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Isolation and characterization of phytochemicals from ethyl acetate fraction of *Solanum nigrum* using GC-MS

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Abstract: *Solanum nigrum* plant has rich therapeutic potential and different developed countries utilize this plant as a chief element for oriental medicinal practice including cancer therapy. The current study determines the isolation and purification of *S. nigrum* bioactive constituents through column chromatography from ethyl acetate extract of the plant followed by the Gas Chromatography-Mass Spectroscopy (GC-MS) to analyze the isolated compounds Different gradient elutions followed by thin layer chromatography of collected fractions were done and structural analysis of the isolated compounds was performed following GCMS analysis, More specifically the compounds were identified as 1, 2 benzene dicarboxylic acid, diisooctyl ester (95%) and as Bis (2-Ethylhexyl) phthalate (84%) along with 3 hydroxy 4 carboxy 2 methyl 6 pyridine (58%) with reference to chemical abstract service which may be responsible for its pharmacological properties. According to the best information available, no documented information exists regarding GC-MS based identification of the isolated chemical compounds from the *S. nigrum*. Present findings will help in exploring the therapeutic potential of 1, 2 benzene dicarboxylic acid, diisooctyl ester (95%), Bis (2-Ethylhexyl) phthalate (84%) and 3 hydroxy 4 carboxy 2 methyl 6 pyridine (58%) will be helpful in the development of new composites in pharmaceutical fields.

Keywords: Bioactive compounds, column chromatography, ethyl acetate, metabolites.

INTRODUCTION

Solanum nigrum (*Solanaceae*) generally known as Makoi or black nightshade, frequently grows as a wild plant in humid environments in diverse types of soils, with dry, stony, shallow, or deep soils, subtropical and tropical regions are the ideal climatic areas for the cultivation of *S. nigrum*. April to May is the best weather for the sowing of the seeds in well-fertilized nursery beds and it has a great role in retrieving as well as degradation of the land. Several ailments such as fever, inflammation, pain, toothache, stomach problems, tonsils, pneumonia, antipyretic, antioxidant, anticancer, diuretic, worm infections, and several tumors (Hameed *et al*., 2017). *S. nigrum* plant is commonly used in oriental practice for cancer therapy in different countries such as China, Africa, and America. Clinical trials of this plant are managing several diseases from ancient times to today (Shakya, 2016). The unripe fruit of the solanum nigrum is free from glycoalkaloids reported in different studies and the other plant parts could be used as therapeutic as they contain solanine a glycoalkaloid in a significant quantity (Kumar *et al*., 2016; Gasti *et al*., 2020).

Leaves and fruit of *S. nigrum* are utilized for medicinal application mostly rather than the other parts of the plant. Extract of leaves is used widely for many health manifestations which include joint pains (gout and rheumatic) skin problems mainly dermatitis and have anti-tuberculosis potential (Jabamalairaj *et al*., 2019). Leaves can be used in nausea, nervous disorder problems, dropsy and able to produce Diaphoresis. (Sunitha *et al*., 2017) The fruit and flower of the *S. nigrum* are used as a tonic in cough and they are remedy for diuretics, bronchitis and pulmonary tuberculosis (Zahara *et al*., 2019). The fruit juice of the plant is used in hydrophobia, convulsions, ophthalmopathy and as an antidiarrheal (Hameed *et al*., 2017). It is effective in lowering the risk of heart disease especially in curing cardiopathy, nephropathy, leprosy, dropsy, hemorrhoids, ophthalmopathy and general debility (Mehmood *et al*., 2021; Kunwar *et al*., 2021). *S. nigrum* is known for its antiproliferative activity on different cell lines including the hepatocellular cell line (HepG2) and cervical cancer cell line (Nawaz *et al*., 2021). *S. nigrum* is nontoxic and safe but normal temperature cannot remove few toxic compounds as high temperature as the decomposition temperature of 250°C is required for the removal of toxic glycoalkaloids (Moyo *et al*., 2020). Fruits of *S. nigrum* are cooked as a vegetable (Jaradat *et al*., 2016).

Therapeutic domains of pharmaceuticals research include oncology and infectious diseases. Metabolic diseases treatment requires the extraction of new bioactive molecules from the plants (Newman and Crag., 2016). About 120 new drugs are available in the market synthesized from microorganisms, terrestrial plants, invertebrates, terrestrial vertebrates, and aquatic

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organisms (Khaleed *et al*., 2021). Medicinal plants have antioxidant potential due to the presence of numerous chemical compounds (Hameed and Akhtar, 2018; Campisi *et al*., 2019).

Gas chromatography mass spectrometry (GC-MS) a systematic system has been developed firmly and recognized as a vital technique for the profiling of metabolites from plants (Mutale *et al*., 2020; Sivaraj *et al*., 2020). GC-MS analysis is important in chemotaxonomic studies and phytochemical analysis of medicinal plants with biologically active components (Shaheen and Ahmed, 2020). A thorough literature review on the plant in the investigation has revealed that so far there is no available information worldwide, linked to the possible chemical components of "*Solanum nigrum*".

The bioactive compounds from plants can be used as a chief molecule in the treatment of different ailments and help in drug invention. The current study was designed to isolate and identify the bioactive components using GC-MS analysis. Bioactive compounds identified in the present study in *S. nigrum* can be used to develop possible novel drugs against emerging infectious diseases with relatively less cost.

MATERIALS AND METHODS

Methodology

Sample collection and preparation of fractions

The whole plant of *S. nigrum* (Mako, Nightshade) glabrous to slightly hairy with appressed non-glandular hairs identified by taxonomist (GC.Herb.Bot.3748) used in this study was locally collected from botanical Jinnah Garden, Lahore, Pakistan. Plant bearing fruit weighed 6.5 kg after the collection was shade dried and crushed to fine powder. About 5 kg of the plant was soaked into 20 liters of analytical grade ethanol (Absolute) at room temperature for 14 days followed by filtration and evaporation. Further, it was fractionated in ethyl acetate and was subjected to column chromatography.

Column chromatography

Analytical grades chemicals used in column chromatography were purchased from Sigma Aldrich (Germany) and Merck (USA). Ethyl acetate extract of *S. nigrum* was subjected to column chromatography using different solvent systems (*n*-hexane, dichloromethane, petroleum ether, and methanol). All collected fractions were pooled based on the polarity of eluted solvents. Glass column (15 cm × 4 cm) and silica gel as stationary phase was used in chromatography. A cotton plug was positioned on the bottom of the column and slurry was made with silica gel. Silica gel was used to pack the column by using the wet packing method and dispensed into the column to form silica bed. The slurry was dispensed on the topmost of the column followed by different solvent system of increasing polarity from *n*-hexane, dichloromethane, ethyl acetate to methanol (different combinations) and eluted gradiently. Different fractions were collected and subjected to TLC. Based on similarity, fractions were combined and the solvent was recovered via rotatory evaporator. A number of fractions were collected after pooling based on TLC patterns. Among all two fractions were selected (SN1 and SN2) based on purity for further analysis.

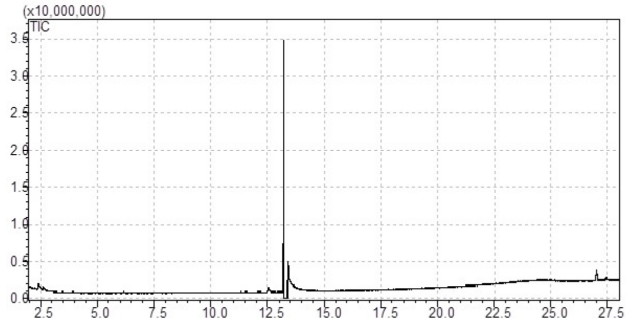

Fig. 1a: Gas chromatography-mass spectrum (GC-MS) analysis chromatogram of isolated compounds from *S. nigrum* showing one promising peak (1a). Time and relative abundance plotted along x axis and y axis respectively

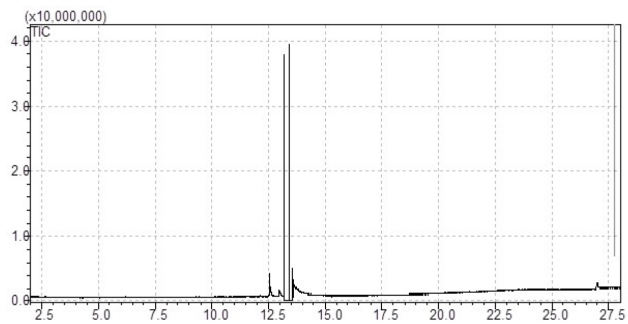

Fig. 1b: Gas chromatography-mass spectrum (GC-MS) analysis chromatogram of isolated compounds from *S. nigrum* showing two distinctive peaks (2a and 2b). Time and relative abundance plotted along x axis and y axis respectively.

Gas Chromatography / Mass Spectrometry analysis (GC\MS)

Gas chromatography within mass spectrometry investigation was done by QP2010- Shimadzu, Japan. EI mode at 70 eV with SLB5 column (30 m x 0.25 mm x 0.25µm Sigma-Aldrich, Germany) having 20 minutes temperature program of 190-325°C at 5°C/min were the operational conditions of the equipment. The temperature of the injector was maintained at 280°C, the flow rate of the carrier gas (helium) was 0.8mL/min, and the split ratio was 1:90. Structural identifications of compounds were performed based upon retention time. Identification of obtained mass spectrum was done using the database of National Institute of Standards and Technology (NSIT). NIST database contains more than 62,000 patterns of identified components. The unknown compounds spectra

Table: Characterization of isolated compounds of *Solanum nigrum*

Peak	Compound chemical name / Common name	Molecular weight (Da)	Molecular formula	CAS (Chemical Abstract Service)
1	1,2-benzenedicarboxylic acid, diisooctyl ester. (Diisooctyl phthalate)	390	$C_{24}H_{38}O_4$	27554-26-3
2a	3 hydroxy 4 carboxy 2 methyl 6 pyridine	323	$C_{17}H_{25}NO_5$	76433-12-0
2b	Bis(2-ethylhexyl) phthalate (Phthalic acid bis)	390	$C_{24}H_{38}O_4$	117-81-7

obtained from *S. nigrum* extract were compared with the standard mass spectra already stored in NIST library (NISTII) as known components.

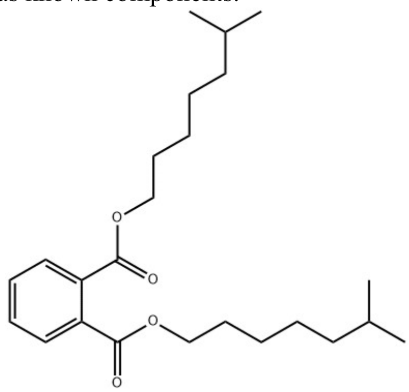

Fig. 1: Structure profiling of the compounds identified as 1,2-benzenedicarboxylic acid, diisooctyl ester from the *S. nigrum* using GC-MS analysis

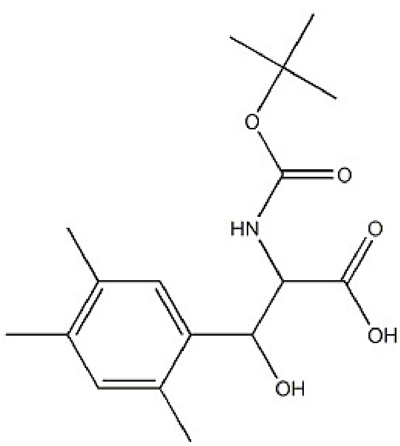

Fig. 2a: Structure profiling of the compounds identified as 3hydroxy 4carboxy 2methyl 6pyridine from the *S. nigrum* using GC-MS analysis.

RESULTS

GC-MS analysis of isolated SN1 established fraction has revealed the presence of 1, 2 benzene dicarboxylic acid, diisooctyl ester (95%) (fig. 1) and Bis (2-Ethylhexyl) phthalate (84%) (fig. 2b) along with 3 hydroxy 4 carboxy 2 methyl 6 pyridine (58%) (fig. 2a) as extracted chemical compounds were present. These isolated compounds are identified with their molecular formula, molecular weight, retention time (RT), and concentration (peak area %) of the matching compounds. Chromatogram of GC-MS analysis of isolated compounds from *S. nigrum* showed the presence of 1 major peak for SN1 as well as 2 major peaks for SN2 and their related components.

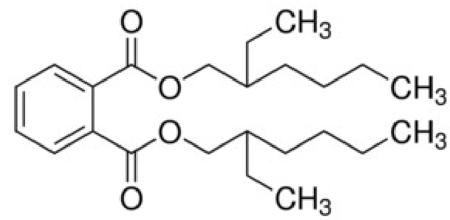

Fig. 2b: Structure profiling of the compounds identified as Bis (2-ethylhexyl) phthalate from the *S. nigrum* using GC-MS analysis.

DISCUSSION

Secondary metabolites which are the important and rich constituent of many medicinal plants include phenol, saponins, tannins, terpenoids, alkaloids, flavonoids and glycosides. Gas chromatography and mass spectrum can characterize these metabolites as they are considered to be primary source of biological and pharmaceutical activities. *S. nigrum* is known for several therapeutic properties such as antioxidant, antimicrobial, antioxidant, antilipidemic, antitumor, hepatoprotective, antiarthritic, antinicotinic, anti-inflammatory and antieczemic (Jabamalairaj *et al.,* 2019).

The present investigation emphasizes the isolation and purification of *S. nigrum* constituents through column chromatography from ethyl acetate extract of the plant followed by the Gas Chromatography-Mass Spectroscopy to analyze the isolated compounds. Interpretation of GCMS analysis was done using the database of NIST having about 62000 patterns (Hameed *et al*., 2015; Hussein *et al*., 2016).

The obtained spectrum of unknown components isolated from the *S. nigrum* was compared to the spectrum of identified compounds present in the NIST database. Compounds identified in the present study as 1, 2 benzene dicarboxylic acid, diisooctyl ester (95%) and Bis (2-Ethylhexyl) phthalate (84%) along with 3 hydroxy 4 carboxy 2 methyl 6 pyridine (58%) have been reported for different biological activities from different plant species and can be helpful in exploring new therapeutic agents (Abbas *et al*., 2014). Benzene dicarboxylic acid and diisooctyl ester (95%) have been reported for their antimicrobial and antifouling properties (Ingole *et al*., 2016; Khan *et al*., 2020; Takua, 2020). Bis (2-Ethylhexyl) phthalate is the commercial plasticizer used in therapeutic devices as dialysis bags, nasogastric tubes, IV catheters, intravenous tubing and bags, blood bags, transfusion tubing, and air tubes (Bouattour *et al*., 2020; Bider *et al*., 2020).

By standard characterization and optimization, these compounds could be beneficial for humans as their presence attributes to the antioxidant, anticancer, antimicrobial and anti-inflammatory, anxiolytic, antinociceptive and antimicrobial properties (Xiang *et al*., 2018). Bioactive compounds have different therapeutic properties in many disease, such as pancreatitis, respiratory disease, colon injury, cardiac, and neuro-degenerative complications. These compounds also show antipruritic, antifungal, and antibacterial activities and also beneficial in the management of skin infections (Jasmin *et al*., 2015; Meng *et al*., 2020)

Synergistic effects and additives phenomena are frequently vital to the bioactivity of different plant extracts but the activity of the purified compound is lost in some cases. It is supposed that crude organic, as well as purified aqueous fractions from the plants, are highly active biologically than the single isolated compound because of the synergistic effects. (Muhammad *et al*., 2016; Vural *et al*., 2020; Usman *et al*., 2020; Khaleed *et al*., 2021).

A comprehensive study was performed to determine the diversity of different solanum species (*S. villosum, S. nigrum, S. coagulans, S. schimperianum, S. incanum L, S. glabratum, S. torvum, S. sisymbriifolium, S. macracanthum* and *S. dulcamara*) from the south-western area and was assessed for its phytoconstituents. The determination was constructed on the variances in the metabolites of the fractions of diverse species using GC-MS analysis. Their results discovered a number of phytochemicals at different retention times (Rani *et al*., 2017).

CONCLUSION

It can be concluded from our study that the ethyl acetate fraction of *S. nigrum* contains novel compounds of pharmacological importance. Identified compounds from *S. nigrum* plant in this study can be used as potential candidates in drug development to overcome different emerging diseases.

Study limitation and future plan

Further biological assays are required to explore more therapeutic benefits of the identified compounds in the present study. More biological properties of identified compounds need to be explored in the future.

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