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Phytochemical Identification And Isolation Of Active Constituents Of *Curcuma Longa* And *Solanum Nigrum*

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ABSTRACT

Turmeric is the common name for this substance, or "haldi." This well-known spice's biological name is *Curcuma longa*, also known as turmeric, is a member of the ginger family, Zingiberaceae. *Solanum nigrum* Linn. also known as *Solanum nigrum* var. *virginicum* L. (in China). *S. nigrum* can be used as a medicine and tastes bitter, is of cold property and slightly toxic, and belongs to the lung and kidney meridians. The present research was based on the phytochemical identification and isolation of active constituents of *Curcuma longa* and *Solanum nigrum*. In the end of January month 2023, the fresh rhizomes of *Curcuma longa* and fresh leaves of *Solanum nigrum* were harvested and collected from the Lucknow region, Uttar Pradesh, IN. After authenticated by the botanist at CSIR – National Botanical Research Institute (NBRI) UP. with reference no. (PDSH/LWG/Authentication/Ang./2023-24/25) on 11/10/2023. In extraction of *Curcuma longa*, 25g coarse powder of Turmeric rhizome was extracted using chloroform (400ml) for 8 hours. In extraction of *Solanum nigrum*, 25g of coarse powder of leaves of *Solanum nigrum* was extracted in methyl alcohol (350ml) for 8 hours. Column chromatography and TLC techniques were used in isolation of active constituents. Mass chromatography was utilized in identification of isolated compounds. In *Curcuma longa* extraction, the percentage yield was found to be as 13.72%; in *Solanum nigrum*, the percentage yield was found to be as 22.4%. It concluded that *Curcuma longa* and *S. nigrum* have numerous active constituents including alkaloids, glycosides, steroids, proteins, saponins, and polysaccharides.

Keywords: *Curcuma longa*, *Solanum nigrum*, active constituents, column chromatography, TLC and Mass spectroscopy.

INTRODUCTION

Turmeric is the conventional term used to refer to this chemical, sometimes known as "haldi." *Curcuma longa*, often known as turmeric, is a widely recognized spice that belongs to the Zingiberaceae family, which is also known as the ginger family. In India, turmeric is employed for several therapeutic purposes in Ayurvedic, Unani, and Siddha medicines, as well as in culinary applications [1]. India is both the primary producer and consumer of turmeric, a therapeutic spice. Turmeric is widely used as a culinary pigment, preservative, and flavoring agent [2]. The Food and Agriculture Organisation of the United Nations [3] reports that the USA imports about 2400 metric tonnes of turmeric annually for consumer consumption. Turmeric is mostly produced in India, although it is also cultivated in Bangladesh, Pakistan, Sri Lanka, Taiwan, China, Burma (Myanmar), and Indonesia in Asia. Turmeric is cultivated in several countries in the Caribbean and Latin America, including Jamaica, Haiti, Costa Rica, Peru, and Brazil [4]. Turmeric is derived from the rhizomes of *Curcuma longa*, which undergo a process of cooking, drying, cleaning, and polishing [5].

Taxonomy

Kingdom: Plantae

Order: Zingiberales

Family: Zingiberaceae

Genus: *Curcuma*

Species: *longa*



a) Whole plant



b) flower



b) Rhizome and its powder

Fig 1. Different parts of *Curcuma longa*

The entire rhizomes are collected following the harvest. The rhizomes are supplied intact [6]. They frequently have a finger-like appearance, with dimensions ranging from 2 to 8 cm in length and 1 to 2 cm in width, featuring bulbs and cracks [7]. The dried rhizomes undergo additional

processing to produce turmeric powder. 'Alleppey' and 'Madras' are distinct cultivars of turmeric that are renowned for their production in various regions of India. Alleppey is mostly sourced from the United States and typically has a volatile oil content ranging from 3.5% to 5.5% and a curcumin content ranging from 4.0% to 7.0% [8]. Turmeric is commonly employed as a therapeutic agent for a range of ailments due to its inclusion of volatile oils and the active compound curcumin. Due to its anti-inflammatory properties, it is extensively utilized [9][10]. Additionally, it possesses antioxidant capabilities that surpass those of vitamins E and C [11]. The effectiveness of the rhizome extract in treating cancer has been studied. The antimicrobial action, which includes the ability to inhibit the growth of bacteria and fungi, is one of the additional activities observed [12]. Turmeric exhibits cardioprotective effects by lowering cholesterol levels and inhibiting platelet aggregation [13][14]. Furthermore, scientific research has demonstrated the efficacy of turmeric in the treatment of several ailments such as Alzheimer's disease, rheumatoid arthritis, and cancer [15].

Solanum nigrum Linn., also referred to as *Solanum nigrum* var. *virginicum* L. (in China). *S. nigrum* is widely spread throughout China, occurring frequently in close proximity to fields, wastelands, and villages. Furthermore, it exhibits a wide distribution in temperate to tropical climates in Europe, Asia, and America. *S. nigrum* possesses medicinal properties, characterized by its bitter taste, frigid nature, and mild toxicity. It is classified under the lung and kidney meridians. Chinese folk medicine and traditional Chinese medicine (TCM) have gained extensive clinical knowledge in the utilization of *S. nigrum* [16]. *S. nigrum*, in its entirety, possesses beneficial properties in promoting the dispersion of blood clots and reducing swelling, as well as in eliminating heat and toxins. It has been widely employed for centuries in the treatment of various ailments such as canker sores, skin eczema, urinary tract infections, bacterial dysentery, prostate issues, and chronic bronchitis [17].



a) whole plant



b) flowers and fruit

Fig 2. Different parts of *S. nigrum*

In addition, in modern clinical practice, *S. nigrum* is commonly combined with other drugs for the treatment of cancers, such as lung cancer, cervical cancer, breast cancer, esophageal cancer, stomach cancer, liver cancer, and bladder cancer. In other Asian countries, such as Japan and India, it has also been documented for the treatment of tumors. Ripe berries of *S. nigrum* are sweet and salty and were reported to have been used as a famine food in China in the 15th

century [18]. In India, the leaves and berries of this plant are commonly consumed as food or vegetable after cooking [19][20].

Taxonomy

Kingdom: Plantae

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species: *nigrum*

S. nigrum is a yearly, upright, herbaceous plant that reaches a height of 0.25–1 m. The plant possesses a taproot system characterized by a well-developed primary root and is frequently hardened and woody. The stem lacks inconspicuous edges, displaying a green or purple hue, and is almost smooth or covered in fine hairs. The leaf has an ovate shape, measuring 2.5–10 cm in length and 1.5–5.5 cm in width. Its tip is briefly pointed. The cuneate leaf has a wedge-shaped base that is broad and slopes downward towards the petiole. It is characterized by irregular, wavy coarse teeth that are present throughout the leaf or on each side. The leaf is smooth or sparsely covered with soft hairs on both sides and has five to six veins on each side. The petiole, or leaf stalk, measures approximately 1–2 cm in length. The scorpion-tailed inflorescence is located outside the axil and consists of 3–6–(10) flowers. The overall length of the pedicel ranges from approximately 1 to 2.5 cm, while the pedicel itself measures around 5 mm in length and is either smooth or covered in fine hairs. The calyx is diminutive, with a shallow cup form, measuring approximately 1.5–2 mm in diameter. The teeth are oval in shape, with a rounded tip, and the junction between the two teeth at the base is inclined. The corolla is white, the tube is concealed within the calyx and measures less than 1 mm in length, while the 5-parted crown is approximately 2.5 mm long. The lobes have an oval shape and measure around 2 millimeters in length. The filaments are brief, the anthers are yellow, measuring approximately 1.2 mm in length, and are roughly four times longer than the filaments. Additionally, the apical hole is oriented inward. The ovary is shaped like an oval and has a diameter of approximately 0.5 mm, while the style measures around 1.5 mm in length. The bottom portion of the central region is adorned with white hairs, while the stigma is diminutive and the skull is contoured. The fruit has a spherical shape, with a diameter of approximately 8 mm, and turns black as it reaches maturity [21]. The seeds are predominantly ovoid, measuring approximately 1.5–2 mm in diameter, and exhibiting compression on both sides.

Phytochemical research conducted in recent decades has verified that the entire *S. nigrum* herb has steroidal saponins, steroidal alkaloids, flavonoids, coumarin, lignin, organic acids, volatile oils, polysaccharides, and various other constituents. The crude extract of *S. nigrum* and certain chemicals described earlier have been scientifically proven to possess diverse actions, such as anticancer, antioxidative, anti-inflammatory, hypotensive, neuroprotective, immunomodulatory, antibacterial, and liver protecting activities. The research focus lies particularly on the anticancer properties of steroidal saponins and steroidal alkaloids. Drug researchers are hopeful to discover potential antitumor drugs from these components [22].

MATERIALS AND METHODS

Chemicals and equipments

Fresh rhizomes of *Curcuma longa*, Fresh leaves of *Solanum nigrum*, methyl alcohol, chloroform, Toluene, Ethyl acetate, Cylindrical chromatographic column, separating funnel, Silica gel, Silica

sand, Iodine chamber, UV light detector, TLC plate developing chamber, Capillary tubes, TLC plates, and magnetic stirrer.

Collection, identification and authentication of the plant species

In the end of January month 2023, the fresh rhizomes of *Curcuma longa* and fresh leaves of *Solanum nigrum* were harvested and collected from the Lucknow region, Uttar Pradesh, IN. CSIR – National Botanical Research Institute (NBRI) Lucknow UP with reference no. PDSH/LWG/Authentication/Ang./2023–24/25 on 11/10/2023. These were washed, dried under shade. These were crushed into coarse powder; weighed and used for extraction.

Extraction processes

Both the herbal powders were extracted through Soxhlet apparatus, separately.

- In extraction of *Curcuma longa*, 25g coarse powder of Turmeric rhizome was extracted using chloroform (400ml) for 8 hours.
- In extraction of *Solanum nigrum*, 25g of coarse powder of leaves of *Solanum nigrum* was extracted in methyl alcohol (350ml) for 8 hours.

Isolation of active constituents by column chromatography

The sample powder, which had been dried by exposure to air, was extracted using an appropriate solvent and subsequently dried under vacuum before being loaded onto the column. The quantity of the sample must be quantified to determine the yield of the intended extract. Gradient solvent system: A gradient solvent system, ranging from non-polar to highly polar solvents, offers optimal elution and separation of diverse organic components included in plant-based organic extracts. Nevertheless, the quantity of solvent required may vary depending on the quantity of the material that needs to be purified.

A cylindrical glass column holding a stationary phase (silica gel) is slowly filled with a liquid solvent. The mobile phase moves downward through the column due to the force of gravity or external pressure. This approach is employed for the purification of chemicals from a mixture. After the column has been prepared, the sample is inserted into the upper part of the column. Subsequently, the mobile solvent is permitted to descend through the column. The chemicals in the mixture exhibit varying affinities for the stationary phase and mobile phase, resulting in their separation and elution at varied time intervals or degrees. Thus, the process of isolating chemicals from the mixture is accomplished. The different chemicals are separated into fractions and subsequently examined to determine their structure [23].

Thin Layer Chromatography (TLC) method [24]

- TLC provides partial separation of both organic and inorganic materials using thin-layered chromatographic plates especially useful for checking the purity of fractions.
- Each fraction is applied onto activated TLC plates using a capillary tube, positioned 1/2 inch away from the lower edge of the plate. The plate is then placed in a developing chamber that contains an appropriate solvent system. The plate is left in the chamber for a specific amount of time, until the developing solvent reaches the top edge of the plate.
- The plate is removed from the developing chamber, dried, and the solvent front is indicated using a lead pencil. Compound bands/spots observed on TLC chromatoplate can be identified

through visual inspection using UV light (254nm), an iodine chamber, or by applying a spray reagent (vanillin–sulfuric acid) to determine the presence of certain compounds.

- The visualized spots of the components in the chromatoplate are marked and the R_f value of each spot is calculated by the formula: $R_f = \text{distance travelled by the sample (cm)} / \text{distance travelled by the solvent (cm)}$.
- TLC plate showing number of bands (compounds) for each fraction can be further purified using high performance liquid chromatography.
- Based on the nature of the compounds, further spectral analyses such as Infrared, Gas Chromatography and Mass spectrometry and Nuclear magnetic resonance can be performed to elucidate the chemical structure of target compounds.

Identification of active constituents

➤ GC–MS analysis

The entire plant extracts of *Curcuma longa* and *Solanum nigrum* were analysed using gas chromatography–mass spectrometry (GC–MS) with the Thermo GC–Trace Ultra Version: 5.0 equipment and Thermo MS DSQ II. The apparatus is equipped with a DB 35– MS Capillary Standard non–polar column, which has dimensions of 30 mm in length, 0.25 mm in internal diameter, and a 0.25 µm film coating. The carrier gas utilised is Helium at a modest flow rate of 1.0 ml/min. The injector was operated at a temperature of 250 °C, while the oven temperature was programmed according to the following schedule: The temperature was initially set at 60 °C for 15 minutes, and then it was steadily raised to 280 °C within a period of 3 minutes. The identity of components was determined by utilising the Willey and NIST libraries, as well as comparing their retention indices. The constituents were identified by comparing them with those present in the computer library (NIST and Willey) connected to the GC–MS instrument. The obtained results have been organised in a table.[25].

RESULTS AND DISCUSSION

Percentage yield

In *Curcuma longa* extraction, the percentage yield was found to be as 13.72% when calculated on the basis of its practical yield. In *Solanum nigrum*, the percentage yield was found to be as 22.4% when calculated on the basis of its practical yield. Thus, % yield was observed highest in leaves extract of *Solanum nigrum*.

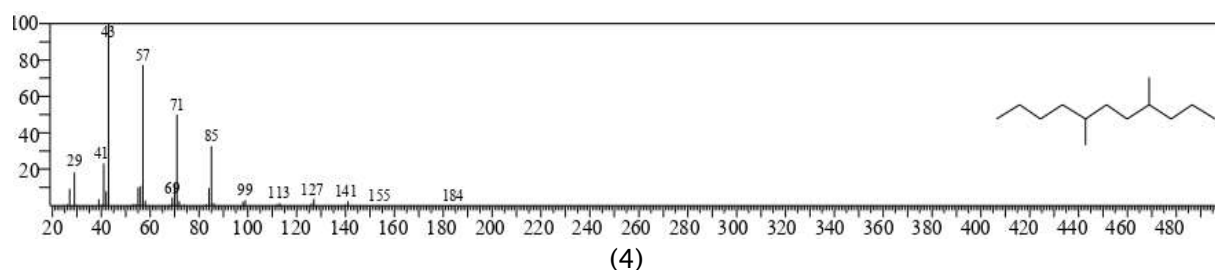
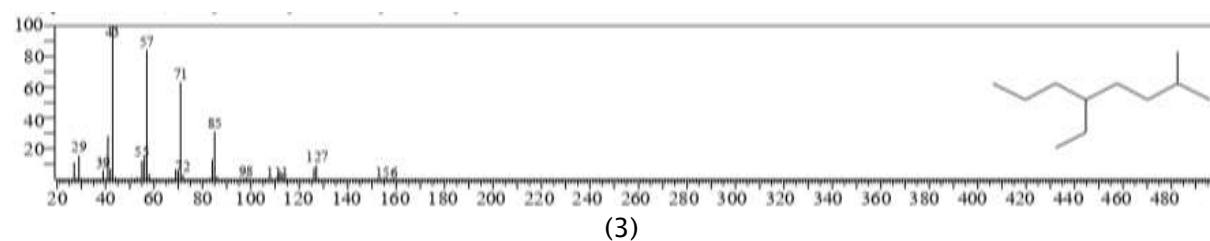
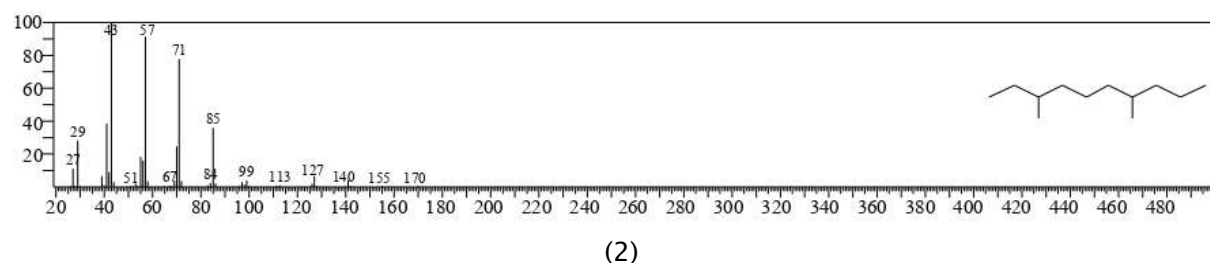
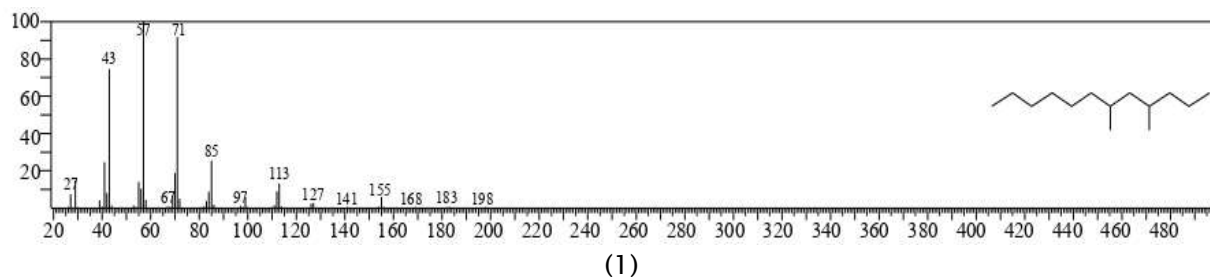
Phytochemical constituents

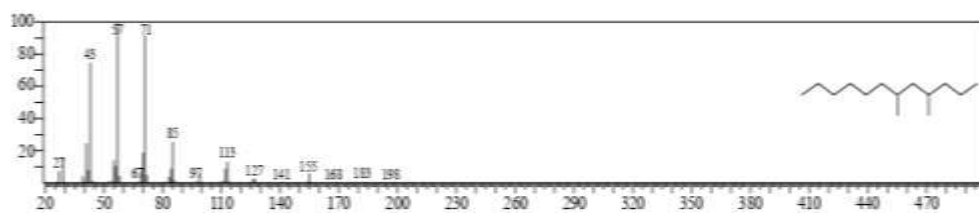
The following table refers the phytochemical constituents isolated from the Turmeric and *S. nigrum* plant extracts:

Table 1. List of major active constituents of (*Curcuma longa*)

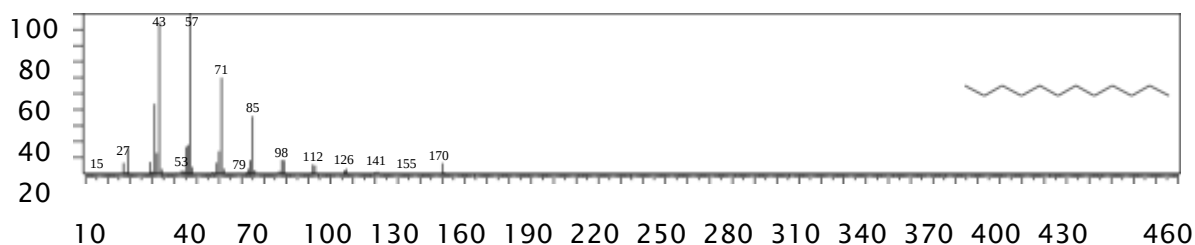
S.N.	Active constituents	Mol. formula	Mol. weight	CAS
1.	4,6-Dimethyldodecane	C ₁₄ H ₃₀	198	61141–72–8
2.	3,7-Dimethyldecane	C ₁₂ H ₂₆	170	17312–54–8
3.	5-Ethyl-2-methyloctane	C ₁₁ H ₂₄	156	62016–18–6
4.	4,7-Dimethylundecane	C ₁₃ H ₂₈	184	17301–32–5
5.	5,6-Dimethyl-nonane	C ₁₁ H ₂₄	156	17302–23–7
6.	Duodecane	C ₁₂ H ₂₆	170	112–40–3
7.	Hexadecane	C ₁₆ H ₃₄	226	544–76–3
8.	Tridecane	C ₁₃ H ₂₈	184	629–50–5

9.	Heptadecane	C ₁₇ H ₃₆	240	629-78-7
10.	2,2,4,4,6,6,8,8,10,10,12,12-Dodecamethylcyclotetrasiloxane	C ₁₂ H ₃₆ O ₆ Si ₆	444	540-97-6
11.	5-amino-1-methyl-, tris(trimethylsilyl) derivative	C ₁₄ H ₃₂ N ₄ O ₃ Si ₃	356	0-00-0
12.	4-Amino-1,2-benzenediol	C ₁₅ H ₃₁ NO ₂ Si ₃	341	0-00-0
13.	Eugenol	C ₁₀ H ₁₂ O ₂	164	97-53-0 M
14.	3-Allyl-2-methoxyphenol	C ₁₀ H ₁₂ O ₂	164	1941-12-4
15.	Copaene	C ₁₅ H ₂₄	204	3856-25-5
16.	5-(3-Hydroxypropyl)-2-pyrrolidinone	C ₇ H ₁₃ NO ₂	143	80243-73-8
17.	Caryophyllene	C ₁₅ H ₂₄	204	87-44-5
18.	1,4,7,-Cycloundecatriene	C ₁₅ H ₂₄	204	0-00-0
19.	Humulene	C ₁₅ H ₂₄	204	6753-98-6
20.	Cycloheptasiloxane	C ₁₄ H ₄₂ O ₇ Si ₇	518	107-50-6

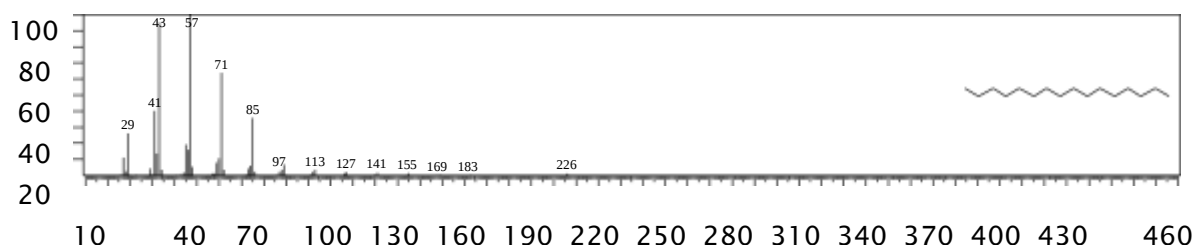
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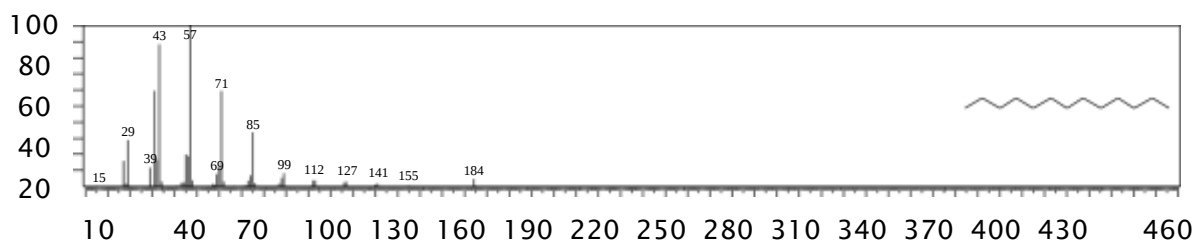
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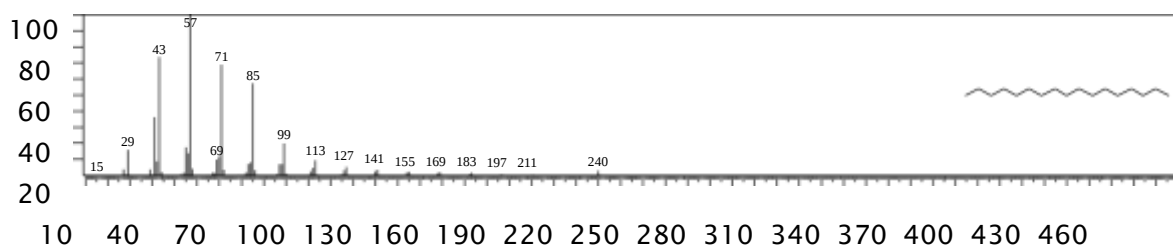
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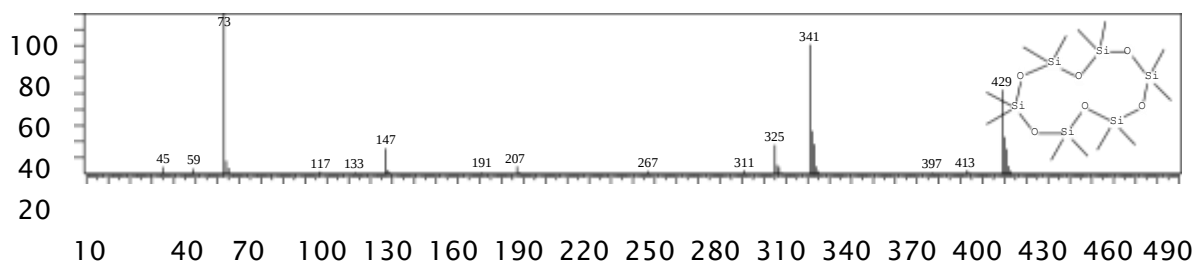
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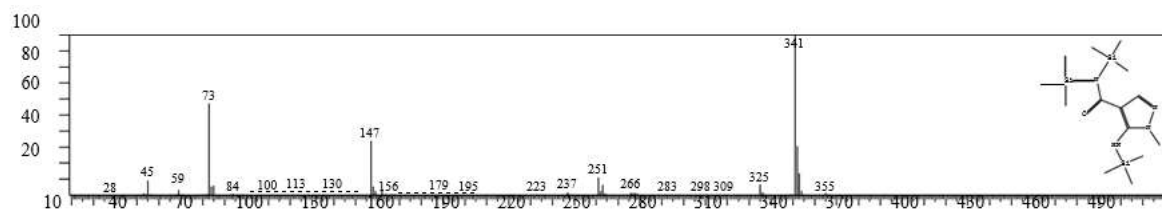
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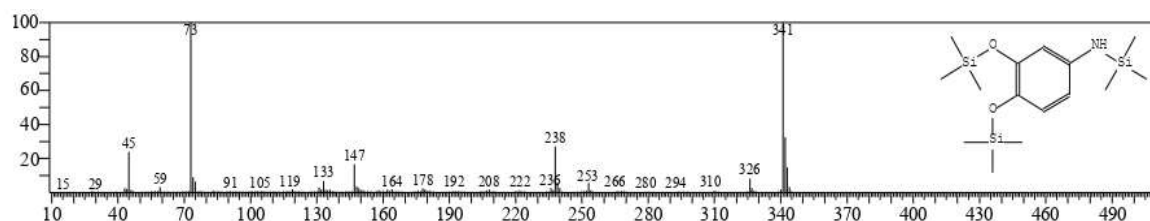
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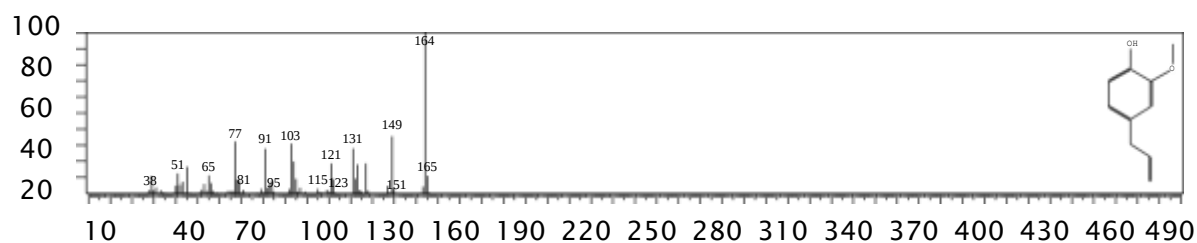
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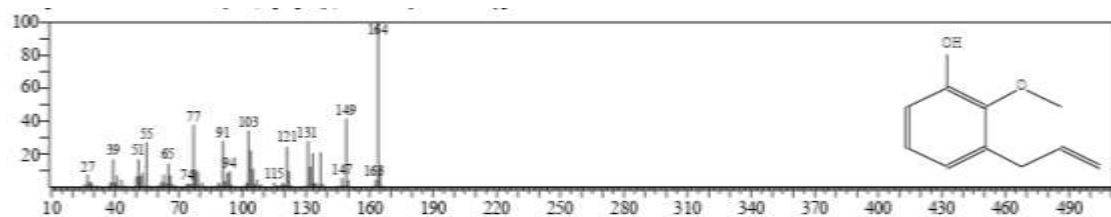
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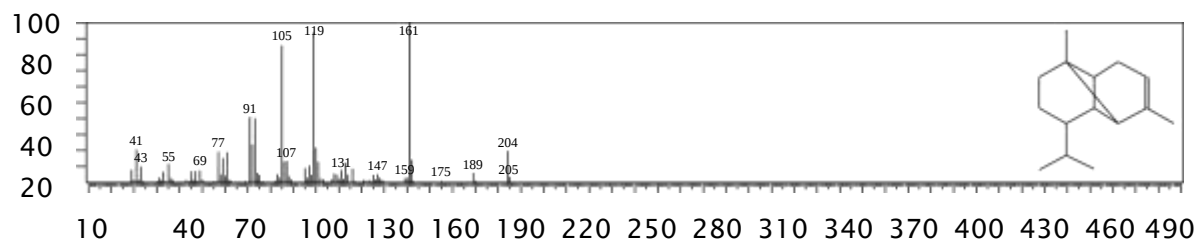
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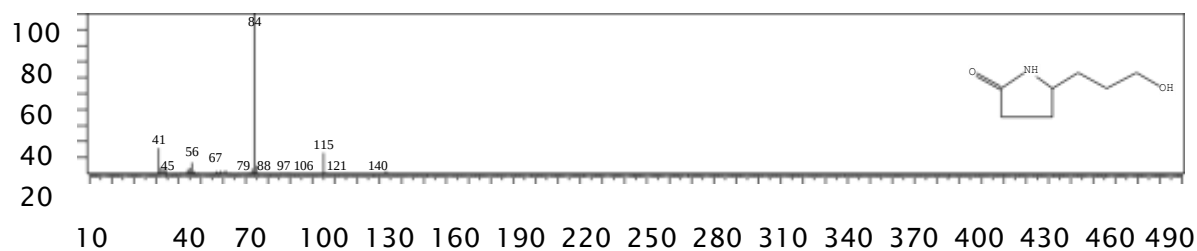
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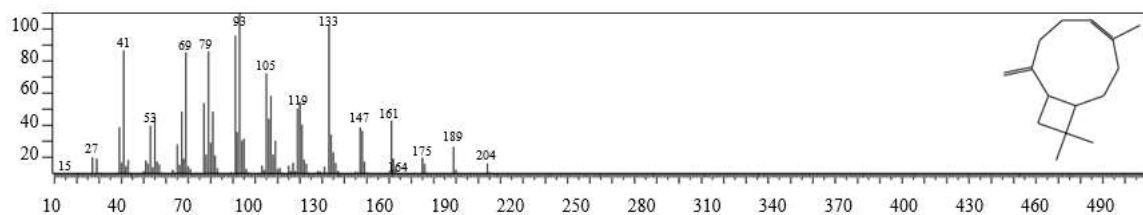
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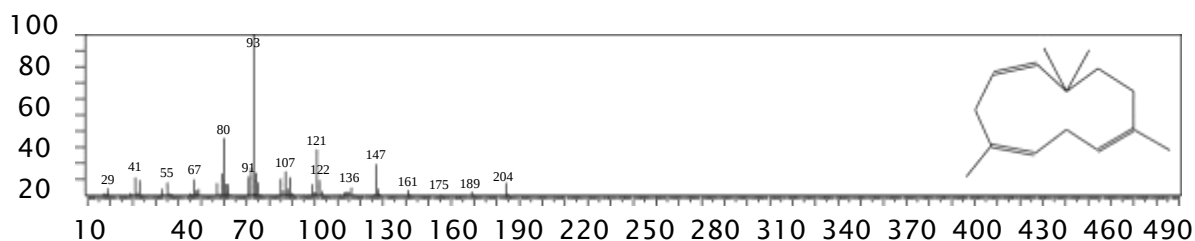
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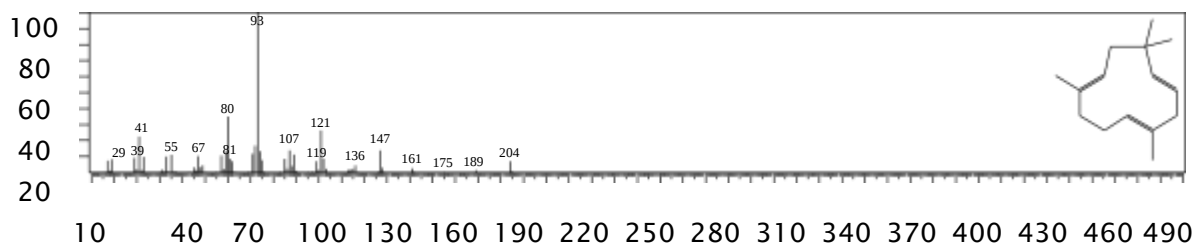
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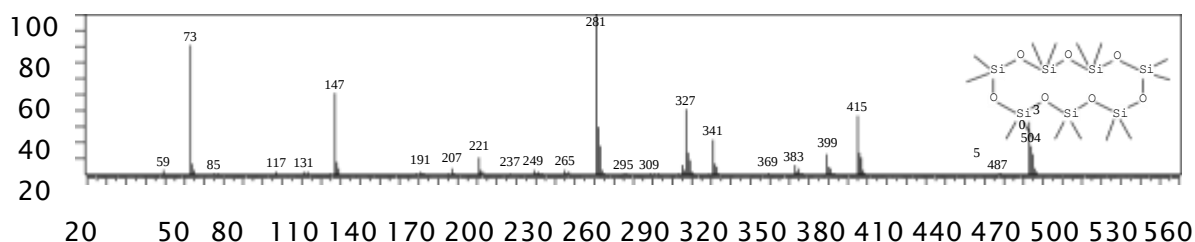
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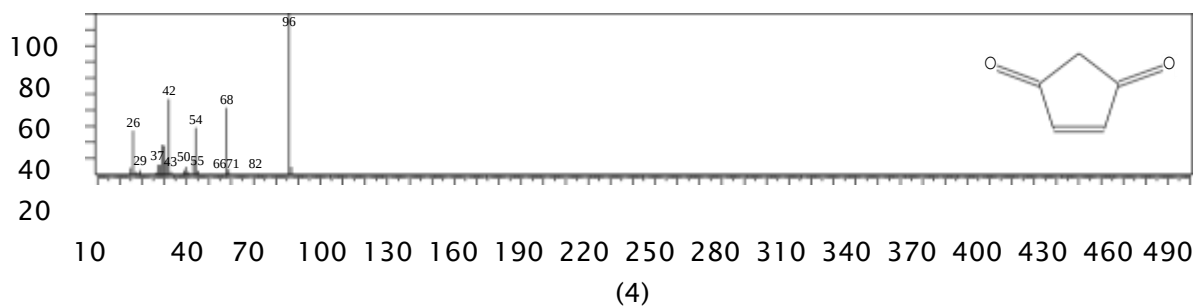
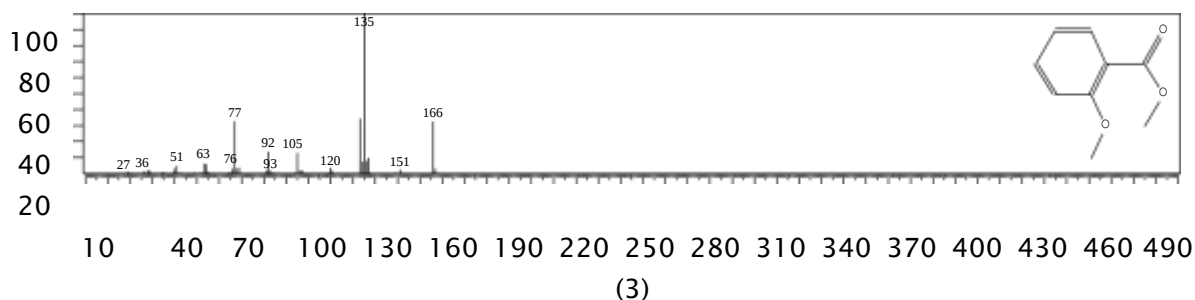
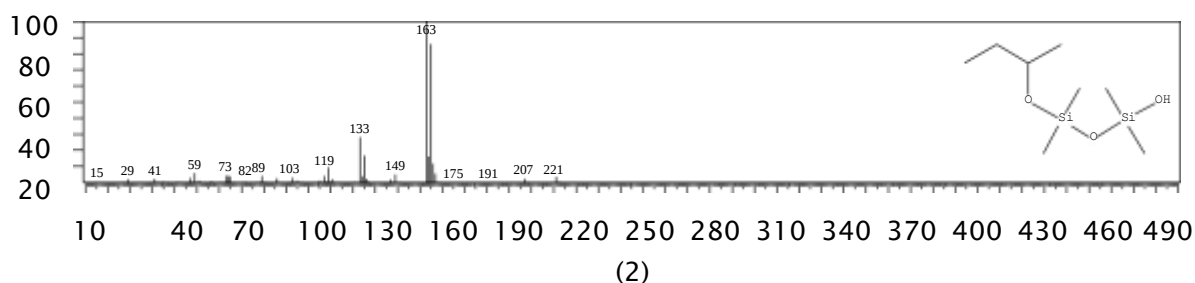
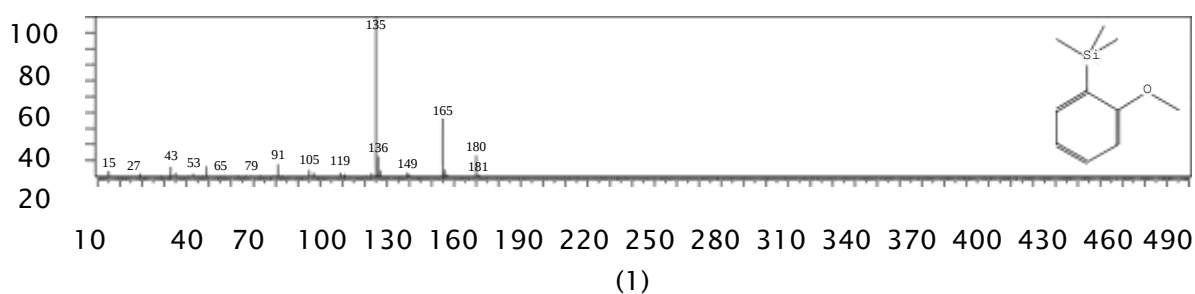


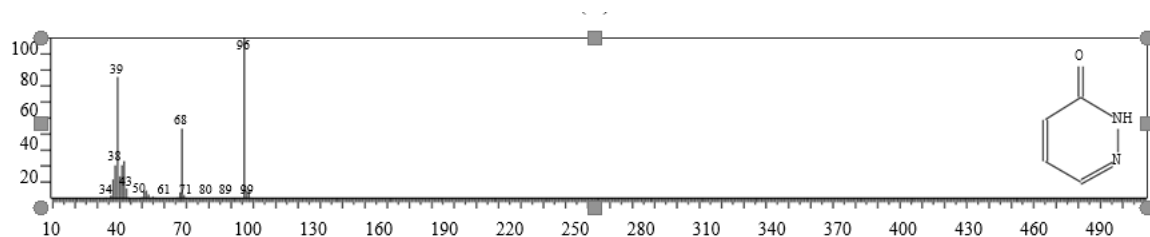
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Table 2. List of major active constituents of *Solanum nigrum*

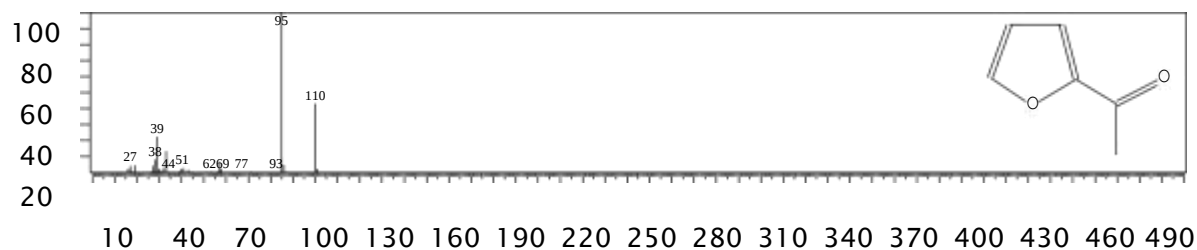
S.N.	Active constituents	Mol. formula	Mol. weight	CAS
1.	Silane	C10H16OSi	180	704-43-8
2.	1,1,3,3-Tetramethyl-3-(1-methylpropoxy)disiloxan-1-ol	C8H22O3Si2	222	0-00-0
3.	Methyl o-methoxybenzoate (Benzoic acid)	C9H10O3	166	606-45-1
4.	4-Cyclopentene-1,3-dione	C5H4O2	96	930-60-9
5.	3(2H)-Pyridazinone	C4H4N2O	96	504-30-3
6.	2-Furyl methyl ketone (Ethanone)	C6H6O2	110	1192-62-7
7.	4,6-Dimethylpyrimidine	C6H8N2	108	1558-17-4
8.	2-Isobutyl-3-methylpyrazine	C9H14N2	150	13925-06-9
9.	Carbamic acid	C9H11NO2	165	1129-41-5

10.	1,4-Benzenediamine	C ₆ H ₈ N ₂	108	106-50-3
11.	Cyclopentanone	C ₅ H ₈ O	84	120-92-3
12.	14-Methylcholesta-2,8-dien-6-yl acetate	C ₃₀ H ₄₈ O ₂	440	56293-01-7
13.	Dimethyl sulfone	C ₂ H ₆ O ₂ S	94	67-71-0
14.	2-Hydroxy-2-cyclopenten-1-one	C ₅ H ₆ O ₂	98	10493-98-8
15.	(S)-Methyl 2,3-dihydroxypropanoate	C ₄ H ₈ O ₄	120	10303-88-5
16.	2-Methyl-5-formylfuran	C ₆ H ₆ O ₂	110	620-02-0
17.	Betulinaldehyde	C ₃₀ H ₄₈ O ₂	440	13159-28-9
18.	Anthraergostatetraenol benzoate	C ₃₅ H ₄₆ O ₂	498	0-00-0

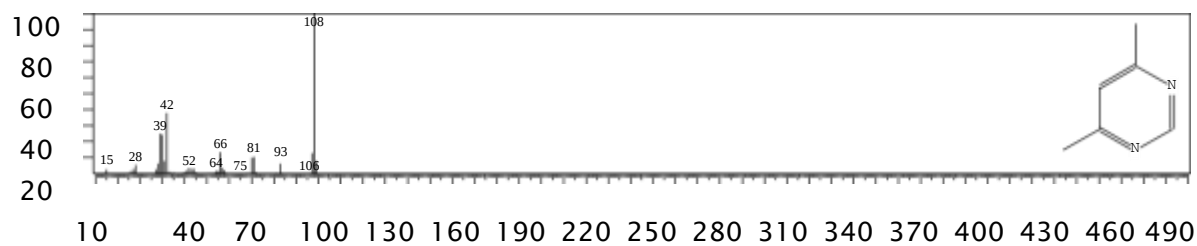
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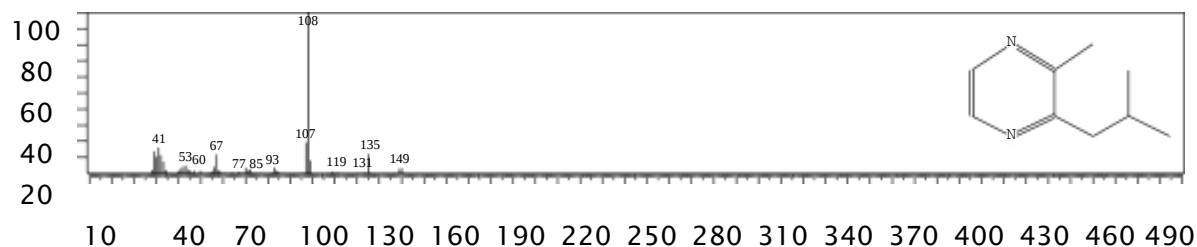
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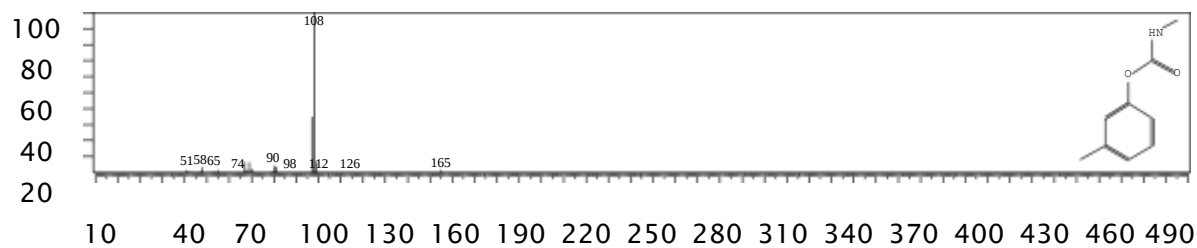
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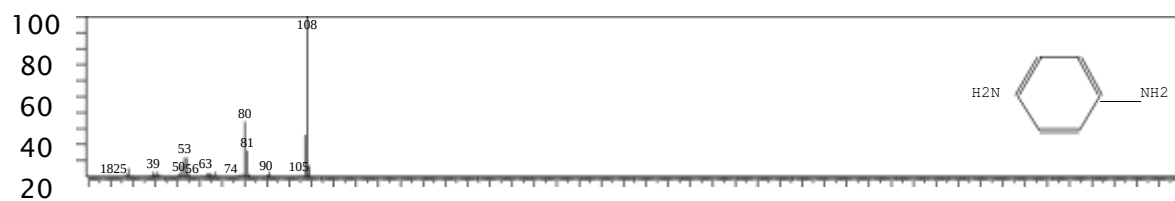
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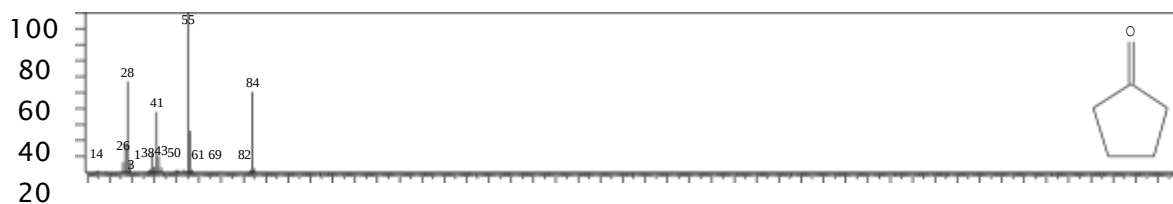
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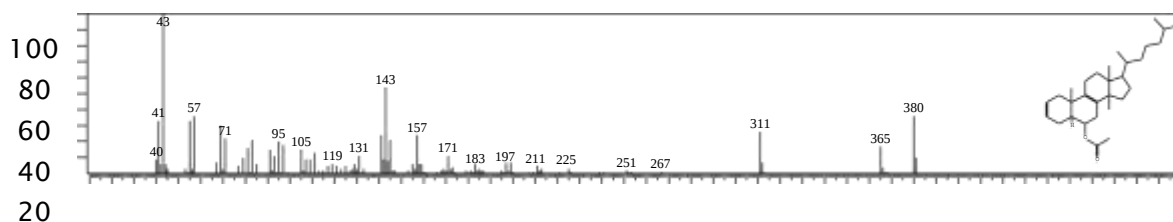
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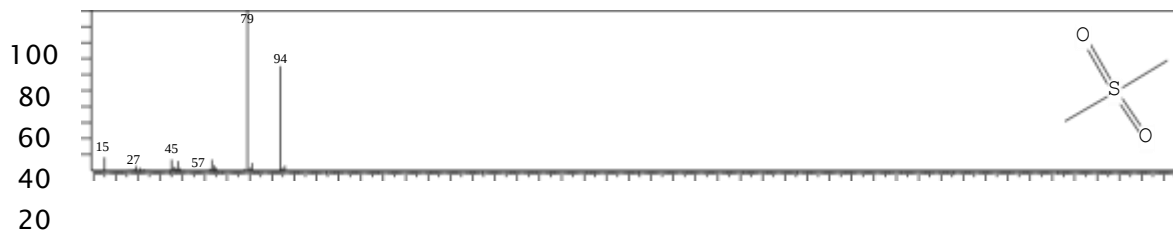
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(10)



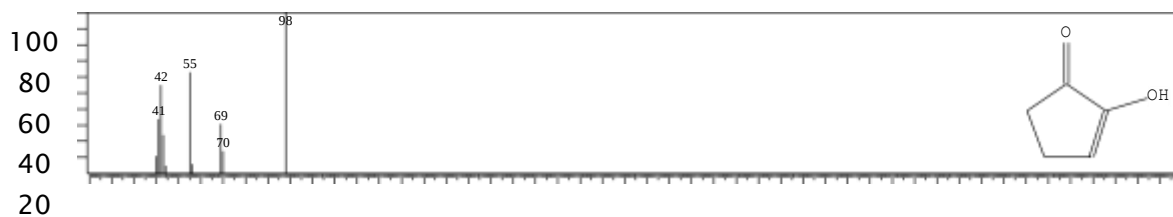
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(11)



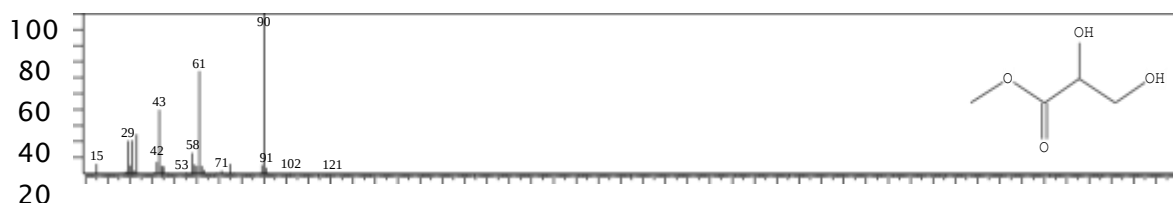
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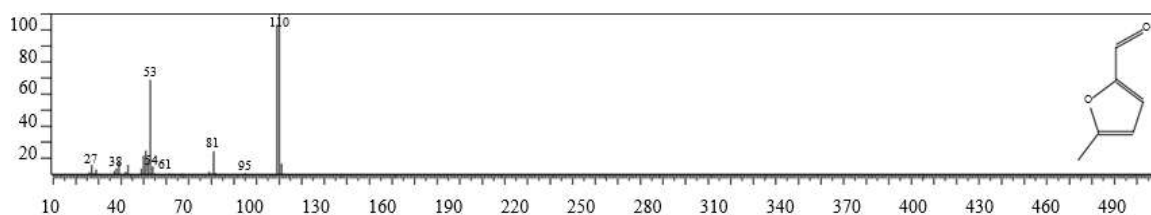
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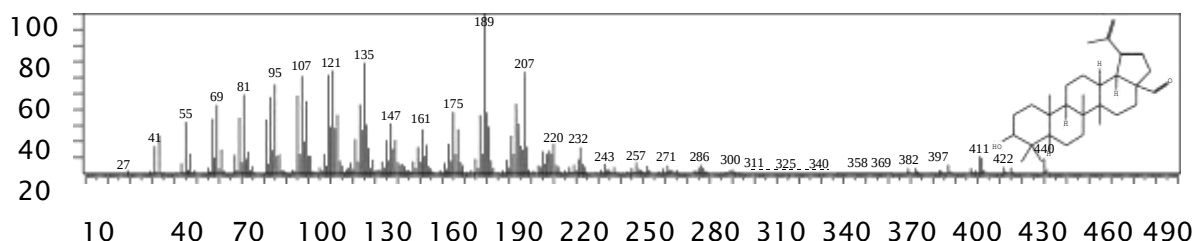
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(14)



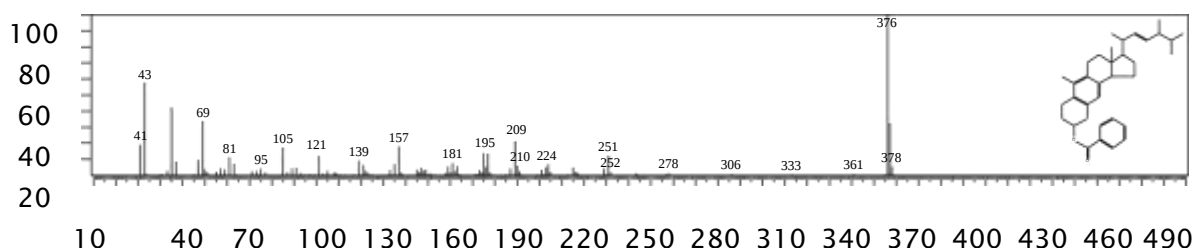
10 40 70 100 130 160 190 220 250 280 310 340 370 400 430 460 490
(15)



(16)



(17)



(18)

It is imperative to explore alternative treatments such as naturally occurring physiologically active phytochemicals. Phytoconstituents with proven efficacy can exert diverse effects on various diseases and have the potential to be utilized as natural supplements in pharmacology to manage a range of infectious diseases in the future. Many synthetic medications have severe adverse effects that are only considered acceptable as a last resort treatment for terminal conditions like cancer [26].

Given the detrimental impacts of synthetic antibiotics, the process of isolating, purifying, and characterizing new varieties of plant secondary metabolites could serve as a safer substitute for synthetic drugs. Furthermore, the process of extracting, isolating, identifying, and determining the bioactive compounds present in plant materials, which are composed of several components, continues to pose challenges. The majority of these compounds need to undergo purification using a mix of several chromatographic techniques and other purification methods in order to separate bioactive molecules [27].

Chromatographic techniques have been effectively employed to separate and purify physiologically active chemicals from a wide range of samples. Column chromatography is a highly popular and extensively utilized method for separating and characterizing both organic and inorganic substances. It has the potential to be very beneficial in the chemical analysis of complicated extracted materials. This study demonstrated the effective use of column-chromatographic techniques to separate and extract physiologically active secondary metabolites from plant extracts.

CONCLUSION

Since ancient era, *Curcuma longa* has been utilized in the food as colouring agent and having various biological properties i.e., antioxidant, analgesic, anti-inflammatory & antipyretic etc. *Solanum nigrum* has attracted great interest as a medicinal and edible herb because of its rich nutrition and wide range of pharmacological activities. It concluded that *Curcuma longa* and *S. nigrum* have numerous active constituents including alkaloids, glycosides, steroids, proteins, saponins, and polysaccharides.

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CONFLICT OF INTEREST

'None' conflict of interest was declared by the authors.

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