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Characterization of Secondary Metabolites via LC-MS Analysis of DCM Extracts of *Solanum nigrum*

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Plants synthesize many secondary metabolites as a part of their normal metabolic activity to prevent themselves from predators but researchers have demonstrated their use for the treatment of various human ailments. These metabolic activities can be measured using techniques like Liquid Chromatography-Mass Spectroscopy etc. The present study reports the presence of various secondary phytochemicals such as flavonoid luteolin, oleuropein glucoside, alkaloid veremivirine, Myristic acid, Trimethylsilyl glycolic acid etc. in the DCM extracts of *Solanum nigrum*.

Key words: *Solanum nigrum*, DCM Extracts, LC-MS, Phytochemicals.

Solanum nigrum Linn (Solanaceae) commonly called as 'Black nightshade' is an annual weed that grows up to 60 cm tall, branched, erect, growing wild in wastelands and crop fields. Alternate leaves are ovate, deep green with an indented margin and acliminate at the tip[1]. It has been extensively used in traditional medicine in India and other parts of world to cure liver disorder, chronic skin ailments (psoriasis and ringworm), inflamatory conditions, fever, eye diseases, hydrophobia, cough and dropsy[2-3]. The phytochemical studies revealed that the plant contains glycoalkaloids, steroidal glycosides, steroidal saponin, steroidal genin, tannin, alkaloids and polyphenolic compounds[4]. It is also reported to have antitumour activity, hepatoprotective agent, diuretic and antipyretic[5-7]. The main objective of present study is to find out the various phytochemicals from the DCM extracts of *Solanum nigrum* using spectroscopic technique LC-MS.

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MATERIALS AND METHODS

Collection of Plant Materials

The plants of *Solanum nigrum* were collected from various places of Kathua district of J&K and identified by Dr. Rajesh Manhas of Botany Department of University of Jammu.

Preparation of Plant Samples

The live plants collected were washed thoroughly under running tap water, then were rinsed in distilled water and shade dried without any contamination for 3-4 weeks. The plant materials are then crushed and soxhlated with various solvents in soxhlet apparatus to prepare various extracts. The DCM extracts were than subjected to Column chromatography and the brown solid fraction obtained from 5:3 petroleum ether: dichloromethane was further subjected to LC- MS for the identification of various chemical constituents.

RESULTS AND DISCUSSION

LC-MS Spectrometry

The various components present in the plant were analyzed using LC-MS spectroscopy from IIIM Jammu. The instrument used is Agilent 1100LC which is coupled with Bruker make mass spectrometer model Esquire 3000. Liquid Chromatography includes Binary Gradient pump with online degasser, autosampler with capacity of 100 samples, column oven and PDA detector. Mass Spectroscopy involves sample inlet system: Liquid chromatographic system and syringe pump inlets. Ionization sources are ESI and APCI constitutes the API ionization sources and the ion trap analyzer with MS_n facility. The instrument is used for the mass determination of pure molecules and LC-MS studies provide information about the number of molecules and their atomic masses present in the mixtures. The instrument is used for qualitative as well as quantitative analysis.

Characterisation of peaks

Peak 1

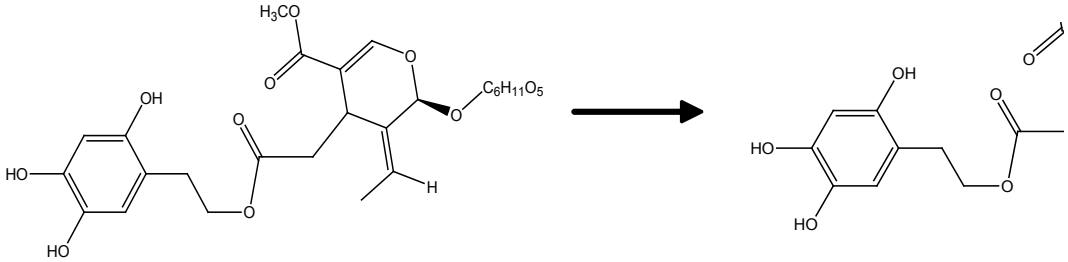
Corresponds to 2-hydroxymethylene-3-methyl pentane dioic acid 1-methyl ester 5-(3, 4, 5- trihydroxy-6-phenethyl oxy tetrahydropyran-2-yl-methyl ester). Peak 453 – $[M+H]^+$, 475 – $[M+Na]^+$, 476 – $[M+Na+H]^+$ [8].

Peak 2

Corresponds to 2-methyl-4,6-dinitro phenol. Peak 197 – $[M]^+$, 609 – $[3M+H_2O+H]^+$, 564 – $[3M-CO]^+$.

Peak 3

Corresponds to Oleuropein glucoside. Peak 701 – $[M]^+$, 702 – $[M+H]^+$, 753 – $[M+2H_2O+OH]^+$, 359 – $[M-C_{16}H_{24}O_9]^+$ [8]

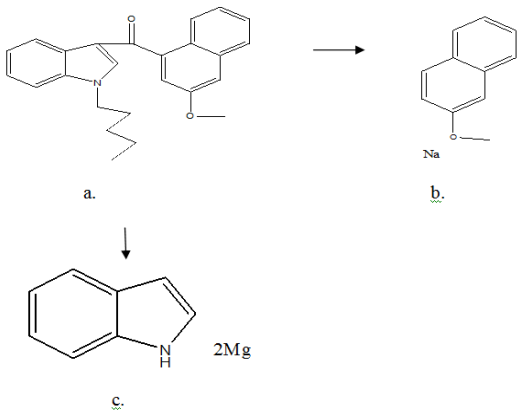


$C_{25}H_{32}O_{14}$ $C_{16}H_{24}O_9$

Fragmentation pattern

Peak 4

Corresponds to (3-Methoxy- naphthalene-1-yl)-(1- pentyl-1H-indol-3 yl)-methanone. Peak a. 371 – $[M+H]^+$, b. 169 – $[C_{10}H_9ONa]$, c.141 $[C_8H_7N + 2Mg]$[9].



a. b. c.

Structure and Fragmentation Pattern

Peak 5

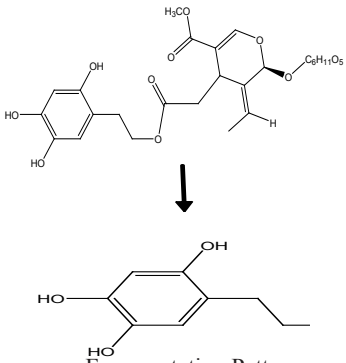
Corresponds to 1-(3-Methoxy-naphthalene-1-yl)-pent-2-en-1-one. Peak 239 – $[M^+]$, 371 – corresponds to its fragment (3-methoxy-naphthalene-1- yl)-(1-pentyl-1H-indol-3-yl)-methanone, 172 belongs to 3-hydroxy-Naphthalene-1-carbaldehyde.

Peak 6

Corresponds to coumarin compound 6-O-demethyl salutaridine $[C_{18}H_{19}NO_4]$. a. Peak 314 – $[M+2H]^+$, b. 315 – $[M+3H]^+$, c. 169 – $[M-C_8H_{10}O_4$ i.e. M- mass of 2-oxo-5, 6-dihydro-2H-pyran-3-carboxylic acid ethyl ester], d. 501 – $[2M-C_6H_8O_3]^-$ i.e. 2M- 2-Formyl acrylic acid ethyl ester [10].

Peak 7

Corresponds to Oleic acid. a. Peak 284 – $[M+H]^-$, 285 – due to isotope effect, b. Peak 147 – corresponds to fragment $C_7H_{14}O_3$ (2-hydroxy heptanoic acid).



Fragmentation Pattern

Fragmentation Pattern:

Peak 8. a

M/Z at 314 corresponds to 10-oxo-2, 3, 5, 6-tetrahydro-1H, 4H, 10H-11-oxa-3a- azobenzo [de] anthracene-9-carboxylic acid ethyl ester **b.** Peak at 315 is due to isotopic effect **c.** Peak at 501 corresponds to $[2M-C_6H_8O_3]$ i.e 2M- Mass of 2-formyl acrylic acid ethyl ester.

Peak 9. a

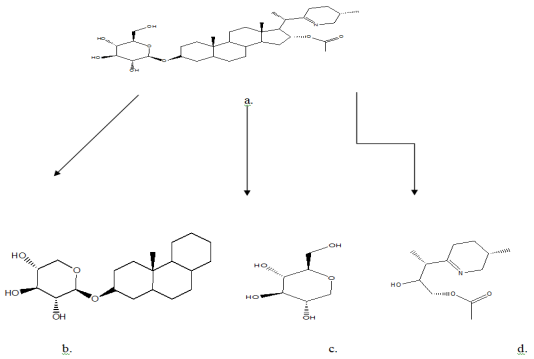
M/Z at 351 corresponds to retrorsine [11]. **b.** M/Z at 352 is due to isotopic effect.

Peak 10. a

M/Z at 557 corresponds to 2'-chloro-4'-(2-(2,4-di-tert-pentyl phenoxy) butyryl amino)-5'-hydroxy benzanilide [12].

Peak 11

Corresponds to steroidal alkaloid havanine $(C_{35}H_{57}NO_8)$ [13]. a. Peak at 619 corresponds to $[M+H]^+$, b. Peak at 353 corresponds to $[M-C_{15}H_{11}O_3]$ i.e. it corresponds to the fragment [2-(4a-Methyl tetradeca hydro-phenanthrene-2-yl oxy) tetrahydropyran-3, 4, 5-triol, c. Peak at 165-$[M- C_{29}H_{45}O_3 +2H]^+$ i.e. belongs to 2-hydroxy methyl –tetrahydropyran-3,4,5-triol, d. Peak at 227 goes to $[M-C_{23}H_{34}O_5+H]^+$ i.e. belongs to Acetic acid 2-hydroxy-3-(5-methyl-3,4,5,6-tetrahydropyridin-2-yl)-butyl ester.



Structure and Fragmentation Pattern

Peak 12

Corresponds to O-(3, 5-dichloro-2,4-dihydroxy benzoyl) benzoic acid. **a.** 327 – $[M+H]^+$, **b.** 328 – $[M+2H]^+$, **c.** 329 – $[M+3H]^+$ (M+2 Peaks of Halogen isotopes), **d.** Peak at 124 is due to $[M-C_7H_2Cl_2O_3 + 2H]$.

Peak 13

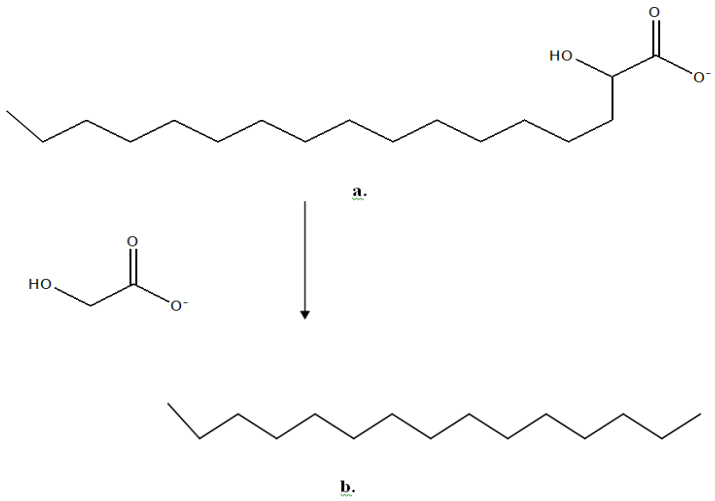
Corresponds to linoleic acid. a. 280 – $[M-H]^-$, b. 279 – $[M]^+$, c. 302 – $[M+Na]^+$, d. 301 – $[M-H+Na]^+$.

Peak 14

Corresponds to tetracosanoic acid ($C_{24}H_{48}O_2$). **a.** 368 – [ESI-MS], b. 311 – $[M-C_4H_8]^+$ i.e. icosanoic acid, c. 310 – $[M-C_4H_8+H]^+$, d. 288 – $[C_{19}H_{40}+H_3O+OH]^-$, e. 289 – $[C_{19}H_{40}+H_3O+2H]^-$[14].

Peak 15

Corresponds to 2-hydroxy hexadecanoate. a. Peak at 286 – $[M+H]^+$, b. 212 – $[M- C_2H_3O_3^-+H^+]$ [15].



Structure and Fragmentation Pattern

Structure and Fragmentation Pattern

Peak 16

M/Z 757 corresponds to Delphinidin-3-cis-coumaroyl rutinoside-5-glucoside [16].

Peak 17

M/Z 417 corresponds to alkaloid veremivirine. **a.** Peak 417 is due to $[M+H]^+$, **b.** Peak 579 – [M-H+ 3-Hydroxy-3-methyl glutaric acid]$^+$.

Peak 18

M/Z 415 corresponds to phenolic compound 1-acetoxy pinoresinol. **a.** 415 – $[M+H]^+$, **b.** 416 – $[M]^+$ **c.** 437 – $[M+Na+H]^+$ [17].

Peak 19. a

M/Z 539 Corresponds to 7-Methoxy-Salcolin B [22].**b**. Peak 701 – $[2M-C_{18}H_{21}O_7 + CO]$ [18].

Peak 20. a

M/Z 227 corresponds to Myristic acid $[M]^+$, **b.** Peak 228- $[M+H]^+$ [19].

Peak 21. a

M/Z 537 corresponds to trimethylacetyl delcosine $[M+H]^+$,**b.** 538 – $[M+2H]^+$, **c.** 509 corresponds to delcosine, **d.** 504 – $[M-OCH_3+3H]^+$, **e.** 595 – $[M+2C_2H_5]$[20].

Peak 22. a

M/Z 274 corresponds to 10, 13-dimethyl-hexadecahydrocyclopenta [a] phenanthrene $[M+H]^+$ **b.** 275 – $[M+2H]^+$ **c.** 318 – $[M+COOH]^+$ **d.** 319 – $[M+COOH+H]^+$ **e.** 301 – $[M+CO^+]$[21].

Peak 23. a

M/Z 149 corresponds to Trimethylsilyl glycolic acid $[M+2H]^+$ **b.** 150 – $[M+3H]^+$ **c.** 167 – [M+OH] **d.** 391 – $[2M+H+C_7H_7^+]$ **e.** 392- $[2M+2H+C_7H_7^+]$.

Peak 24. a

M/Z 338 corresponds to Trans-13-docasenoic acid ($C_{22}H_{42}O_2$) $[M^+]$ **b.** M/Z 339 – $[M+H]^+$ **c.** 138 – Corresponds to fragment Sodium heptanoate[22].

Thus it is possible to isolate large number of secondary metabolites from *Solanum nigrum*

CONCLUSION

A large number of secondary metabolites are present in *Solanum nigrum* which are having great therapeutic value used to combat many diseases and to boost the immune system.

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