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LC-MS analysis of a potent medicinal plant *Solanum nigrum* L. variants black and orange fruits

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Abstract
The plant *Solanum nigrum* L. traditionally claimed in the treatment of mouth ulcers and is used in cooking like spinach. The main aim of the present work is to investigate the phytoconstituents by Liquid Chromatography - Mass Spectrometry (LC-MS) analysis. For analysis hydroethanolic extracts were used. The spectrum interpretation and fragmentations identification were carried out using a spectrum database for organic compounds in SDBS application. They showed the presence of five and seven constituents in variant black and orange fruits respectively. The analysis revealed the presence of same phytoconstituents like Solasonine, Solamargine, and Triterpenoids derivatives in both variants.

Keywords: phytoconstituents**,** *solanum nigrum*, solanaceae, LC-MS, solasonine and solamargine

Introduction

Medicinal herbs are of great importance to the health of human. The medicinal properties of these plants lie in some phytoconstituents that produce a definite biochemical action on human system. Nowadays, the plants are studied and used as a base line for identification of the novel compounds that to treat various illnesses around the world. So the researcher interested to discovering new drug from the plant. The plant *Solanum nigrum* L. is herbaceous annual herb which belongs to the family, the plants are usually 10 to 15cm long with a tender green, smooth stem. It is widely found in river bund, wet wood, waste land, quagmire, old field, road side and in wet cultivated land. Our field observations and in earlier work of (Dhasmana *et al.*, 2007) [4] revealed the presence of variations among *Solanum nigrum* populations. Three variants *viz*. Black, Orange and yellow fruited were observed. In Tamil Nadu and Pondicherry, black and orange fruited variants were observed dominantly. Both variants have been used as green and for treating diseases like ulcer (mouth and stomach) under a vernacular name Manathakkali or Milakuthakkali. Forming a basic food and medicine and have been used one for the other without prejudice (Jayanthi 2021) [10]

It is used in fever, hepatitis, stomach complaints and dysentery. The plant juice is administered for ulcer and other skin diseases. The fruits are used as stimulant, appetite laxative, for treating asthma and "excessive thirst. Leaf and berry decoctions are used to cure liver related problems including jaundice. It is widely used in oriental medicine because it is utilized to be antioxidant, antitumorogenic, anti-inflammatory, diuretic, hepatoprotective, and antipyretic activity Nyeem *et al.* (2016) [15] and also confirmed plant which inhibits cervical carcinoma through Chinese experiments.

This genus *Solanum* is medicinally important for the presence of steroidal alkaloids mainly solasodine and other related glycosides (Giulietti 1991) [8]. Eltayeb *et al.* 2003 [5], quantitatively estimated the content of Solasonin and Solamargin through an optimized isolation method (HPTLC) in various part of *Solanum incanum* plants at different stages of development. The steroidal glycoalkaloids (solasonine) and aglycone (solasodine) from *Solanum nigrum* through HPLC and GC-MS analysis were analysed and 5.85 mg/g of solasodine was 5.85 and the aglycone solasodine with significantly higher amount (75.94%) was reported by Gheewalaa *et al.* (2013) [7].

With the above background, the present work has been initiated with a view to establish pharmacopoeial standards for *Solanum nigrum* L. variants whole plants. The main object of this study is to identify the phytoconstituents through LC- MS. Because no reports available on LC-Ms of this variants.

Materials and methods

In present investigation the whole plants of *Solanum nigrum* L variants black and orange fruit (Plate-1&2) has been selected and were collected during same season and time (March – April 2021) from Thirumalairayan Pattinam, 609 606, Karaikal Region, Pondicherry, Union Territory of South India.

Preparation of extract

The hydroethanolic extract was prepared using ethanol. The whole plants were dehydrated inan oven at 40 ^{O} C, ground and macerated in ethanol for 72 hrs. This was filtered and condensed. The collected extracts were used for this present analysis.

Habit **Fruiting twig**

Plate 1: Habit of *Solanum nigrum* L. variant (Black fruited)

Habit **Fruiting twig.**

Plate 2: Habit of *Solanum nigrum* L. variant (Orange fruited)

Plant identification

Floras like Gamble,1957 [6]; Matthew, 1983 [13]; Nair and Henry, 1983 [14];Anonymous, 1992 [1], Chatterjee, 1994 [2]; Kirtikar and Basu, 1935 [11]; the collected plant specimens were identified and confirmed with the help of type specimens available in the Herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamilnadu. In addition, as per the method of Jain and Rao 1976 [9]; the herbarium were prepared and deposited in Tamil University Herbarium TUH-300(A) – *Solanum nigrum* L. (black fruited). TUH-300(B) – *Solanum nigrum* L. (orange fruited).

LC-MS Analysis (Dass, 2007) [3]

The LC-MS System consisted of a liquid chromatography (LC–10 ADVP, Shimadzu). System controller (SCL–10 AVP,Shimadzu, MS detector LC MS-QP 8000 Shimadzu) and injector (7725 I Reodyne) with 2µl loop.LC-MS solution (Shimazu) software was used to control the LC-MS system and for data processing. Chromatographic separations were performed on an Ascentis Express C_{18} Column 50 x 2.1mm, 2.7µm, supelco, USA) packed by 2.7µm fused core particles that comprise a 1.7µm solid core and a 0.5 µm pocus shell. For the separations, a gradient of mobile phase A-2v/v/% formic – acid in methanol: water = 7:93-and mobile phase B-2v/v% formic acid in methanol was used. The gradient profile was set as follows. 0.00 min 0% B eluent, 10.00 mm 90% B eluent, 10.01 min 0% B element and 15.00 min 0% B eluent. The flow rat was 0.2ml min^{-1}, the column temperature was ambient. The column outlet was connected to the electrospray sample inlet. The electrospray source was operated in positive mode and the interface conditions were as follows; capillary voltage of 5kV, CDL voltage of 5 V, CDL temperature of 250° C and deflector voltage was 2.0kV. Mass Spectrophotometer conditions (cone and collision energy) were optimized by direct infusion of the standards. SIM (Selected Ion Monitoring) acquisition mode was used for the analysis in order to detect only specific mansions during the analysis.

Results

LC-MS

The Liquid Chromatography Mass Spectroscopy (LC-MS) of hydroethanolic ethanol extract of *Solanum nigrum* black fruited shown in Fig.1 and mass spectrum detected constituents is given in table-1. It was observed that the different peaks was obtained at different retention times like 21.3followed by 21.6, 35.9, 38.8, 41.5 belonging to the compounds Solasonine, Solamargin, Daidzin, Chrysoriol-hexose and Triterpenoid derivative. The molecular peak (base peak) at m/z 884.5 was solasonine, at m/z 868.5 was solamargine at m/z 417.3 was diadzin at m/z 579.3 was Chrysoriol hexose and at m/z 663.4 was tritrepenoid.

The chromatogram obtained for variant orange fruited shown in Table 2 (Fig.2). The molecular peak (base peak) at m/z 461.7 is good agreement with the compound gingerol).The fragmentation has showed molecular peak (base peak)at m/z 884.5 was Solasonine.The mass spectrum showed the molecular peak (base peak) at m/z 868.5 was solamargine. The compound showed molecular peak (base peak) at m/z 414.3 was diosgenin.The fragmentation has also showed the value at at m/z 277.2, m/z 333.1, m/z 663.4 which are good agreement with moroctic acid,Sanguinarine

and tritrepenoid respectively. The structure of bioactive compounds and molecular formulas. The chemical constituents obtained from LC-MS showed known common biological constituents in both variants.

Table 1: Major biological compounds identified in LC-MS from hydroethanolic extract of *Solanum nigrum* L. variant black fruit

S.No	Name of the Compounds	RT	Peak
1.	Solasonine	21.3	884.5
2.	Solamargine	21.6	868.5
3.	Daidzin	35.9	417.3
4.	Chrysoriol – hexose	38.8	579.3
5.	Triterpenoid derivative	41.5	663.4

RT- Retention time

Table 2: Major compounds identified in LC-MS from hydroethanolic extract of *Solanum nigrum* L. variant orange fruit

S. No	Name of the Compounds	RT	Peak
1.	Gingerol	2.0	461.7
2.	Solasonine	2.7	884.5
3.	Solamargine	2.8	868.5
4.	Diosgenin	3.6	414.3
5.	Moroctic acid	33.8	277.2
6.	Sanguinarine	38.5	333.1
7.	Tritrepenoid	39.8	663.4

RT- Retention time

Total Ion Chromatogram (Positive mode)

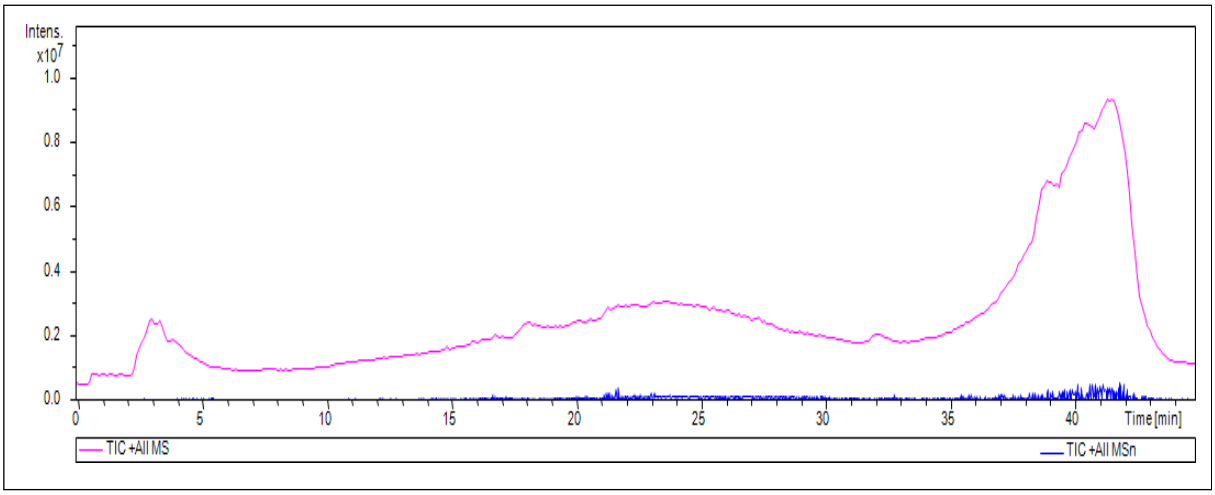

Fig 1: LC-MS analysis of hydroethanolic of *Solanum nigrum* L. variant black fruited

Total Ion Chromatogram (Positive mode)

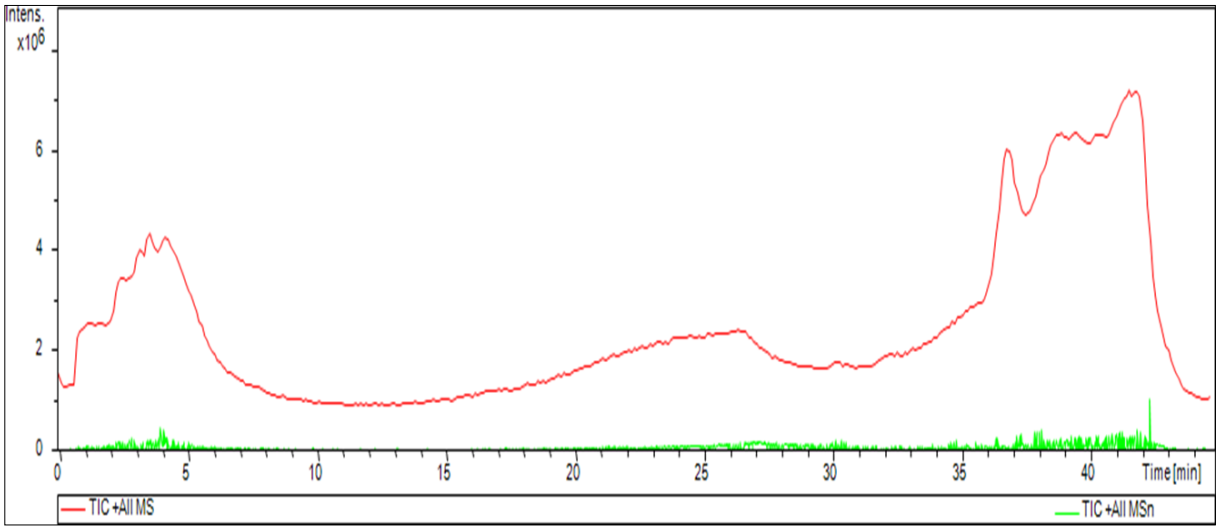

Fig 2: LC-MS analysis of hydroethanolic extract of *Solanum nigrum* L. variant orange fruit

Discussion

The integration of herbal medicine into modern medical practices, including treatments for various infectious diseases, must take into account the interrelated issues of quality, safety, and efficacy (Schwartsmann *et al*., 2002) [17]. Quality is the paramount issue because it can affect the efficacy and/or safety of the herbal products being used. Current products quality ranges from very high to very low due to intrinsic, extrinsic and regulatory factors. Despite the common belief that phytocompounds are safe, they all have inherent risks just like synthetic compounds. LC-MS is one of the best techniques to identify the constituents of volatile matter, long chain and branched chain hydrocarbons, alcohols acids, esters etc.

The Liquid Chromatography Mass Spectroscopy (LC-MS) of hydroethanolic ethanol extract of *S. nigrum* variant black

fruited shown in Fig. 1; Table -1. The results of variant orange fruit shown in Fig. 2; Table 2. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Major biological compounds like Solanine, Solamargine, Triterpenoids daidzin, chrysoriol-hexose, diosgenin, gingerol, moroctic acid and sanguinarine have been identified in both variants. But the content of Solanine and solamargine differ quantitatively among the variants.

The identified constituents from LC-MS are used in industries in various applications like flavour, antioxidant, anti-inflammatory, antimicrobial and cancer preventive (Sermakkani and Thangapandian, 2012).In addition the identified compounds possess many biological properties. For instance, Chrysoriol hexose (R/T 36.6) compound is found in alfalfa chrysoerior is widespread flavones, belongs to the family of flavonols. These are compounds that have the 3-hydroxy flavones backbone with anticancer, antioxidant, antitumor, chemopreventive, pesticidal and sunscreen properties. Daidzin (R/T 36) can be used as antioxidant, antiestrogenic, anticarcinogenic, antiatherogenic and antiosteoporotic activity. The compound Triterpepoid derivative (R/T 41.5) hypocholesterolemic, nematicide, pesticide, lubricant activities and plasticizer compound -1, 2 - benzenedicarboxylic acid (R/T 20.91) have antioxidant and anti inflammatory properties.

Gingerol is one among the seven compounds identified in the present study. Its analogues have a favorable toxicity profile, but are cytotoxic towards a range of cancer cell lines including blood cancer and lung cancer, Senwal *et al*. (2015) [18]. Gingerol has been investigated *in vitro* for its effect on cancerous tumors of the bowel, breast tissue, ovaries and pancreas, (Lee *et al.*, 2008 and Park *et al.*, 2006) [12, 16]. These compounds were found to have potential antioxidant and anticancer activities.

Sanguinarine is also one among the seven compounds identified in LC-MS analysis. It has been shown to exhibit antibiotic, antiapoptotic, anti-fungal, anti-inflammatory and anti - angiogenic functions. Sanguinarine belongs to the family of Benzoquinolines. These are organic compounds containing benzene fused to a quinoline ring system.

In future detailed exploration of phytoconstituents by quantification and characterization with medicinal properties would be accomplished with pharmacological studies may be reliable source of medicine for the future generation.

Summary

The plants are important source of potentially useful structures for the development of new therapeutic agents.In the present study, not many reports are available on the LC-MS analysis of this variants. Furthermore the mass spectrum obtained from liquid chromatography showed the molecular peaks. These compounds are agreement with Diadzin, chrysoriol hexose and triterpenoid derivative in variant black fruited. The compound gingerol, mortotic acid, sanguinarine and triterpenoids derivative in variant orange fruited. The present findings concluded that, *Solanum nigrum* L. variants have great potential phytoconstituents which leads to pharmaceutical application.

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