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COMPARISON OF *IN-VITRO* ANTIOXIDANT, ANTI-INFLAMMATORY ACTIVITIES AND GC-MS PROFILES OF *NIGELLA SATIVA* AND *TRIGONELLA FOENUM-GRAECUM* SEEDS

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Keywords:

Antioxidant, Anti-inflammatory, Radical scavenging, Minimum inhibitory concentration, Bioactive compounds

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ABSTRACT: Inflammation, activated by oxidative stress, is the leading cause of all chronic diseases. Our culinary practices should include antioxidant and anti-inflammatory foods to combat these problems. Spices in India have been used traditionally not only as flavour enhancers in food but also as natural therapeutic agents in the prevention and treatment of a wide variety of ailments owing to the presence of an array of bioactive principles. The aim of the present study was to compare the *in-vitro* antioxidant, anti-inflammatory, and GC-MS profiles of *Nigella sativa* and *Trigonella foenum-graecum* seeds. *In-vitro* antioxidant and anti-inflammatory assays like DPPH (2, 2 – diphenyl - 1- picrylhydrazyl), Phosphomolybdenum, Ferric reducing antioxidant power, Superoxide radical scavenging assay, and Human Red Blood Cell stabilization assay was conducted to analyze the antioxidant and anti-inflammatory activities. *Nigella sativa* seeds were observed to have more antioxidant properties than *Trigonella foenum-graecum* seeds in all the assays. Maximum antioxidant potential was observed for *Nigella sativa* seeds in superoxide radical scavenging assay with lowest IC_{50} value of 17.28 µg/mL. The anti-inflammatory property was found to be higher in *Trigonella foenum-graecum* seeds when compared to *Nigella sativa* seeds. IC_{50} value of *Trigonella foenum-graecum* seeds was found to be 54.59 µg/mL when compared to *Nigella sativa* seeds with an IC_{50} value of 98.85 µg/mL. Since, both antioxidant and anti-inflammatory potentials are important in improving chronic diseases, both *Nigella sativa* and *Trigonella foenum-graecum* seeds should be used in sufficient amounts in our daily diet to combat diseases.

INTRODUCTION: Over the decades, scientists have found that different parts of the plant contain unique therapeutic potential in curing many diseases. The growing interest in natural products is due to the side effects of the long-term use of commercially available antibiotics.

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Several novel compounds from many plant sources have been identified to contribute to antioxidant and anti-inflammatory properties. Natural sources should be identified and included in the diet to reap the health benefits [1].

Spices have been an important part of traditional medicine and Indian cuisine from ancient times. Not only have they been used in food as preservatives, taste, and flavour enhancers, but also used in treating certain diseases [2]. The aim of the study was to compare the antioxidant and anti-inflammatory properties of *Nigella sativa* (black cumin) and *Trigonella foenum-graecum*

(fenugreek) seeds of Indian origin. Bioactive compounds especially, phenols and flavonoids from natural sources, vary in quantity according to their geographical location [3]. Both black cumin and fenugreek seeds have been proven to be effective in asthmatic patients, which is a unique property possessed by very few natural sources owing to the presence of the high amount of antioxidants present in sample [4-5].

The comparative study between *Nigella sativa* and *Trigonella foenum-graecum* seeds of Indian origin is sparse. This study also deals with many antioxidant assays, which help us to obtain a greater understanding of its antioxidant properties from different perspectives.

Nigella sativa seeds, commonly known as black cumin are grown in Mediterranean countries, Middle East, Eastern Europe, and Western Asia. In Persian cuisine, these seeds are used as a spice in foods like bread, yogurt, pickles, and salads.

They have been traditionally used in treating many diseases in the Mediterranean and Middle Eastern countries like Iran, Pakistan, India, Saudi Arabia, Syria, and Turkey [6].

Trigonella foenum-graecum is distributed worldwide and is mainly found in India, China, parts of Europe, Africa, Australia, North America, and South America. Scientists have proven fenugreek seeds to be useful in treating diabetes, hypercholesterolemia, cancers, and many other non-communicable diseases [7].

MATERIALS AND METHODS:

Preparation of the Extract: Indian varieties of *Nigella sativa* (black cumin) seeds and *Trigonella Foenum-Graecum* (fenugreek) seeds were bought from local markets in Chennai, Tamil Nadu.

The plant material was identified and authenticated from the Department of Botany, RTM Nagpur University, Nagpur (authentication no.10451). Methanolic extracts of both the samples were prepared by macerating the ground seeds in methanol for 72 h.

After 72 h, the supernatant was filtered through Whatman's filter paper no. 8. The filtrate was evaporated in the open air to obtain the condensate. Stock solution (1 mg/mL) was prepared in methanol using the condensate.

The stock solution was used for antioxidant and anti-inflammatory assays.

Determination of *In-vitro* Antioxidant Activity: DPPH (2, 2 – diphenyl – 1 - picrylhydrazyl) assay, phosphomolybdenum assay, ferric reducing antioxidant power assay, and superoxide radical scavenging assay were conducted to evaluate the antioxidant properties of *Nigella sativa* and *Trigonella foenum-graecum* seeds.

DPPH Assay: DPPH (2, 2 – diphenyl – 1 - picrylhydrazyl) assay was first described by Blois (1958). DPPH is a stable free radical which becomes paired off in the presence of an antioxidant by hydrogen atom transfer. DPPH radical is reduced to DPPH-H form, which is responsible for the decolourization (yellow color) [8]. Various concentrations of the samples were taken from the stock solution of *Nigella sativa* seeds (0, 20, 40, 60, 80, 100, 120 µg/mL) and *Trigonella foenum-graecum* seeds (50, 100, 150, 200, 250 µg/mL). It was mixed with 0.1mM of DPPH solution and 1 mL of methanol. This was done in triplicates and was repeated for standard quercetin. The solution was incubated for 30 min in a dark cupboard. Absorbance was read at 517 nm. The percent radical scavenging activity was calculated using the formula: Absorbance of Sample *

Percent inhibition = Absorbance of control - Absorbance of control × 100 / Absorbance of control

Phosphomolybdenum Assay: This method was initially described by Prieto, Pinedo, and Anguilar (1999). This method is based on the reduction of Mo (VI) to green phosphate complex, Mo (V). Various sample concentrations were added with 1 mL reagent containing 4mM of ammonium molybdate, 28 mM sodium phosphate, and 600 mM concentrated sulphuric acid. The solution was incubated at 95 °C for 90 min in a water bath. The absorbance was read at 695 nm[9]. The samples were taken in triplicates and were compared with standard quercetin. The percent reduction was calculated using the formula:

Percent inhibition = Absorbance of sample - Absorbance of control × 100 / Absorbance of sample

Ferric Reducing Antioxidant Power Assay: This assay was initially described by Oyaizu (1986). Ferric Reducing Power Assay (FRAP) works on the principle of reduction of Fe^{3+} to Fe^{2+}. Various concentrations of the samples were mixed with 1 percent (w/v) potassium ferricyanide solution, 1 mL of 0.2 M phosphate buffer. The phosphate buffer was kept at pH 6.6. The solution was incubated at 50 °Cin a water bath for 30 min. After incubation, 500 µL of 10 percent of TCA (Trichloroacetic acid) solution, 100 µL 0.1 percent (w/v) Ferric Chloride solution was added. The solution was shaken, and absorbance was read at 700 nm[10]. The samples were taken in triplicates and were compared with standard quercetin. The percent reduction was calculated using the formula:

Percent reduction = Absorbance of the sample - Absorbance of control × 100 / Absorbance of sample

Superoxide Radical Scavenging Activity: Superoxide Radical Scavenging Assay was performed according to a previously described method by Fontana, Mosca, and Rosei (2001). Non- enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide produces superoxide radicals which can be compared to biological radicals in the body. This reduces Nitro Blue Tetrazolium (NBT) to purple formazan. 1 mL of methanol, 200 µL of freshly prepared riboflavin, 200 µL of EDTA, and 100 µL NBT was added in all the test tubes containing different concentrations of the sample (20, 40, 60, 80, 100, 120 µL).

The solution in the test tubes was illuminated in UV-lamp for 15 minutes. Absorbance was read at 590 nm[11]. The samples were taken in triplicates and were compared with standard quercetin. The percent radical scavenging activity was calculated using the formula:

Percent inhibition = Absorbance of control - Absorbance of sample × 100 /Absorbance of control

Determination of *In-vitro* Anti-Inflammatory Activity: Anti-inflammatory activity was assessed using Human Red Blood Cell Stabilization assay (similar to lysosomal membrane) which determines the stability of HRBC membrane by the sample extracts to predict anti-inflammatory activity [12].

Human Red Blood Cell Stabilization Assay: Anti-inflammatory activity was performed by Human Red Blood Cell stabilization assay. Human Red Blood Cell stabilization assay uses erythrocytes in blood as a model system for determining the anti-inflammatory activity. Hemolysis occurs when there is lysis of the membrane lipid bilayer [13]. In this assay, inflammation produced is heat-induced. 1 mL blood was collected from a healthy human volunteer and transferred into centrifuge tubes. Tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with an equal amount of saline. The supernatant was discarded. The volume of blood was reconstituted as 10 percent (v/v) suspension with normal saline.

1 mL of the sample with different concentrations was mixed with 1 mL of 10 percent RBC suspension. The only saline was added to the control tube. The test tubes containing the reaction mixture were incubated in a water bath at 56 °C for 30 min. At the end of incubation, tubes were cooled under running tap water and centrifuged at 2500 rpm for 5 min. The absorbance of the supernatant was taken at 560 nm. This was done in triplicates. The percentage inhibition of hemolysis was calculated as follows:

Hemolysis (%) = Absorbance of control - Absorbance of sample × 100 / Absorbance of control

Gas Chromatography-Mass Spectrometry: Gas Chromatography-Mass Spectrometry was conducted to determine the presence of bioactive compounds responsible for the antioxidant and anti-inflammatory properties. An HP-5 column (Agilent technologies 6890 N JEOL GC Mate II GC-MS model with 30 m × 0.25 mm i.d with 0.25 µm film thickness) was injected with methanolic extract of *Nigella sativa* seeds. The chromatographic conditions used included a carrier gas (helium) with a flow rate of 1 mL/min an injector which operated at 200 °C and column oven temperature, temperature maintained as 50-250 °C with a rate of 10 °C / min injection mode. Ionization voltage was kept at 70 eV and ion source temperature was maintained at 250 °C. Interface temperature wasat 250 °C with mass range of 50-600 mass units [14]. The same procedure was repeated for *Trigonella foenum-graecum* seeds.

Identification of Bioactive Compounds: National Institute Standard and Technology (NIST) database was used for the interpretation of the GC-MS

spectrum. It was compared with the spectrum of known compounds in the NIST library.

RESULTS AND DISCUSSION:

Determination of Antioxidant Activity: DPPH (α, α-diphenyl-β-picrylhydrazyl) assay is one of the simplest and preliminary steps in the determination of antioxidant activity. DPPH is a highly unstable molecule that requires electron transfer or hydrogen atom transfer for it to become stable. Its ability to react with even the weak antioxidants makes it efficient in determining the free radical scavenging activity. Even though DPPH method results are comparable with other antioxidant assays, more antioxidant assays need to be performed to confirm the radical scavenging activity. Both *Nigella sativa* and *Trigonella foenum-graecum* seeds samples showed antioxidant activity, but the IC_{50} was higher for the latter. The percent radical scavenging activity or inhibition of *Nigella sativa*, *Trigonella foenum-graecum* seeds, and standard quercetin in DPPH scavenging assay is represented in **Tables 1A** and **1B**.

TABLE 1A: DPPH SCAVENGING ASSAY OF NIGELLA SATIVA AND *TRIGONELLA FOENUM-GRAECUM* SEEDS

S. no.	Concentration (µg/mL) of *Nigella sativa* Seeds	Concentration (µg/mL) of *Trigonella foenum-graecum* Seeds	Percent Inhibition (*Nigella sativa* Seeds)	Percent Inhibition (*Trigonella foenum-graecum* Seeds)
1	Control	Control	0	0
2	20	50	11.16 ± 0.11%	4.02 ± 0.18%
3	40	100	19.53 ± 0.07%	20.11 ± 0.44%
4	60	150	23.78 ± 0.17%	39.65 ± 0.56%
5	80	200	32.88 ± 0.30%	48.46 ± 0.14%
6	100	250	44.53 ± 0.32%	52.68 ± 0.54%
7	120	300	54.61 ± 0.09%	54.59 ± 0.27%

TABLE 1B: DPPH SCAVENGING ASSAY OF STANDARD QUERCETIN

S. no.	Concentration (µg/mL)	Percent inhibition
1	Control	0
2	2	44.02 ± 0.23%
3	4	55.28 ± 0.21%
4	6	80.06 ± 0.19%
5	8	83.36 ± 0.59%
6	10	87.34 ± 0.31%
7	12	90.12 ± 0.25%

IC_{50} is the minimum inhibitory concentration required to scavenge 50 percent of the free radicals. The IC_{50} of methanolic extract of *Nigella sativa* seeds was found to be 109.86 µg/mL, which is much lesser when compared to *Trigonella foenum-graecum* seeds with IC_{50} of 237.8 µg/mL. Lower the IC_{50}, greater the radical scavenging activity. Maximum radical scavenging activity was observed to be 54.61 ± 0.09% at 120 µg/mL and 54.59 ± 0.27% at 300 µg/mL for *Nigella sativa* seeds and *Trigonella foenum-graecum'* seeds, respectively. IC_{50} of standard quercetin was found to be 0.62 µg/mL. The maximum antioxidant capacity of standard quercetin was found to be 90.12 ± 0.25% at 12 µg/mL.

Determination of Total Antioxidant Capacity by Phosphomolybdenum Assay: Phosphomolybdenum assay is a positive test to detect the presence of ascorbic acid, some phenolics, alpha-tocopherol, and carotenoids [14].

In certain studies, it has been observed that the fruit extracts which showed the highest DPPH assay value had the lowest phosphomolybdenum value [15]. A similar observation was noted in the case of *Trigonella foenum-graecum* seeds. The percent reduction of *Nigella sativa*, *Trigonella foenum-graecum* seeds, and standard quercetin in phosphomolybdenum assay is represented in **Table 2A** and **2B**.

TABLE 2A: PHOSPHOMOLYBDENUM ASSAY OF *NIGELLA SATIVA* AND *TRIGONELLA FOENUM-GRAECUM* SEEDS

S. no.	Concentration (µg/mL) of *Nigella sativa* Seeds	Percent Reduction (*Nigella sativa* Seeds)	Percent Reduction (*Trigonella foenum-graecum* Seeds)
1	Control	0	0
2	20	43.13 ± 1.16%	31.76 ± 2.67%
3	40	84.02 ± 0.25%	78.35 ± 0.45%
4	60	89.58 ± 0.14%	88.44 ± 0.14%
5	80	91.26 ± 0.12%	93.37 ± 0.10%
6	100	93.8 ± 0.11%	93.74 ± 0.09 %
7	120	94.31 ± 0.29%	94.75 ± 0.08%

TABLE 2B: PHOSPHOMOLYBDENUM ASSAY OF STANDARD QUERCETIN

S. no.	Concentration (µg/mL)	Percent Reduction
1	Control	0
2	2	50 ± 3.78%
3	4	66.66 ±2.26%
4	6	84.16 ±1.09%
5	8	87.5 ± 1.04%
6	10	89.47 ± 0.67%
7	12	91.66 ± 0.61%

The IC_{50} value of *Nigella sativa* seeds was found to be 23.13 µg/mL and 25.52 µg/mL for *Trigonella foenum-graecum* seeds, respectively. Maximum reduction was found to be 94.31 ± 0.29% and 94.75 ± 0.08% at 120 µg/mL for *Nigella sativa* and *Trigonella foenum-graecum* seeds, respectively. IC_{50} value of standard quercetin was found to be 2 µg/mL.

Ferric Reducing Antioxidant Power Assay: Ferric Chloride works on the principle of electron transfer leading to a reduction from Fe^{3+} to Fe^{2+}in the presence of 2, 4, 6-trypyridyl-s-triazine. This assay is useful for detecting redox compounds with potentials of less than 700 nm. FRAP helps to detect compounds that help in hydrogen atom transfer particularly thiols like glutathione and proteins [16]. *Nigella sativa* is known to improve oxidative stress by modulating glutathione reductase [17]. The percent reduction of *Nigella sativa*, *Trigonella foenum-graecum* seeds, and standard quercetin in ferric reducing antioxidant power assay is represented in **Tables 3A** and **3B**. IC_{50} value was found to be 19.63 µg/mL and 59.88 µg/mL for *Nigella sativa* and *Trigonella foenum-graecum* seeds, respectively. Maximum reduction was found to be 82.57 ± 0.53% and 76.35 ± 0.11% at 120 µg/mL for *Nigella sativa* and *Trigonella foenum-graecum* seeds, respectively. IC_{50} of standard quercetin was found to be 2.13 µg/mL.

TABLE 3A: FERRIC REDUCING ANTIOXIDANT POWER ASSAY OF *NIGELLA SATIVA* AND *TRIGONELLA FOENUM-GRAECUM* SEEDS

S. no.	Concentration (µg/mL) of *Nigella sativa* Seeds	Percent Reduction (*Nigella sativa* Seeds)	Percent Reduction (*Trigonella foenum-graecum* Seeds)
1	Control	0	0
2	20	50.93 ± 0.73%	25 ± 0.82%
3	40	61.55 ± 0.33%	43.16 ± 0.06%
4	60	66.54 ± 0.34%	50.1 ± 0.48%
5	80	69.34 ± 0.18%	52.83 ± 0.69%
6	100	77.29 ± 0.18%	61.39 ± 0.40%
7	120	82.57 ± 0.53%	76.35 ± 0.11%

TABLE 3B: FERRIC REDUCING ANTIOXIDANT POWER ASSAY OF STANDARD QUERCETIN

S. no.	Concentration (µg/mL)	Percent Reduction
1	Control	0
2	2	46.77 ± 0.20%
3	4	58.22 ± 0.17%
4	6	74.61 ± 0.17%
5	8	76.42 ± 0.11%
6	10	76.76 ± 0.11%
7	12	80.23 ± 0.12%

Superoxide Radical Scavenging Assay: Superoxide radical is an important radical species in chemical and biological systems [18] PMS/NADH system (phenzine methosulfate nicotinamide adenine dinucleotide) generates superoxide radicals which reduce NBT (Nitro blue Tetrozolium) into a purple formazan [19].

The percent radical scavenging activity or inhibition of *Nigella sativa*, *Trigonella foenum-graecum* seeds, and standard quercetin in superoxide radical scavenging assay is represented in **Tables 4A** and **4B**.

TABLE 4A: SUPEROXIDE RADICAL SCAVENGING ASSAY OF *NIGELLA SATIVA* AND *TRIGONELLA FOENUM-GRAECUM* SEEDS

S. no.	Concentration (µg/mL) of *Nigella sativa* Seeds	Percent Inhibition (*Nigella sativa* Seeds)	Percent Reduction (*Trigonella foenum-graecum* Seeds)
1	Control	0	0
2	20	57.87 ± 1.12%	33.02 ± 0.99%
3	40	62.96 ± 0.50%	36.69 ± 1.00%
4	60	71.75 ± 0.05%	56.42 ± 0.36%
5	80	73.61 ± 0.66%	59.17 ± 0.10%
6	100	75.92 ± 0.40%	61.92 ± 0.60%
7	120	79.62 ± 0.36%	68.94 ± 0.08%

TABLE 4B: SUPEROXIDE RADICAL SCAVENGING ASSAY OF STANDARD QUERCETIN

S. no.	Concentration (µg/mL)	Percent Reduction
1	Control	0
2	2	11 ± 1.50%
3	4	43.42 ± 0.06%
4	6	49.84 ± 1.02%
5	8	84.09 ± 0.60%
6	10	86.23 ± 0.57%
7	12	88.99 ± 0.29%

IC_{50} value was found to be 17.28 µg/mL and 53.17 µg/mL for *Nigella sativa* and *Trigonella foenum-graecum* seeds, respectively. Maximum reduction was found to be 79.62 ± 0.36% and 68.94 ± 0.08% at 120 µg/mL for *Nigella sativa* and *Trigonella foenum-graecum* seeds, respectively. IC_{50} value of standard quercetin was found to be 6.01 µg/mL.

Minimum Inhibitory Concentration: Fig. 1 shows the minimum inhibitory concentration of antioxidant assays for *Nigella sativa* seeds, *Trigonella foenum-graecum* seeds and standard quercetin.

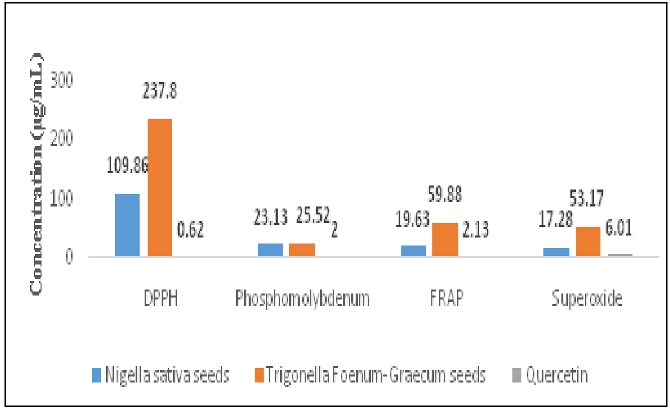

FIG. 1: MINIMUM INHIBITORY CONCENTRATION OF *NIGELLA SATIVA* SEEDS, *TRIGONELLA FOENUM-GRAECUM* SEEDS AND STANDARD QUERCETIN

Nigella sativa seeds were found to have higher antioxidant properties than *Trigonella foenum-graecum* seeds in all the assays, namely DPPH assay, phosphomolybdenum assay, ferric reducing antioxidant power assay, and superoxide radical scavenging assay.

Human Red Blood Cell Stabilization Assay: The anti-inflammatory property was found to be higher for *Trigonella foenum-graecum* seeds when compared to *Nigella sativa* seeds. It is represented in **Fig. 2**. The effect was found to be concentration-dependent. There was an increase in the percentage inhibition of hemolysis with concentration.

IC_{50} was found to be 98.97 µg/mL and 45.79 µg/mL for *Nigella sativa* and *Trigonella foenum-gaecum* seeds, respectively. Maximum reduction was found to be 53.22 ± 2.21% and 62.73 ± 0.08% at 120 µg/mL for *Nigella sativa* and *Trigonella foenum-graecum* seeds, respectively.

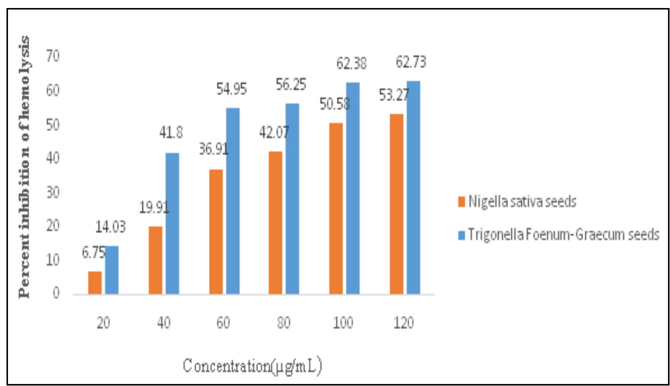

FIG. 2: HUMAN RED BLOOD CELL STABILIZATION ASSAY OF *NIGELLA SATIVA* AND *TRIGONELLA FOENUM-GRAECUM* SEEDS

GC-MS Analysis of Methanolic Extract of *Nigella Sativa* Seeds: GC-MS analysis of *Nigella sativa* seeds revealed the presence of many compounds that have antioxidant and anti-inflammatory properties. **Fig. 3** represents the GC spectrum of *Nigella sativa* seeds, and **Table 5** represents bioactive compounds identified in the methanolic extract of *Nigella sativa* seeds. The compounds were identified to be 2, 5- Bis (4-hydroxyphenyl) - 1, 3, 4-oxadiazole; Perlolyrine (2-Dehydrocarbonylflazin); 4 - Hydroxy – 3 - methoxybenzyl alcohol; Tetradecanoic acid, 12-methyl - methyl ester; Trans – 3 - (o-hydroxyphenyl) 1-phenyl) - 2 propene – 1 - one; 2 methyl - 6 nitro - 4 quinolinol; 5 – Ethyl – 2 - [4-(4-ethylcyclohexyl)phenyl]pyrimidine and Pregn-5 – en – 20 - one, 3 - hydroxy - Antioxidant and anti-inflammatory properties have been reported by these compounds in many studies [20-23].

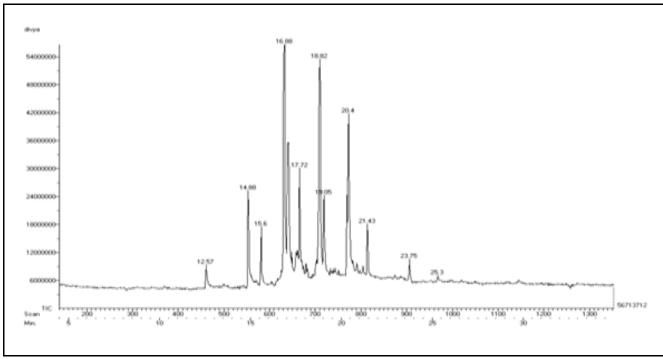

FIG. 3: GC SPECTRUM OF *NIGELLA SATIVA* SEEDS

TABLE 5: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *NIGELLA SATIVA* SEEDS

S. no.	Retention Time	Compound Name	Molecular Weight (g/mol)	Molecular Formula	Structure
1	16.88	Flavone	222.24	$C_{15}H_{10}O_2$	
2	18.82	2,5-Bis(4-hydroxyphenyl)-1,3,4-oxadiazole	254.24	$C_{14}H_{10}N_2O_3$	
3	20.4	2-Dehydrocarbonylflazin	264.28	$C_{16}H_{12}N_2O_2$	
4	17.72	Trans-3-(o-hydroxyphenyl)1-phenyl)-2 propene-1-one	224.25	$C_{15}H_{12}O_2$	
5	14.88	4-Hydroxy-3-methoxybenzyl alcohol	154.16	$C_8H_{10}O_3$	
6	19.05	Tetradecanoic acid, 12-methyl-, methyl ester	256.42	$C_{16}H_{32}O_2$	
7	15.6	2 methyl – 6 nitro-4 quinolinol	204.18	$C_{10}H_8N_2O_3$	
8	21.43	3 eicosene	280.5	$C_{20}H_{40}$	
9	12.57	Hexanoic acid	116.16	$C_6H_{12}O_2$	
10	23.75	5-Ethyl-2-[4-(4-ethylcyclohexyl)phenyl]pyrimidine	294.4	$C_{20}H_{26}N_2$	
11	25.3	Pregn-5-en-20-one, 3-hydroxy-	316.5	$C_{21}H_{32}O_2$	

GC-MS Analysis of Methanolic Extract of *Trigonella foenum-graecum* Seeds: GC-MS analysis of *Trigonella Foenum-Graecum* seed revealed the presence of many compounds that have antioxidant and anti-inflammatory properties. **Fig. 4** rg*Graecum* seeds and **Table 6** represents bioactive compounds identified in the methanolic extract of *Trigonella foenum-graecum* seeds. GC-MS analysis showed number of phenolic compounds like 3-(2-methyl 6-methoxyphenyl-3H quinazolinone, 2 – amino – 6 – benzotriazol - 2 - yl-4-methyl-phenol and Benzamide, 2-amino-N-[2-(1-methylethyl)phenyl]- and Phenol, 2,4-bis-(1,1-dimethylethyl), flavone, Phenol, 2,4-bis-(1,1-dimethylethyl) and Phenol 2 propyl. A compound known as phytol was also found to be present in the sample which helps synthesize Vitamin E and K in the body [24]. Alpha - terpeinol also possesses antioxidant and anti-inflammatory activities [25].

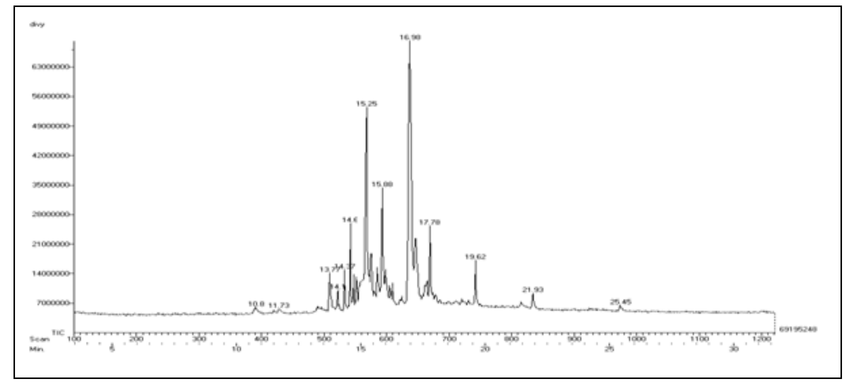

FIG. 4: GC SPECTRUM OF *TRIGONELLA FOENUM-GRAECUM* SEEDS

TABLE 6: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *TRIGONELLA FOENUM-GRAECUM* SEEDS

S. no.	Retention Time	Compound Name	Molecular Weight (g/mol)	Molecular Formula	Structure
1	16.98	3-(2-methyl 6-methoxyphenyl-3H quinazolinone	266.29	$C_{16}H_{14}N_2O_2$	
2	15.25	2-amino-6-benzotriazol-2-yl-4-methyl-phenol	240.26	$C_{13}H_{12}N_4O$	
3	15.88	Benzamide, 2-amino-N-[2-(1-methylethyl)phenyl]-	254.33	$C_{16}H_{18}N_2O$	
4	17.78	1-eicosene	280.5	$C_{20}H_{40}$	
5	14.6	flavone	222.24	$C_{15}H_{10}O_2$	
6	19.62	9-Octadecynoic acid	280.4	$C_{18}H_{32}O_2$	
7	14.37	Phenol, 2,4-bis-(1,1-dimethylethyl)	310.5	$C_{22}H_{30}O$	
8	13.77	Nonanoic acid, 1-methylethyl ester	200.32	$C_{12}H_{24}O_2$	
9	14.1	Thujopsene	204.35	$C_{15}H_{24}$	
10	21.93	Phytol	296.5	$C_{20}H_{40}O$	
11	10.8	Phenol 2 propyl	136.19 g/mol	$C_9H_{12}O$	
12	11.73	Alpha- terpineol	154	$C_{10}H_{18}O$	
13	25.45	Elaidic acid, isopropyl ester	324.54	$C_{21}H_{40}O_2$	

CONCLUSION: Both *Nigella sativa* and *Trigonella foenum-graecum* seeds were found to have antioxidant and anti-inflammatory properties. *Nigella sativa* seeds were observed to have a higher antioxidant property in all the assays, whereas *Trigonella foenum-graecum* seeds were found to have higher anti-inflammatory properties when compared to *Nigella sativa* seeds. Phenolic compounds possesses high antioxidant and anti-inflammatory properties. GCMS profiles of *Nigella sativa* and *Trigonella foenum-graecum* seeds revealed the presence of many phenolic compounds, which makes it important to be included in our daily diet.

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CONFLICTS OF INTEREST: Nil

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