50}O_{4}Si_{2}</td>
<td>458</td>
<td>458.324762</td>
<td>Figure 10</td>
</tr>
<tr>
<td>9</td>
<td>2-Pyrrolidinecarboxylic acid-5-oxo, ethyl ester</td>
<td>C_{7}H_{11}NO_{3}</td>
<td>157</td>
<td>157.073894</td>
<td>Figure 11</td>
</tr>
<tr>
<td>10</td>
<td>1-Dodecanamine, N.N-dimethyl</td>
<td>C_{14}H_{31}N</td>
<td>213</td>
<td>213.24565</td>
<td>Figure 12</td>
</tr>
<tr>
<td>11</td>
<td>Cyclooctasiloxane, hexadecamethyl</td>
<td>C_{16}H_{40}O_{8}Si_{8}</td>
<td>592</td>
<td>592.15033</td>
<td>Figure 13</td>
</tr>
<tr>
<td>12</td>
<td>5-keto-2, 2-dimethylheptanoic acid, ethyl ester</td>
<td>C_{11}H_{20}O_{3}</td>
<td>200</td>
<td>200.141245</td>
<td>Figure 14</td>
</tr>
<tr>
<td>13</td>
<td>Cyclodecasiloxane, eicosamethyl</td>
<td>C_{20}H_{60}O_{10}Si_{10}</td>
<td>740</td>
<td>740.187912</td>
<td>Figure 15</td>
</tr>
<tr>
<td>14</td>
<td>9.12.15-Octadecatrienoic acid</td>
<td>C_{18}H_{30}O_{2}</td>
<td>278</td>
<td>278.22458</td>
<td>Figure 16</td>
</tr>
<tr>
<td>15</td>
<td>Octadecanoic acid</td>
<td>C_{18}H_{30}O_{2}</td>
<td>284</td>
<td>284.27153</td>
<td>Figure 17</td>
</tr>
<tr>
<td>16</td>
<td>Octadecanamide</td>
<td>C_{18}H_{30}O_{2}</td>
<td>284</td>
<td>284.27153</td>
<td>Figure 18</td>
</tr>
<tr>
<td>17</td>
<td>Octadecanal</td>
<td>C_{18}H_{30}O_{2}</td>
<td>284</td>
<td>284.27153</td>
<td>Figure 19</td>
</tr>
<tr>
<td>18</td>
<td>Octadecyl, 3-ethyl-5-(ethylbutyl)</td>
<td>C_{26}H_{54}</td>
<td>366</td>
<td>366.422552</td>
<td>Figure 20</td>
</tr>
<tr>
<td>19</td>
<td>N-Acetyl-L-tryptophan ethyl ester</td>
<td>C_{15}H_{10}N_{2}O_{3}</td>
<td>274</td>
<td>274.131742</td>
<td>Figure 21</td>
</tr>
<tr>
<td>20</td>
<td>Ethyl iso-allocholate</td>
<td>C_{26}H_{40}O_{5}</td>
<td>436</td>
<td>436.318874</td>
<td>Figure 22</td>
</tr>
<tr>
<td>21</td>
<td>Phthalic acid, di(2-propylpentyl)ester</td>
<td>C_{24}H_{44}O_{5}</td>
<td>390</td>
<td>390.27701</td>
<td>Figure 23</td>
</tr>
<tr>
<td>22</td>
<td>17-(1.5-Dimethyl[hexyl]-10.13-dimethyl-2.3.4.7.8.9.10.11.12.13.14.15.16.17-tetradecahydro-1H</td>
<td>C_{27}H_{54}O_{2}</td>
<td>386</td>
<td>386.354866</td>
<td>Figure 24</td>
</tr>
</tbody>
</table>

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-1-7-(2methyl-propoxy) tetrasiloxane-1, dodecanoic acid, 3-hydroxy-, ethyl ester, cyclopentasiloxane-ocamethyl, cyclopentasiloxane, dodecanedioic acid, bistert-butyldimethylsilyl 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.

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

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 antioxidant 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
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.
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

phytochemical analysis of some important medicinal and aromatic plants. 3(5-6):188-195.


