



STUDY OF ANTIMICROBIAL POTENTIAL, CHEMICAL COMPOSITION AND FREE RADICAL SCAVENGING PROPERTY OF *NIGELLA SATIVA* SEED COLD PRESSED OIL AND N-HEXANE EXTRACT FROM DIFFERENT GEOGRAPHIES

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ABSTRACT

Plants are blessings to the human being. *Nigella sativa* seed is known as seeds of blessings in Islamic literature by Prophet Mohammad. Although there are studies available on specific medicinal property of *Nigella sativa* seed of particular region, there is no comparative study available on the antimicrobial, Chemical composition and Free radical scavenging property of *Nigella sativa* seed from different geographies. In this study, we collected the *Nigella sativa* seeds from various geographies like India, Pakistan, Tunisia, Egypt, Saudi Arabia, Turkey, and Oman. All seeds were crushed separately and pressed in pressing mill to expel seed oil. N-hexane extract was prepared by Soxhlet extraction for 4 hours. N-hexane Extracts obtained were concentrated and dried. All seven bacterial cultures were procured from ATCC (American Type Culture Collection). ATCC cultures used were *Pseudomonas aeruginosa* (ATCC9027), *Salmonella Typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC6538), *Bacillus Cereus* (ATCC 10876), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404). Disc diffusion method was used to test the Antimicrobial property of all seven microorganisms using Müller-Hinton agar. *Nigella sativa* seed oil and n-hexane showed strong antimicrobial property against Gram Positive bacteria like *Staphylococcus aureus* (ATCC6538), *Bacillus Cereus* (ATCC 10876) and Low antimicrobial property against Gram Negative bacteria like *Pseudomonas aeruginosa* (ATCC9027), *Salmonella Typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 8739) and Yeast and Mold *Candida albicans* (ATCC 10231) while No antimicrobial activity was shown *Aspergillus brasiliensis* (ATCC 16404). It is observed that *Nigella sativa* seed oil and n-Hexane seed extract from India, Tunisia, and Pakistan were having strong antimicrobial activity against studied microbes. Further GCMS study of chemical composition indicate that *Nigella sativa* variety from Indian subcontinent and Tunisia showed rich active contents like Thymoquinone and phenolic compounds. The DPPH assay of Cold pressed seed oil of *Nigella sativa* complements the chemical composition. Free radical scavenging property of Indian subcontinent and Tunisia varieties are higher than their Middle Eastern counter parts.

KEY WORDS

Nigella sativa, Antimicrobial, Antioxidant, Plant medicines

Introduction

In the recent past, there are the lot of studies published which talks about antimicrobial properties of *Nigella sativa*. *Nigella sativa* seed was studied for its antimicrobial activity against multi-drug resistant *Staphylococcus aureus*, which was isolated from diabetic wounds of 34 patients. *Nigella sativa* oil

exhibited antibacterial activity against the *Staphylococcus aureus* isolates [1]. In a study where Shaaban HA et al., studied the antibacterial activity of Essential oil (EO) of *Nigella sativa* extracted from crude oil in water-based microemulsion showed strong antibacterial activity against six pathogenic bacteria when studied using agar well diffusion method. Results

indicated that *Nigella Sativa* Essential Oil micro emulsion was found to be most effective against *S. aureus*, *B. cereus* and *S. Typhimurium* at tested concentration Essential oil of 100 µg/well. The activity of Essential oil micro emulsion was observed to be higher than Ceftriaxone solution against *S. typhimurium* at 400 µg/well and well comparable activity against *E. coli* at 500 µg/well. *Nigella sativa* essential oil microemulsion did not show any activity against *L. monocytogenes* and *P. aeruginosa* [2]. Randhawa MA et al. studied the antimicrobial activity of nanoparticulated amphotericin-B, ketoconazole, and thymoquinone against *Candida albicans* yeasts and *Candida* biofilm. Nanoparticles were prepared using the ball milling technique. It observed to be 5 to 20 nm in size with quasi-spherical morphology. Nano-sized active were found 3 to 4 times more effective against *Candida* yeasts and *Candida* biofilm [3]. Mahmoudvand H et al. evaluated the antifungal property of essential oil and various extracts of *Nigella sativa* and its most active ingredient Thymoquinone against pathogenic dermatophyte strains *Trichophyton menta agrophytes*, *Microsporum Canis* and *Microsporum gypseum* using disk diffusion method and estimation of minimum inhibitory concentration (MIC) of extracts using broth microdilution method. The study indicated that the essential oil and various extracts of *Nigella sativa* and Thymoquinone have strong antifungal effects on dermatophytes like *T. mentagrophytes*, *M. canis* and *M. gypseum* [4]. Sarwar A and Latif Z studied the antibacterial activity of *Nigella sativa* oil against twenty bacterial strains, isolated and purified from different environmental sources. *Salmonella* strains resistant to Ceftriaxone and Ciprofloxacin antibiotics were studied for antibacterial activity of natural extracts of *Nigella sativa* oil. *Nigella sativa* seed oil was found to be more effective against Ceftriaxone and Ciprofloxacin-resistant *Salmonella* species. They linked the antimicrobial property of *Nigella sativa* seed oil with chemical constituents like thymol, thymoquinone, p-cymene, α-phellandrene, cis-carveol, α-pinene, β-pinene, α-longipinene, trans-anethole, and longifolene [5]. Bakathir HA and Abbas NA. studied the antibacterial effect of *Nigella sativa* seeds. They observed that *Nigella sativa* water extract have inhibition potential against *Staphylococcus aureus* at the concentration of 300 mg/ml with distilled water

(D.W.) as the control. The inhibition zone obtained from Hadramout (HNSGS) was more than with *Nigella sativa* ground seeds from Ethiopia (ENSGS). They reported the practically no inhibition was observed in the growth of *E. coli* and *Enterobacter* [6]. Chaieb K et al., studied the Antibacterial activity of Thymoquinone against Bacterial biofilms using the crystal violet (CV) and 2, 3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2Htetrazolium-5-carboxanilide (XTT) reduction assay. Thymoquinone showed a significant bactericidal activity against the majority of the tested bacteria (MICs values ranged from 8 to 32 µg/ml) especially Gram-positive cocci (*Staphylococcus aureus*-ATCC 25923 and *Staphylococcus epidermidis* - CIP 106510 [7]. Landa P studied the antimicrobial potetial of seed extracts of *Nigella arvensis*, *Nigella hispanica*, *Nigella damascena*, *Nigella orientalis*, *Nigella nigellastrum*, *Nigella orientalis*, and *Nigella sativa* obtained by successive extraction with n-hexane, chloroform, and methanol against ten strains of pathogenic bacteria and yeast using the microdilution method. Study indicated Chlorofom extract of *Nigella arvensis* was found to be most potent among all species tested. Chlorofom extract of *Nigella arvensis* inhibited Gram-positive bacterial and yeast strains with MIC values ranging from 0.25 to 1 mg/mL. Further they observed selective inhibitory action of n-hexane extract of *N. orientalis* on growth of *Bacteroides fragilis* (MIC = 0.5 mg/mL). They did not observe any antimicrobial activity for other *Nigella* species [8]. This observation is slightly deviating from many studies referred here. Morsi Nm studied the Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. These isolates comprised 16 gram negative and 6 gram positive representatives. They showed multiple resistances against antibiotics, especially the gram-negative ones. Crude extracts of *Nigella sativa* showed a promising antimicrobial effect against the tested organisms. The most effective extracts were the crude alkaloid and water extracts. Gram-negative isolates were affected more than the gram-positive ones, which is quite unusual with *Nigella sativa* seed extract [9]. In a study, it is seen that *Nigella sativa* seed cake fractions using methanol, ethyl acetate, hexane, and water have significant phenolic content. The total phenolic contents were observed to be 78.8, 27.8, 32.1 and 12.1 mg gallic acid equivalents (GAE) per gram in

Ethyl acetate fraction, Methanolic Extract, Water fraction and Hexane fraction, respectively. Methanolic extract and Ethyl acetate fraction showed highest DPPH followed by Water fraction and Hexane fraction. The extract and fractions exhibited the high effect on reducing the oxidation of β -carotenehydroxyanisole [10].

Shafeeque Ahmad, et al., studied the therapeutic effect of omega six linoleic acid and thymoquinone enriched extracts from *Nigella sativa* seed oil in the mitigation of lipidemic oxidative stress in rats. Pretreatment of hyperlipidemic rats with these test extracts resulted in a significant decrease in the level of lipid peroxidation markers, lipid hydroperoxide, conjugated diene, and malondialdehyde (16– 50%) compared to the hyperlipidemia control rats [11].

In the study, *Nigella Sativa* seed extracts were prepared using optimized Supercritical Fluid Extraction (SFE) and the traditional Soxhlet extraction. The extracts were analyzed for various known antioxidants. *N. sativa* extracts were found to prevent protein carbonyl formation as well as depletion of intracellular glutathione (GSH) in fibroblasts exposed to toluene. Further, partially purified SFE and Soxhlet fractions could prevent loss of hepatic GSH in toluene-induced oxidative stressed Wistar rats as well as in L929 fibroblasts. The antioxidant contents of extract produced with SFE extract are richer as compared to antioxidant content in the extract obtained from Soxhlet approach. The study indicated that the protective effects of *N. sativa* might not only be due to thymoquinone but perhaps other antioxidants [12].

In this study, we attempted to compare the antimicrobial and free radical scavenging properties of *Nigella sativa* seed Oil and n-Hexane extract from different geographies. Further we tried to link concentration of few chemicals with antimicrobial and Free radical scavenging property of cold pressed oil and n-Hexane extract. The micro-organism subjected in this study is quite significant from human health and hygiene point of view. The output of this study is expected to throw significant light on the best source of *Nigella sativa* for commercial use.

Material and method

a) Antimicrobial property

1. Samples

***Nigella sativa* seeds** were collected from 7 countries like India, Pakistan, Tunisia, Egypt, Saudi Arabia, Turkey, and Oman. A portion of the samples were identified and kept in university herbarium for further reference. All samples were well cleaned, washed with water and disinfected with isopropyl alcohol to remove all biological contaminants. Sterilized seeds are dried and kept in cleaned samples bottle at cool and dry places until its use.

2. Solvents used:

N-Hexane solvents were used in the experiments were of analytical grade from M/s Merck.

3. Glassware and heating mantle:

All glassware like Round Bottom Flask, Soxhlet extractor, Allihn Condenser, Interchangeable Joint and Erlenmeyer Graduated Conical Flasks were from M/s Borosil. Weighing Balance and Electrical heater used in experiments were calibrated. All extracts were stored in Borosil chromatographic vials.

4. Microbial strains:

The culture used in the experiments were arranged from American Type Culture Collection (ATCC) , Gram Negative - *Pseudomonas aeruginosa* (ATCC9027), *Salmonella Typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 8739) Gram Positive - *Staphylococcus aureus* (ATCC6538), *Bacillus Cereus* (ATCC 10876) and Yeast and Mould - *Candida albicans* (ATCC 10231), *Aspergillus brasiliensis* (ATCC 16404).

5. Media:

Media used for the experiment were arranged from M/s Hi-media, Mumbai, India. Mueller Hinton Agar (MHA) the media used for performing Zone of Inhibition.

Methods:

All samples of seeds are cleaned, disinfected and dried. All dried samples were crushed using a grinder. 20g of crushed samples of seeds was placed in Soxhlet thimble. 200ml of solvent was used, which was added in Round bottom flask. The heater is set at 55 deg C and Round bottom flask was placed in the heating mantle. All soxhlet extraction was carried out till clear solvent is observed during reflux. For n-Hexane distillation only 4 hours distillation was found to be sufficient.

For *Nigella sativa* seed oil was expelled by pressing the crushed seeds in pressing mill, Oil expelled are collected in clean glass bottles. This Oil was cleaned using muslin cloth.

All samples were kept in refrigerator till future use

Zone of inhibition using Kirby-Bauer Disk Diffusion Susceptibility Test Protocol:

Antimicrobial potential of *Nigella sativa* seed oil and n-hexane extract were studied by zone of inhibition using Kirby-Bauer Disk Diffusion Susceptibility Test Protocol on Mueller-Hinton (MH) agar plate. This is a standardized viable alternative to broth dilution methods. Mueller Hinton Agar (MHA) plates are

prepared as per manufacturer's instruction provided with media. Sample Identification marks are done on location mark on MHA plate to pour sample in define area. 0.1ml Bacterial strain with concentration 10^8 cfu/ml is poured on MHA surface and spread uniformly by sterile L spreader under Laminar Air flow. A sterile disc (size 10mm Φ) is to be placed on MHA plate. Approximately 25-60mg sample is poured on sterile discs and record the sample quantity. Incubation of the MHA plate to be done at 30-35°C for 24 hrs. After completion of incubation period, the measurement of zone of inhibition to be done as shown in Figure 9.



Figure 1 ATCC culture used in the experiment



Figure 2 MHA Media



Figure 3: MHA Plate and marking of sample



Figure 4 Bacterial strain Solution and disc

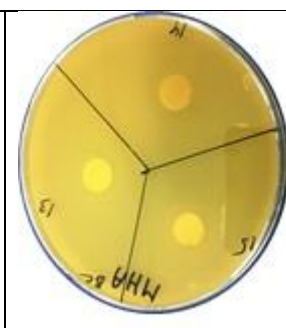


Figure 5 Placing disc on MHA



Figure 6 Sample addition



Figure 7 Incubation



Figure 8 Zone of inhibition

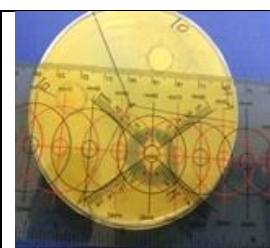


Figure 9 Measuring of Zone of Inhibition

N-hexane solvent was used as negative control while Zinc pyrithion 1.5% solution was used as positive control in the experiment.

b) Antioxidant Property/ radical scavenging assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) was arranged from Sigma-Aldrich (USA). Ethanol was arranged from VWR Chemicals. Free radical scavenging assay (Free radical scavenging activity) of cold pressed seed oil was measured in terms of radical scavenging ability using the stable free radical DPPH. Different concentrations (10µl, 20µl, 30 µl, 40µl & 50µl) of sample were taken and tested for optimum reaction. 50µl of samples was found to be optimal for reaction with 3ml of 0.08% Solution of DPPH along with 10 ml of Ethanol. The tubes were incubated at 25°C for 60 minutes. The absorbance value was recorded at 510 nm using PerkinElmer LAMBDA™ 25 UV/Vis Spectrophotometers. The same procedure was followed for control without the sample [13].

DPPH Scavenging ability (%) = $\frac{[A \text{ control} - A \text{ sample}]}{A \text{ control}} \times 100$

c) Chemical composition analysis

Data Analysis

A) Antimicrobial Potential

1. Sample Identification

Table 1 Cold pressed *Nigella sativa* seed oil sample details

Country	India	Tunisia	Pakistan	Oman	KSA	Egypt	Turkey
Sample ID	1	2	3	4	5	6	7
Reference	NS01	NS03	NS04	N005	N005	N006	N007

Table 2 N- Hexane extract of *Nigella sativa* seed sample details

Country	India	Tunisia	Pakistan	Oman	KSA	Egypt	Turkey
Sample ID	16	14	11	12	13	10	15
Reference	H1	H6	H2	H3	H7	H4	H5

- Media used Müller-Hinton agar plate
- Bacterial concentration: 10^8 cfu/ml
- Photos of MHA plates with Zone of inhibition

Instrument set up was includes Agilent 7890B GC and 5977B MSD, the silica capillary column was used in the Agilent GC-MS. It has a temperature range of 20°C to 280°C, and a bonded and cross-linked stationary phase of low polarity; the small intern diameter, the column length and the possibility of applying high gas flows, these characteristics all together permit a fast-chromatographic separation. The mass spectrometer used has a quadruple mass analyzer and an electron impact ionization source. The ionization source was operated at 70 eV at a temperature of 230°C, while the quadruple was kept at 150°C. The MS detector was use in SCAN and in SIM mode.

Suitable quantity of sample was extracted by ethyl acetate organic solvent. The sample was cleaned using Sodium sulphate, Sodium chloride, PSA and C18 sorbent (C18 cartridges). Sample was concentrated by using Turbo Evaporator and then recon with ethyl acetate solution.

Then sample was injected into Tandem Mass Spectrometry Coupled with Gas Chromatography. The Chromatogram was further studies with NIST 14 Mass Spectral Library.

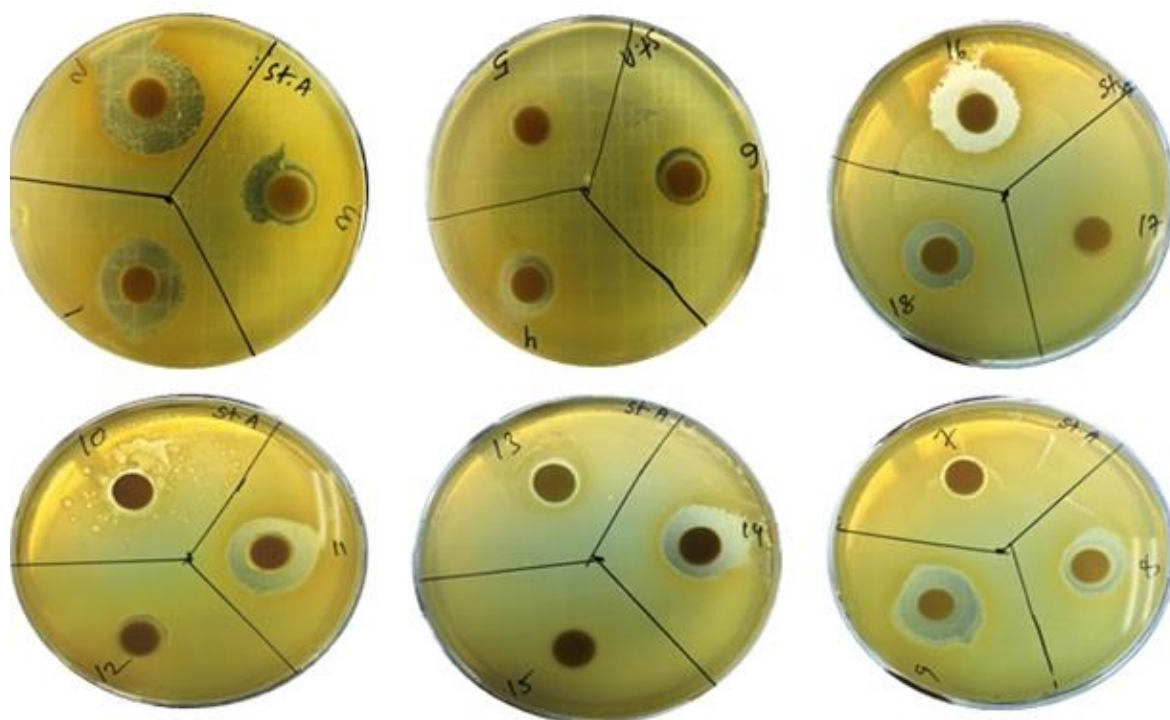


Figure 10 Zone of Inhibition of Cold Pressed *Nigella sativa* seed oil and N-Hexane extract for *Staphylococcus aureus* (ATCC6538)

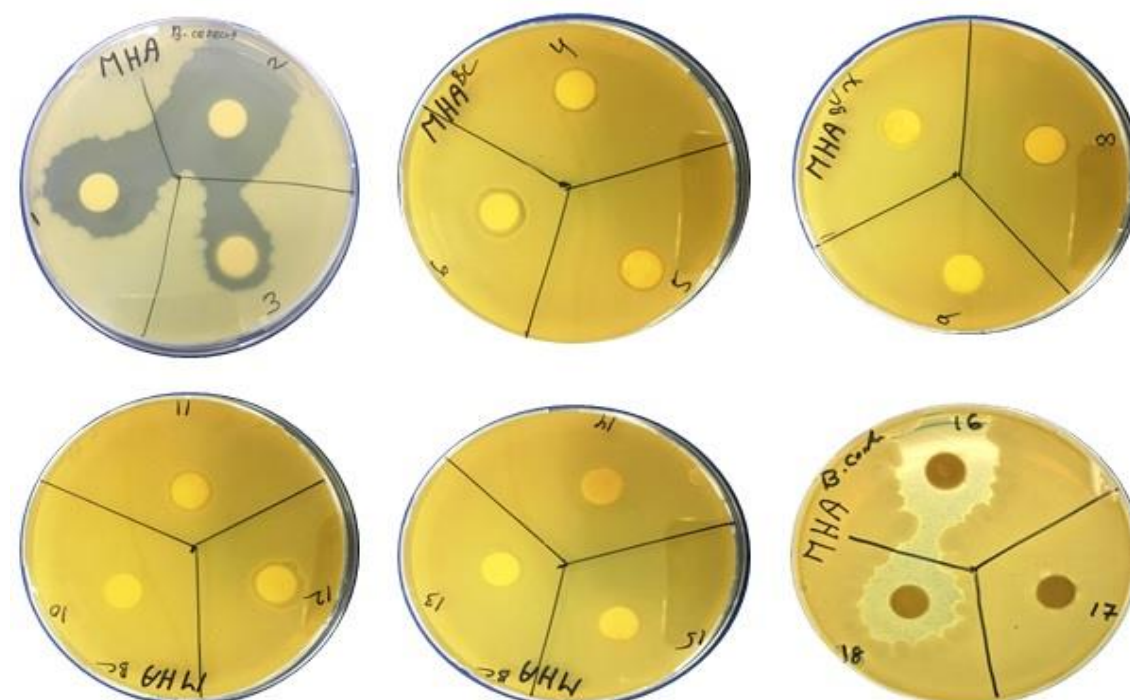
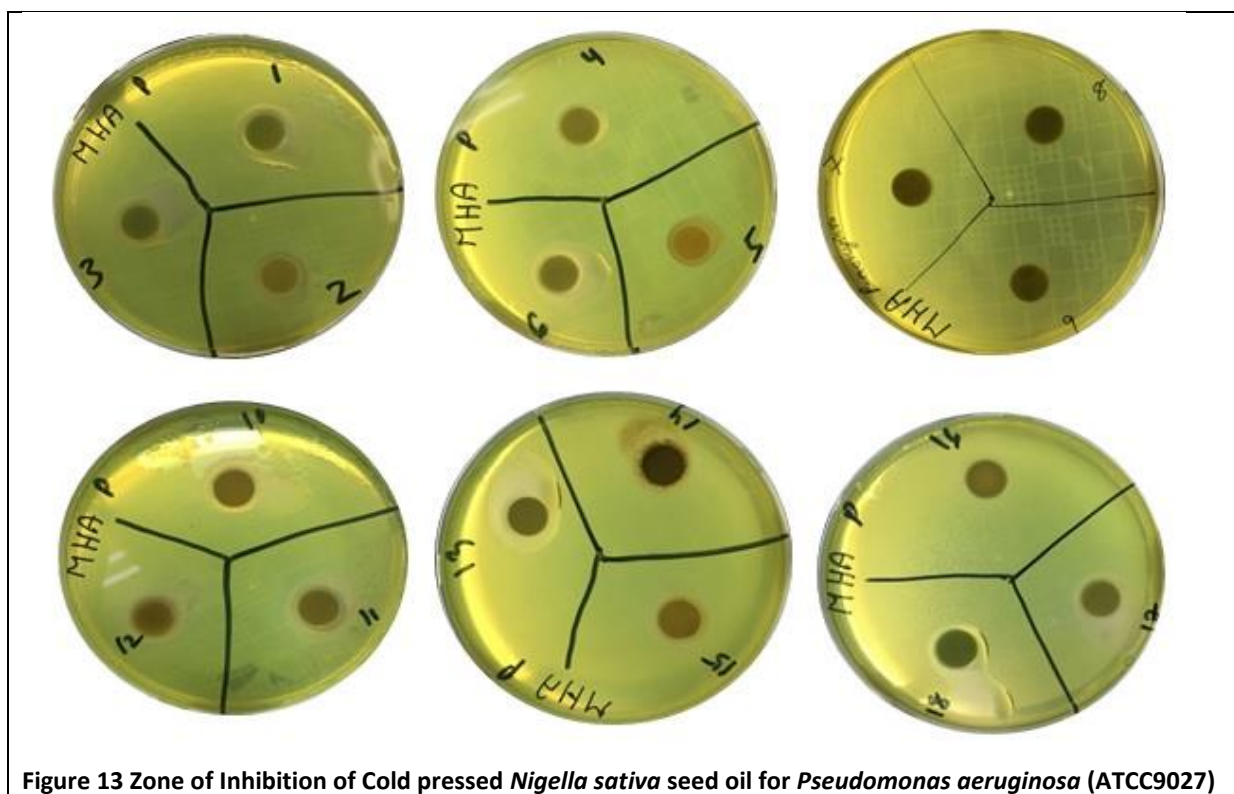
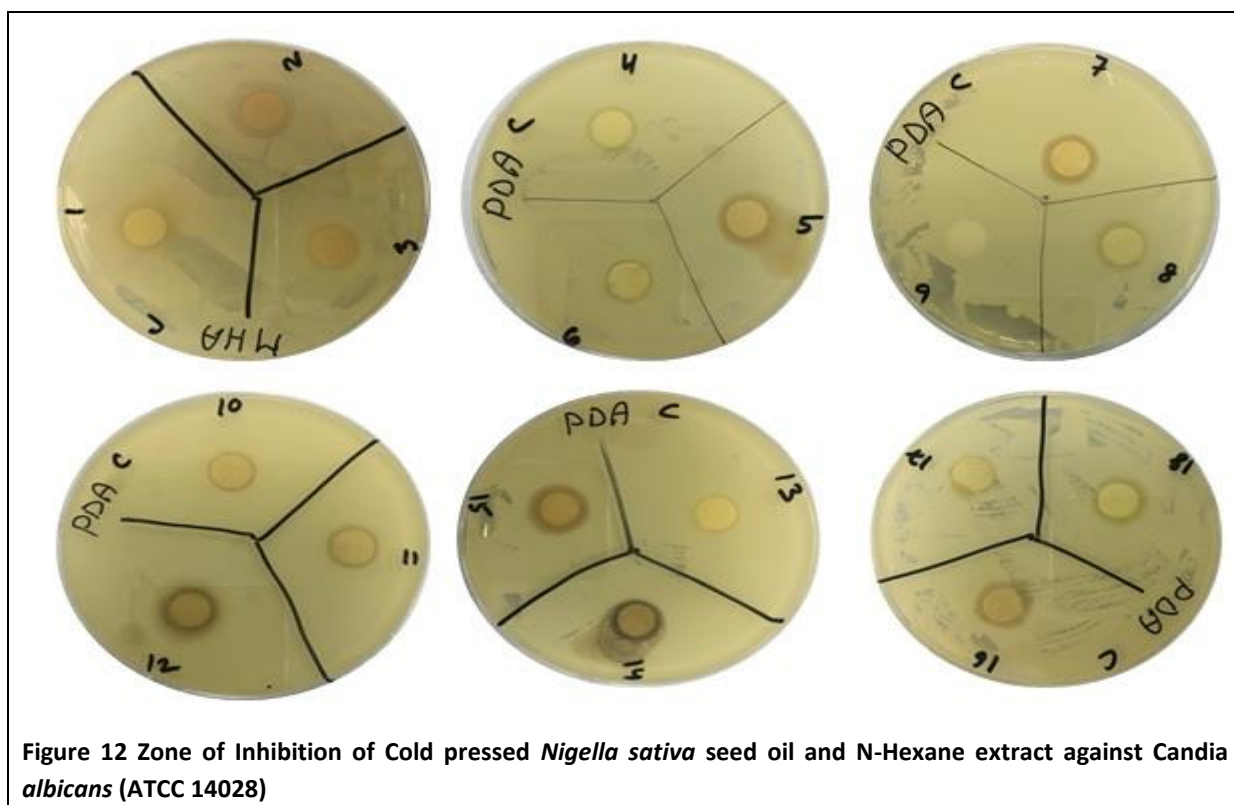


Figure 11 Zone of Inhibition of Cold pressed *Nigella sativa* seed oil and N-Hexane extract against *Bacillus Cereus* (ATCC 10876)



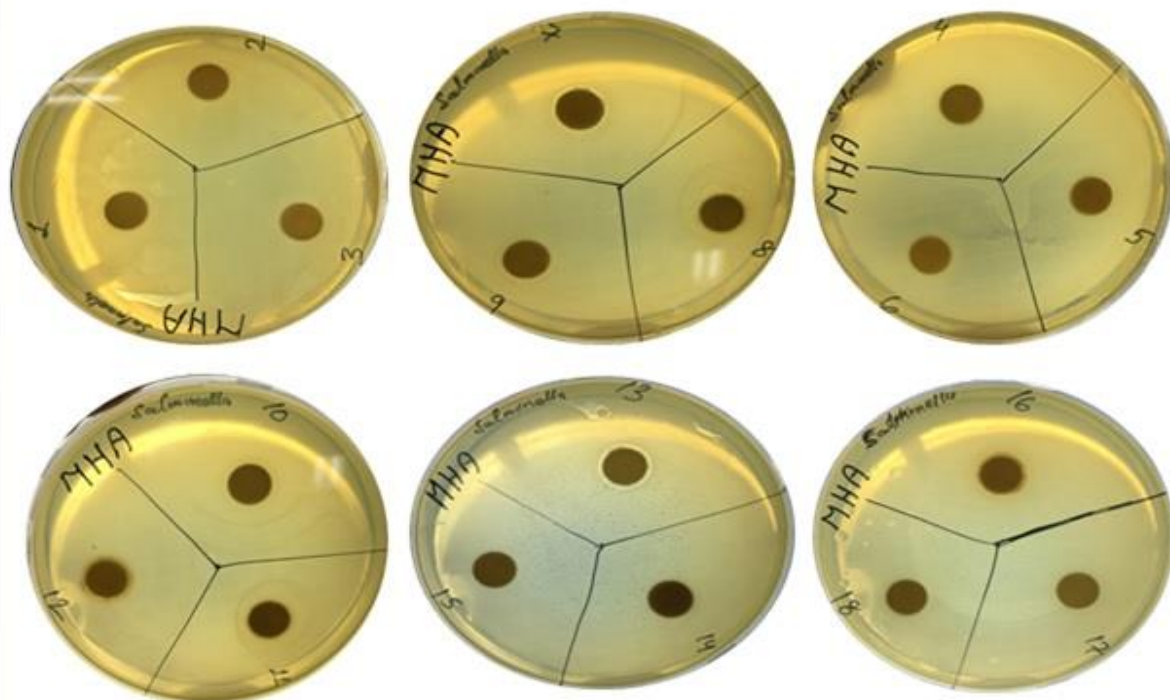


Figure 14 Zone of Inhibition of Cold pressed *Nigella sativa* seed oil for *Salmonella Typhimurium* (ATCC 14028)

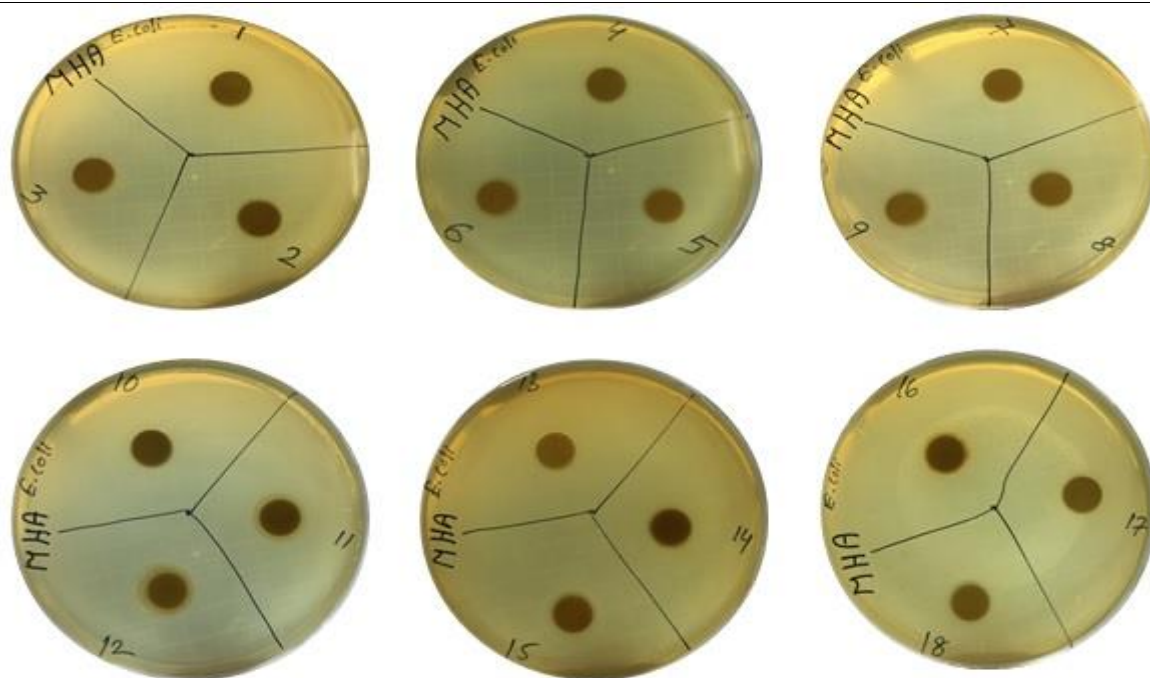


Figure 15 Zone of Inhibition of Cold pressed *Nigella sativa* seed oil for *Escherichia coli* (ATCC 8739)

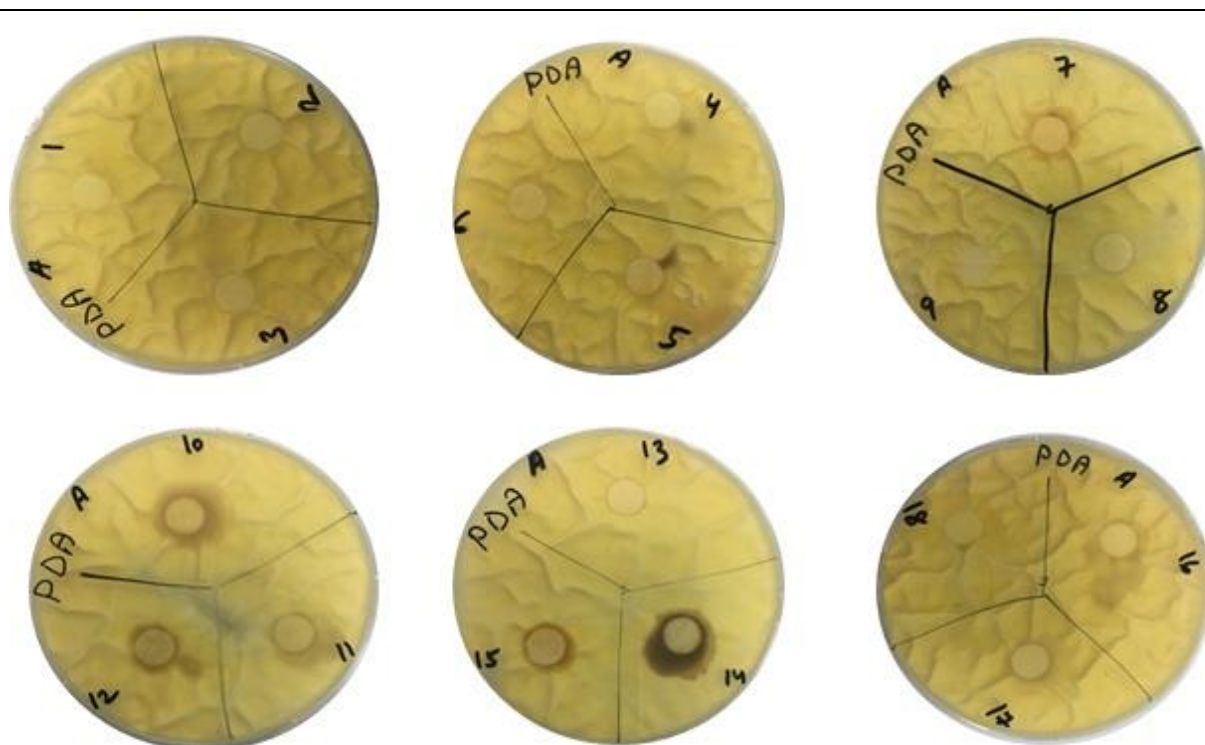
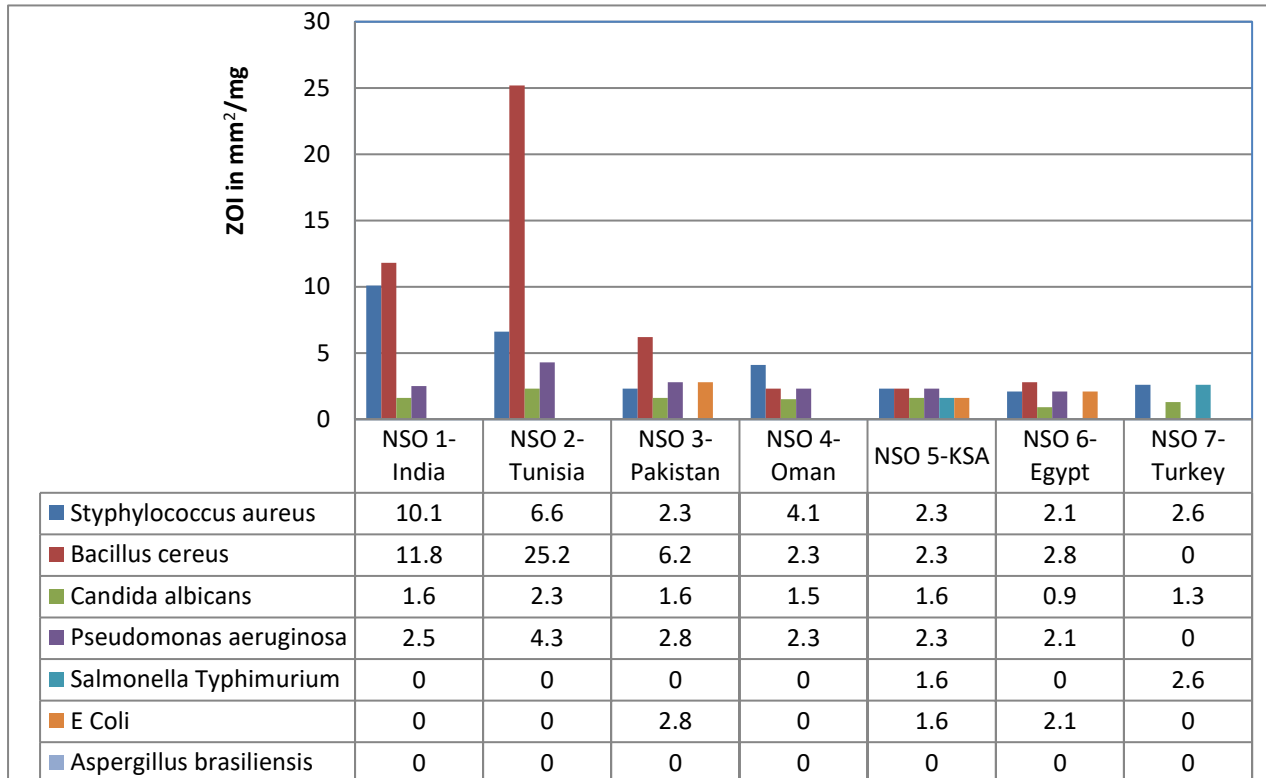


Figure 16 Zone of Inhibition of Cold pressed *Nigella sativa* seed oil for *Aspergillus brasiliensis* (ATCC 16404).

Graph 1 Zone of Inhibition for Cold pressed *Nigella sativa* seed oil against studied microorganism



B) Free Radical scavenging property



Figure 17 : Initial observation with 50µl of samples + 3ml of 0.08% Solution of DPPH + 10 ml of Ethanol.

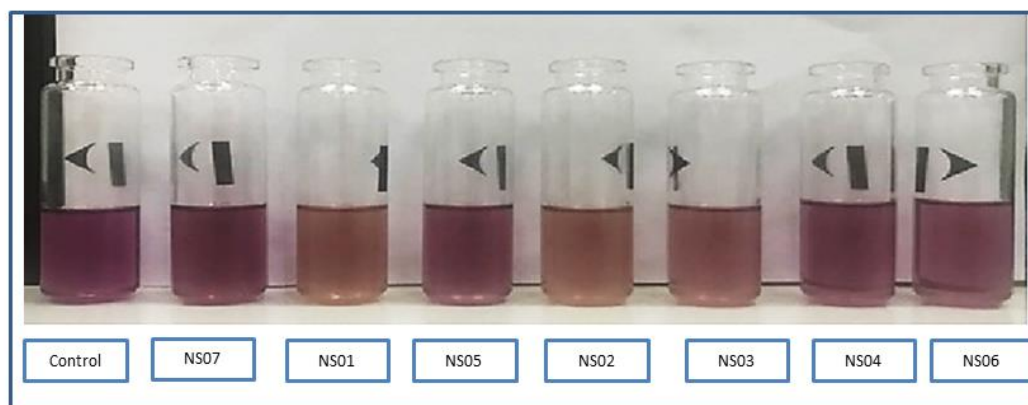
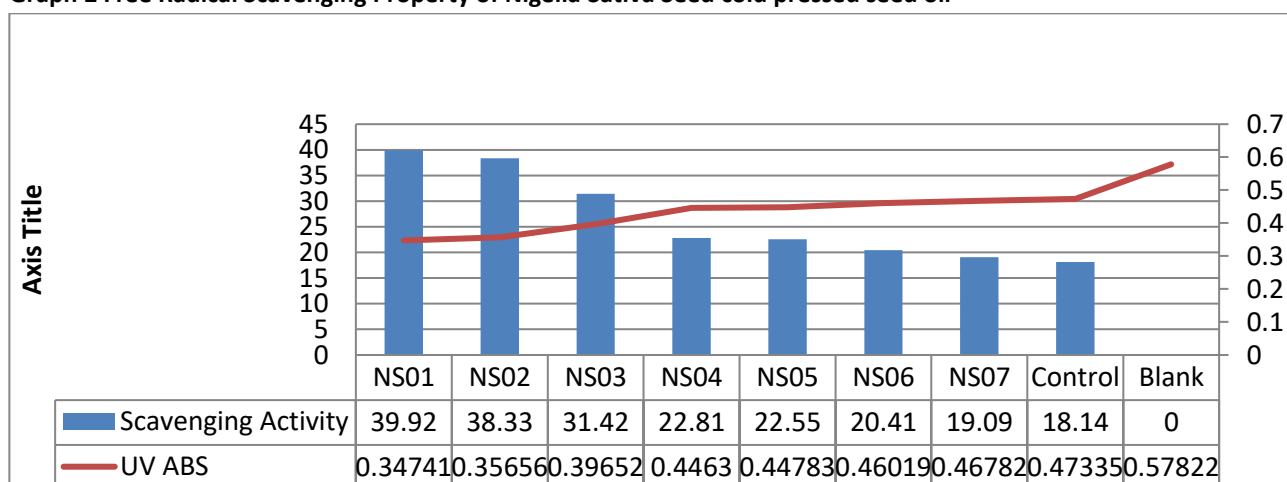


Figure 18 final observation with 50µl of samples + 3ml of 0.08% Solution of DPPH + 10 ml of Ethanol.

Graph 2 Free Radical Scavenging Property of Nigella Sativa Seed cold pressed seed oil



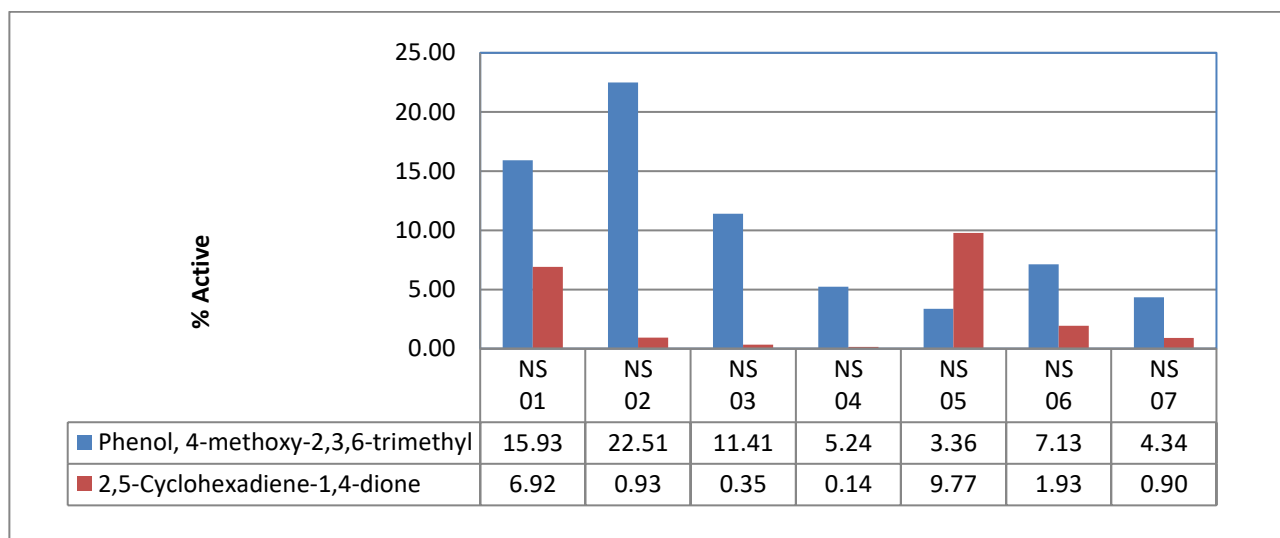
C) Chemical composition

Following are the chromatograms and characterized chemical composition of n-hexane extract of Nigella sativa seed from different geographies

Table 3 Chemical Composition of n-Hexane Extract of Nigella sativa Seed

S.No.	COMPOUND NAME	0019	0023	0022	0020	0024	0025	0031
		NS01	NS02	NS03	NS04	NS05	NS06	NS07
		%	%	%	%	%	%	%
1	N-Methylglycine	20.69	34.00	49.40	36.31	34.40	44.01	38.69
2	Bicyclo[3.1.0]hexane	2.55	1.58	1.09	0.00	3.73	0.67	1.02
3	Benzene, 1-methyl-2-(1-methylethyl)	9.12	8.72	5.28	0.67	14.22	3.73	2.84
4	1,4-Cyclohexadiene	1.34	1.21	0.69	0.00	2.14	0.00	0.00
5	Phenol, 4-methoxy-2,3,6-trimethyl	15.93	22.51	11.41	5.24	3.36	7.13	4.34
6	2,5-Cyclohexadiene-1,4-dione	6.92	0.93	0.35	0.14	9.77	1.93	0.90
7	Myristic acid	1.36	2.07	0.92	0.35	4.21	1.03	0.35
8	1-Hexadecanol	0.00	0.19	0.48	0.15	0.95	0.00	0.00
9	Palmitic acid	0.52	0.81	0.65	30.53	2.56	0.70	0.32
10	Linoleic acid	2.60	2.76	3.02	1.94	2.71	2.92	2.09
11	Hexadecanoic acid	1.08	0.00	0.00	0.79	0.94	3.30	1.94
12	Stigmasterol	0.59	0.56	0.68	0.30	2.25	0.81	0.60
13	β -Sitosterol	1.15	1.59	1.55	0.73	5.15	1.47	1.27
14	9,12-Octadecadienoic acid	24.01	14.47	2.09	4.36	1.15	24.62	1.27
15	1-Heptatriacotanol	0.61	0.80	1.15	0.48	2.47	1.07	22.91
16	9,12,15-Octadecatrienoic acid	1.51	1.65	2.41	0.33	0.82	0.00	0.75
17	Oleic Acid	0.00	0.94	1.14	0.29	0.00	1.56	1.84
18	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one	2.63	0.00	0.00	0.00	0.83	3.52	0.95
19	Bicyclo[3.1.1]hept-2-ene	0.57	1.00	0.30	0.00	0.00	0.00	0.22
20	Others	6.83	6.83	6.83	6.83	6.83	6.83	6.83

Graph 3 Total Phenolic and Thymoquinone contents in the cold pressed seed oil of Nigella sativa



Results

It is observed that cold pressed Oil and n-hexane extract of *Nigella sativa* seeds showed antimicrobial activity against *staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*,

Nigella sativa cold pressed oil and n-hexane showed antimicrobial potential in following rating India Tunisia Pakistan, Oman, KSA, Egypt and turkey.

It is observed that average 25 numbers of chemical compounds are characterized in cold pressed oil of *Nigella sativa* seed extract from different geographies. During this study, we observed that total phenol content are highest in Indian variety followed by Tunisia and Pakistan. During this study, we observed that all *Nigella sativa* cold pressed seed oil showed presence of N-Methylglycine or 2-(Methylamino) acetic acid. This is the first time that N-Methylglycine is report in *Nigella sativa*. N-Methylglycine is gaining important clinically for the treatment of Schizophrenia and depression. The DPPH assay of Cold pressed seed oil of *Nigella sativa* complements the chemical composition. Free radical scavenging property of Indian subcontinent and Tunisia varieties are higher than their Middle Eastern counter parts.

Discussion

Antimicrobial property of *Nigella sativa* seed very well studied but comparasion of antimicrobial property is not very well presented. This research is an attempt to create a benchmark study, which would facilitate the selection of most potential variety of *Nigella sativa*

seeds. Shaaban HA et.al., showed that Essential oil (EO) of *Nigella sativa* has strong antibacterial activity against pathogenic bacteria like *S. aureus*, *B. cereus* and *S. typhimurium* [4]. Chaieb K et al., proved the Antibacterial activity of Thymoquinone. They proved Thymoquinone has significant bactericidal activity against gram positive cocci *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (CIP 106510) [7]. All these earlier studies are complementing our study in terms of antimicrobial property. In this study, also *Nigella sativa* cold pressed seed oil and n- Hexane extracts showed antibacterial property against *Staphylococcus aureus*, which is gram positive bacteria. There are few efforts in past to do benchmark study of antimicrobial property of *Nigella sativa* but the efforts were restricted to single variety [13-14]. This study is a comprehensive effort to compare antimicrobial potential of *Nigella sativa*.

Nigella sativa seed is a rich treasure of bioactive chemicals, In past, there are lot of studies which focuses on Chemical composition of seed oil and various extracts of *Nigella sativa* seed^{15,16} which reports presence of more than 47 chemical compounds out of major phenolic compound were thymol, Thymoquinone, Thymohydroquinone and Dithymoquinone¹⁷. There are attempt to check the chemical compositions of *Nigella sativa* seed oil from specific geographies like Tunisian *Nigella sativa* seed was studied using GC-MS technics showed presence of 30 compounds which mostly consisted of alpha- pinene (13.75 %), limonene (2.55 %), p-cymene (43.58 %), carvacrol (2.53 %) and tymoquinone (1.65 %). Further

study also pointed few differences in terms of percentages and presences of few chemical compounds like Thymoquinone, p-cymene, α -pinene, terpinolene, γ -terpinene (11.2 %), and α -thujene (4.6 %) ⁹⁶. The chemical composition of *Nigella sativa* seed oil cultivated in Morocco was studied for its fatty acid and sterol composition using gas chromatography. The study reported presence of linoleic acid (58.5%), oleic acid (23.7%), palmitic acid (13.1%), β -sitosterol, and stigmasterol. In another study researcher studied the composition *Nigella sativa* seed oil using GC-MS analysis technics, and they identified a total of 32 compounds which includes 9-eicosyne (63.04%), linoleic acid (13.48%), and palmitic acid (9.68%). Seed oil also showed presence of compounds like 2.84% of alkanes and 0.30% sesquiterpene hydrocarbons¹⁸. The current study is in agreement with previously mentioned study, which indicated presences of fatty acids, thymoquinone and phenolic compounds. During this study, new chemical compound N-Methylglycine is newly identified in n-hexane extract and seed cold pressed oil. N-Methylglycine is antipsychotics, which can be used for the treatment of schizophrenia ¹⁹ and treatment of depression ²⁰.

Conclusion

It is observed that, *Nigella sativa* seeds from different geographies have different level of antimicrobial and antioxidant potency. The variation in antimicrobial and antioxidant potency may be linked to typical chemical composition of *Nigella sativa* seed extract. Which is also depending on genetic makeup of particular variety in particular region. Genetic diversity amongst the *Nigella sativa* from India, Pakistan and Tunisia seems to be low, which offer them similar chemotypic characteristic. Contents of chemical further can be impacted by environmental condition in particular region, time of seed harvesting, exposure of stock to high temperature and heat. From this study, we can have concluded that Indian, Pakistani and tunisian variety of *Nigella sativa* seed can be used for further propagation and breeding program.

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