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Original Research Article

GC- MS Analysis of n-Hexane Extract of *Nigella sativa* (Seeds)

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Abstract: Plants and plant-based compounds are the basis of many of the modern pharmaceuticals used today for the treatment of various dreadful diseases. Medicinal plants have been a major source of therapeutic agents since ancient times, to cure human disease. *Nigella sativa* L, an annual herbaceous plant commonly known as Kalonji or Black cumin has been used from centuries for treatment of various ailments. Its seeds have been extensively studied in the last 4-5 decades and these studies have reported it to possess a number of medicinal properties. The present study dealt with extraction of *Nigella sativa* seeds with n-Hexane and GC-MS analysis of the prepared extract. This phytochemical study will be useful in determination of the pharmacological activity of the extract. Major constituents of the extract was found to be trilinolein, thymoquinone and acetoiso vanillone.

Keywords: Gas chromatography-Mass spectrometry – *Nigella sativa* –Extract- Seed.

INTRODUCTION

For centuries, medicinal plants are the basis for the treatment of various diseases. Nearly 80% of people living in developing countries still depend on plant based traditional medicine for their primary health care and almost three fourths of the herbal drugs used worldwide are derived from medicinal plants. However, the quality control of herbal medicine remains challenge owing to the fact that there is a high variability in the active constituents involved. Hence, World Health Organization (WHO) has approved fingerprint technique or standardized extract for quality assurance of herbal medicines. In recent years, phytochemicals are increasingly purported to exert potent beneficial actions to support health and may play a role in reducing synthetic drug use for the treatment of metabolic complications. To this effect, research has focused on the identification and isolation of compounds from natural products. Many plants synthesize compounds that are useful for the maintenance of health in humans and animals, like aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many of these compounds extracted from natural products could be useful lead compound in the production of drugs [1].

Amongst the promising medicinal plant, Kalonji (*Nigella sativa)* a dicotyledonous of ranunculacea is an amazing herb with a rich historical and religious background. The seeds of *N. sativa* are the source of the active ingredient of this plant. The actual importance of *N. sativa* to the Muslims came from the holy saying of the Prophet Mohammed "Prayers and peace be upon him" in the black seed is the medicine for every disease except death. It is the same black seed referred by Prophet Mohammed as a panacea (universal healer), that is a remedy for all ailments but cannot prevent ageing or death [2]. It contains over 100 valuable nutrients including 21 % protein, 38 % carbohydrates and 35 % plant fats and oils [3].The seeds of *N.sativa* L. are the most extensively studied, both phytochemically and pharmacologically. The aqueous and oil extracts of the seeds have been shown to possess antioxidant, anti-inflammatory, anticancer, analgesic and antimicrobial activities. Thymoquinone, the most important constituent of the extracted black seed essential oil, has been shown to be the active principle responsible for many of the seed's beneficial effects and so many pharmacological activities including anticancer activities. Phytochemically; it contains fixed oil, protein, alkaloids, saponin and essential oil. *N. sativa* has been reported to possess potent antioxidant, hepatoprotective, antiparasitic, anticancer, antidiabetic, antimicrobial, analgesic, anti-inflammatory, anti-nociceptive, anti-ulcer, and anti-histaminic etc. [4]. The last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological for secondary metabolite profiling in both plant and non-plant species [5]. So, the present study was aimed to investigate the possible chemical components by preparing the n-hexane extract of *N. sativa* and

identification of the compounds by subjecting it to GC-MS analysis.

MATERIALS & METHODS

Plant material

The seeds of *Nigella sativa* were collected from Kottayam district Kerala, during February 2017 and authenticated by Mr.Jomi Augustin, Head of Department of Botany, St. Thomas College, Palai, Kerala, India.

Preparation of Extract

100 g of seeds were finely powdered. It is subjected to decoction with distilled water. The strong decoction was collected and concentrated. Then that was exhaustively extracted with n-hexane and evaporated to remove the n-Hexane fraction[6, 7]. The oily extract was collected and sent an aliquot quantity for GC-MS analysis.

Preliminary phytochemical screening

The n-hexane extract was tested for alkaloids, quinones, flavonoids, phenols, steroids, tannins, terpenoids, saponins, amino acids, fixed oil, volatile oils, carbohydrates, proteins [8].

Working procedure

One microliter sample was subjected for the study. The Instrument used, Varian CP-3800 Saturn 2200 GC/MS/MS with factor four VF-5MFcolumn.Oven temperature maintained at 100^0C for 1.5 minutes and temperature gradually increased to270^0c at 5^0c per minute and 1µlitre sample was injected for analysis. Helium gas 99.9 % was used as the carrier gas, the flow rate of carrier gas was1ml per minute sample injected temperature was maintained at 250^0c and split ratio is 20 throughout the experiment period. The ionization mass was done with 70 eV. The mass spectra were recorded for the mass range 40-600 m/z for 60 minute. Identification of compound was based on comparison of their mass spectra. As the compound separated, on elution through column were detected in electronic signals. The m/z obtained was calibrated through graph obtained which was called as the mass spectrum graph, which is the finger print of the molecule. The identification of the compound was based on the comparisons of their mass spectra with Nist library.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the n-hexane extract showed the presence of amino acids, proteins, carbohydrates, fixed oil, steroids, flavonoids, alkaloids, phenols, quinones.

Table 1: GC-MS data

Sl.no	Name of the compound	Retention time(min)	Percentage (%)	Molecular formula	Molecular weight
1	m-cymene	9.568	3.152	$C_{10}H_{14}$	134.2182
2	Butyl benzene	10.608	0.434	$C_{10}H_{14}$	134.2182
3	Amyl benzene	13.046	0.346	$C_{11}H_{16}$	148.2447
4	Thymoquinone	14.573	6.248	$C_{10}H_{12}O_2$	164.2011
5	Carvacrol	15.187	2.118	$C_{10}H_{14}O$	150.2176
6	O-eugenol	15.836	2.271	$C_{10}H_{12}O_2$	164.2011
7	Delta-cadinol	16.128	0.0466	$C_{15}H_{26}O$	222.3663
8	Beta caryophyllene,	16.622	0.135	$C_{15}H_{24}$	204.3511
9	Alpha-ylangene	16.967	0.047	$C_{15}H_{24}$	204.3511
10	Acetoiso vanillone	17.766	4.524	$C_9H_{10}O_3$	166.17
11	Laevojunenol	18.504	0.103	$C_{15}H_{26}O$	222.366
12	Myristic acid	19.352	0.134	$C_{14}H_{28}O_2$	228.3709
13	Monoelaidin	21.66	2.293	$C_{21}H_{40}O_4$	356.54
14	Trilinolein	22.11	20.774	$C_{57}H_{98}O_6$	879.3844
15	1-monolinolein	23.49	2.497	$C_{21}H_{38}O_4$	354.524
16	Methyl 12-methyl tridecanoate	19.068	0.134	$C_{15}H_{30}$	242.398
17	10s,11s-Himachala-3(12),4-diene	18.906	0.088	$C_{15}H_{24}$	204.3511
18	Hexadecamethyl Cyclo octasiloxane	18.12	0.072	$C_{16}H_{48}O_8Si_8$	593.23152
19	Cycloocta siloxane hexa deca methyl	18.186	0.088	$C_{16}H4_8O_8Si_8$	593.23152
20	Hexanedioic acid 1,6 diethyl ester	16.555	0.739	$C_{15}H_{24}$	204.3511
21	1,13 tetradecadiene-3-1	15.384	0.02	C14H24O	208.34
22	3,4 dimethyl 6 ethyl phenol	15.077	0.134	$C_{10}H_{14}O$	150.222
23	Ether,p-menth-6-en-2-yl	12.221	0.243	C11H20O	168.28
	TOTAL		46.65%		

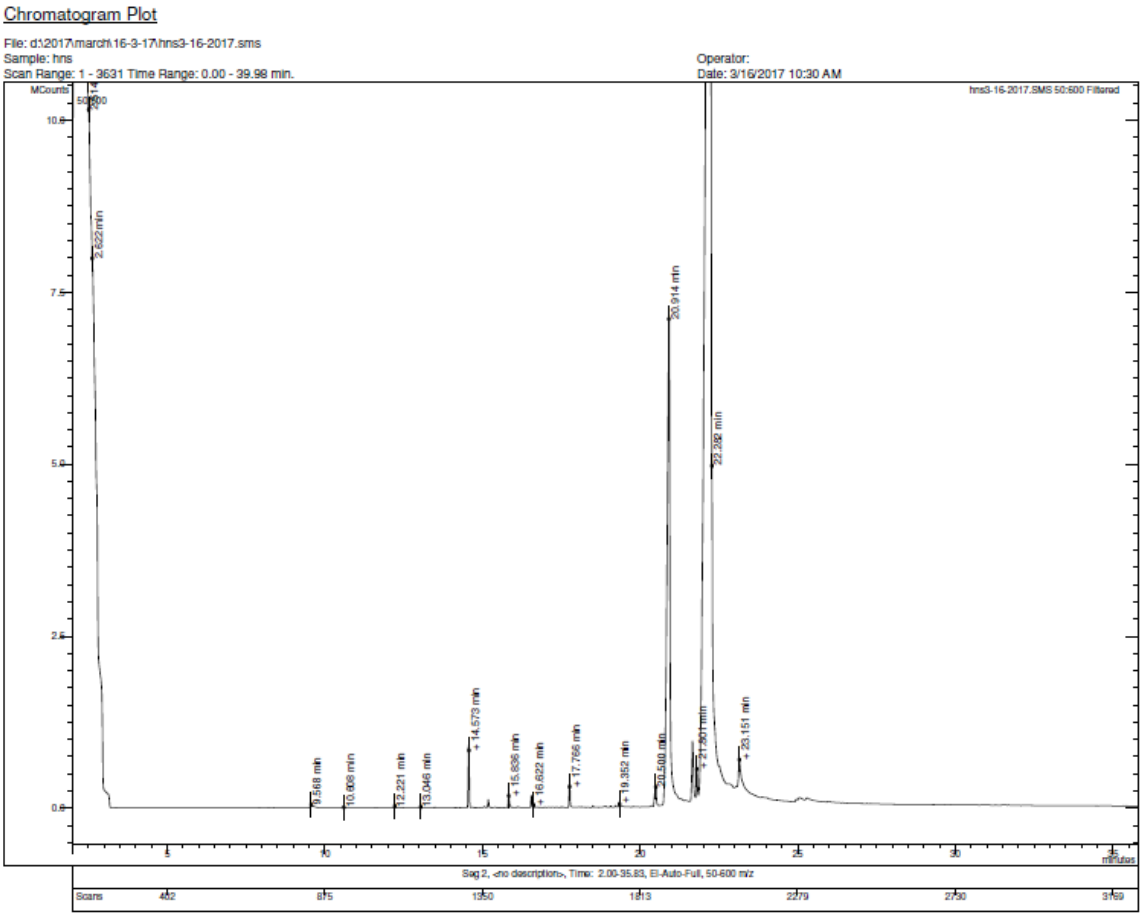

Fig-1: GC-MS chromatogram

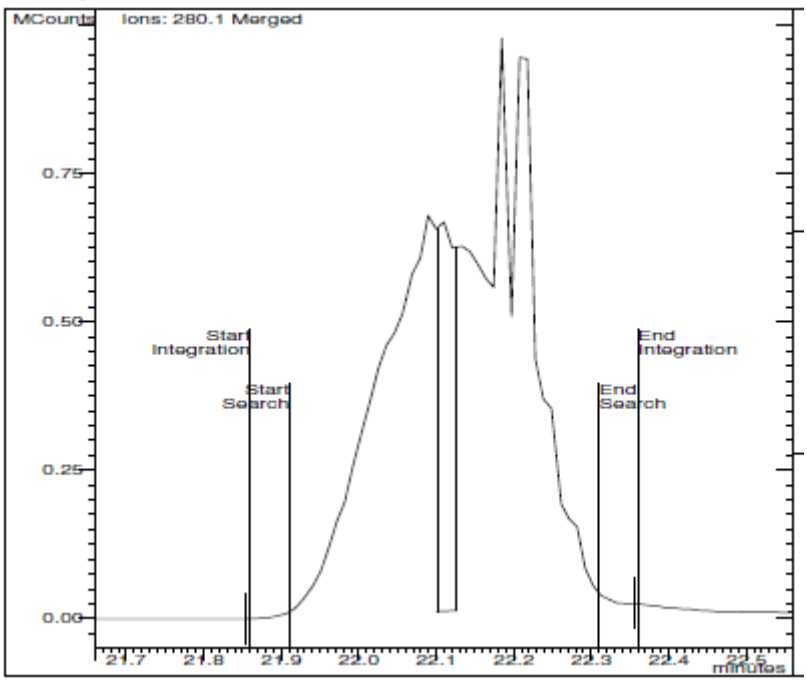

Fig-2: Chromatogram of Trilinolein

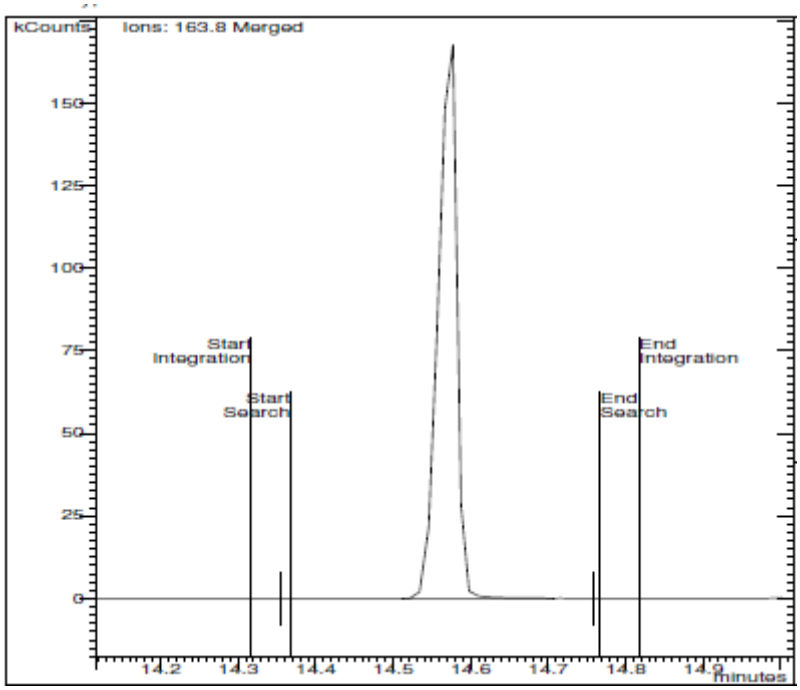

Fig-3: Chromatogram of Thymoquinone

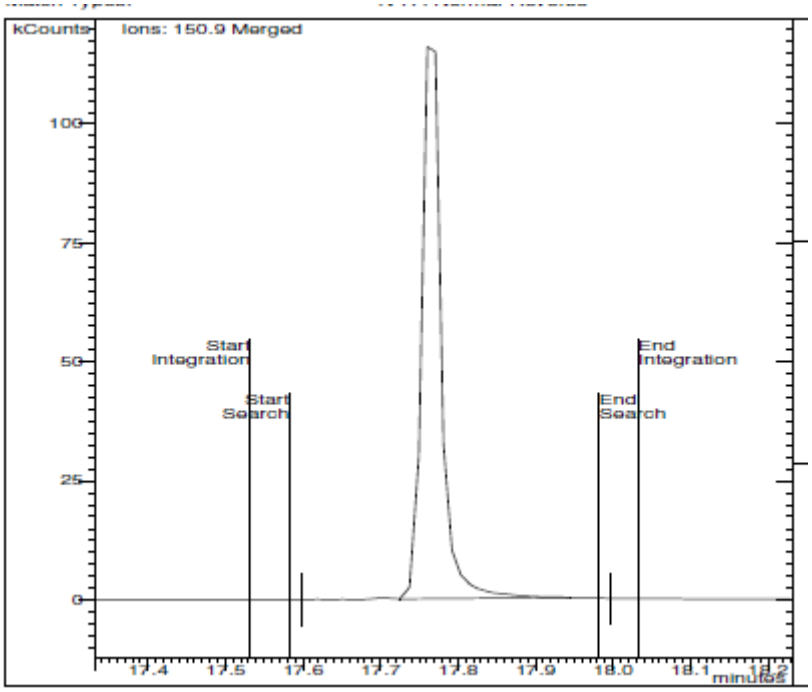

Fig-4: Chromatogram of Acetoiso vanillone

CONCLUSIONS

N. sativa seed and its components are frequently used as a natural remedy for many ailments. In view of its wide range of medicinal uses, the plant has under gone extensive phytochemical studies. The seeds are very rich and diverse in chemical composition. The GC-MS analysis of the prepared extract showed the presence of 49 compounds in which 23 were identified. The percentage of total compounds was found to be 46.646%. The major constituents of this extract are Trilinolein (20.774%), Thymoquinone (6.248%), and Acetoisovanillone (4.524%).

Uses of trilinolein include anti-ischemic, antiarrhythmic, antioxidant and anti-hypertrophic [9].Uses of thymoquinone includes anticancer, pro-apoptotic, anti-inflammatory, chemo preventive [10], anti-arthritic [11] in the treatment of Alzheimer's disease [12] etc. Aceto isovanillone is an aromatic plant ketone [13] .So the extract can be used accordingly.

More work is needed to determine the pharmacokinetics, biochemical, pharmacodynamic and therapeutics of active components and their interactions with modern drugs and importance to human health with sufficient detail. By suitable methods, these compounds can be isolated and studied for various medicinal and biological activities. The results will be promising to the medicinal and pharmaceutical field.

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