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STUDIES ON ANTIBACTERIAL ACTIVITY, ANTIOXIDANT ACTIVITY AND GC-MS ANALYSIS OF *NIGELLA SATIVA* SEEDS

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Keywords:

Nigella sativa, GC-MS analysis, Antioxidant activity, ATCC pathogens and DPPH assay

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ABSTRACT: The *Nigella sativa* seeds were collected from Cuddalore and powdered thoroughly by using mortor and pestle. The *Nigella sativa* seeds were individually extracted with different solvents such as ethanol, methanol, acetone and water. The antibacterial activity of seed extracts were tested against the 6 ATCC pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Clostridium sporogenes*, *Bacillus spizizenii* and *Pseudomonas aeruginosa* using Muller Hinton agar. The ethanol extract of *Nigella sativa* seed showed maximum activity against *Klebsiella pneumoniae* (12 mm) and *Bacillus spizizenii* (12 mm). The methanol extract of *Nigella sativa* seed exhibited minimum activity against *Clostridium sporogenes* (8mm), *Escherichia coli* (8mm), *Pseudomonas aeruginosa* (8mm) and highest activity against *Klebsiella pneumoniae* (20mm). The acetone extract of *Nigella sativa* seed showed highest activity against *Escherichia coli* (20mm). The aqueous extract of *Nigella sativa* seed showed maximum inhibitory activity against *Klebsiella pneumoniae* (23mm). Among the 4 solvent extracts, water extracts of *Nigella sativa* seed showed maximum inhibition. The GC-MS analysis of the *Nigella sativa* seed extract was carried out and it showed the following major phytochemical constituents such as Piperidine, 1-(2-phenylethyl)- , p-cymene, cis-4-methoxy thujane, Thymoquinone, phenol, 2-methyl-5-(1-methylethyl)-, Tricyclo[5.4.0.0(2, 8)]undec-9-ene, 2,6,6,9-tetramethyl-(1R, 2S, 7R, 8R)- , Longifolene, p-cymene-2, 5-diol, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid,9,12-octadecadienoic acid(Z-Z)-, 11-14-Eicoradienoic acid, Glycerol 1-palmitate, 9,12-Octadecadienoic acid (Z, Z)-,2-hydroxy 1-(hydroxymet), Ethanol, 2-(9,12-Octadecadienyloxy)-,(Z-Z) - , beta. -Sitosterol, 2-Dodecen-1-yl(-) succinic anhydride. The methanol seed extract of *Nigella sativa* showed the % of antioxidant activity using DPPH assay was 19.88% where the positive control ascorbic acid showed 98.68%.

INTRODUCTION: Medicinal plants are nowadays known as the richest bioresource of drugs which is used as a traditional medicine for several diseases. It is also used as folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs.

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When compared to the synthetic drugs people mostly prefer the traditional medicines for their use [1]. *Nigella sativa* Linn. is commonly known as black cumin. It belongs to the botanical family of Ranunculaceae.

Mostly in the modern days, the *Nigella sativa* seeds are used for the medicinal and nutritional purposes [2]. It is also widely used in the many modern eastern countries and other parts of the world [3]. In food industries, the *Nigella sativa* seeds are used as a condiment and also a traditional remedy for number of diseases which includes gastrointestinal tract problems, asthma, jaundice, hypertension,

diabetes, eczema, rheumatism and inflammation [4]. The medicinal plants are also the important source of providing the minerals, vitamins and the trace elements to the humans and some of the consumers considered it as the potentially safe drug. The *Nigella sativa* seeds have the antimicrobial activity against drug resistant bacteria [5, 6], methicillin resistant bacteria [7], wound infection causing bacteria [8, 9], acne causing bacteria [10], bacteria causing Otitis media [11], against throat infection causing bacteria [12], Periodontal infection causing bacteria [13], against Chlamydial infections [14], against Gram positive and Gram negative bacterial infections [15-20], fungi [21, 22], virus [23] and parasites. The *Nigella sativa* oil is used as a food preservative and carminative. The seeds of *Nigella sativa* are composed of more than 100 compounds [20].

The *Nigella sativa* plants are commonly present in the Middle East, Europe, Western and Middle Asian countries [7]. *Nigella sativa* (Black cumin) also called Black seed, Black caraway, Roman coriander, kalonji or fennel flower. The *Nigella sativa* seed extract have the antibacterial effect against the bacterial strains such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Clostridium sporogenes, Pseudomonas aeruginosa* and *Bacillus spizizenii*. The *Nigella sativa* seeds extract have the antifungal effect against the fungal strains such as *Aspergillus flavus, Aspergillus niger* and also against the *Candida* species like *Candida albicans* and *Candida tropicalis* [22].

Higher concentration of thymoquinone, carvacrol, t-anethole and 4-terpineol acts as a good scavengers against free radicals which will reduce the possibilities of cell damage and serve as good antioxidants [24-28]. It also possess anticancer potential [29] and possess cytotoxicity [30, 31, 32], Large population of pathogens were highly resistant against the modern day synthetic drugs [5]. This make problems to treat disease and cause side effects. Herbal medicines have thousands of compounds which can act against all forms of diseases [33-37]. In the present study, medicinal plant *Nigella sativa* seeds were selected for its antibacterial activity against the ATCC cultures such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Clostridium sporogenes, Bacillus spizizenii* and *Pseudomonas aeruginosa* and its antioxidant property.

MATERIALS AND METHODS:

Collection and Processing of *Nigella sativa* Seeds: Seeds of *Nigella sativa* were collected from Cuddalore **Fig. 1**. The seeds of *Nigella sativa* were collected and powdered by using the mortor and pestle and stored in a sterile plastic container for further use.

Preparation of Seed Extract: The powder of the *Nigella sativa* seeds were mixed seperately with different solvents such as ethanol, methanol, acetone and water. (10g/100ml) .Then the mixtures were stirred for 3 days using shaker. This method was frequently done to got enough extracts. Finally the mixture were filtered through Whatman no.1 filter paper for the clear collection of extracts and were evaporated in the rotary evaporator and the extract was stored in sterile container for further use.

Preparation of Seed Extract Loaded Sterile Disc: 50 µl of the extract was loaded in the sterile discs and then dried and used for antimicrobial studies against bacterial pathogens.

Collection of ATCC Cultures: ATCC cultures such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus spizizenii, Pseudomonas aeruginosa* and *Clostridium sporogenes* were used.

Antibacterial Activity of Seed Extracts of *Nigella sativa*: The disc diffusion method was used for the antibacterial activity of the seed extract against bacterial pathogens. Ethanol, methanol, acetone and water extracts of *Nigella sativa* seeds were prepared and were tested against clinically important pathogens such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus spizizenii, Pseudomonas aeruginosa* and *Clostridium sporogenes.* These cultures were inoculated in tryptic soy broth and incubated at 37°C for 6 hours and observed for visible turbidity. Then the Muller hinton agar was plated in sterile petriplates and were allowed to solidify. The bacterial cultures prepared in broth were swabbed over agar surface aseptically. Then gently placed the discs containing seeds extract (100mg/ml) of *Nigella sativa* on the agar surface at equal distance. Control plates were kept by using the solvents such as ethanol, methanol, acetone and water loaded

discs and broad spectrum antibiotic disc and incubated at 37°C for 24 hours. Then the zone of inhibition was measured in mm.

GC-MS Analysis: Rxi-5SiL MS column (fused silica) cross bond with 1,4- bis(dimethyl silica) phenylene dimethyl polysiloxane.

(i) Sample elution using 50:1 helium was used carrier gas at 1.0 minute.
(ii) Column temperature 40°C for 2 minute to 310°C minute.
(iii) Time taken for chromotography per sample is 45 minutes.

Analysis of the Phytocomponents in *Nigella sativa* Seeds using GC-MS Technique: GC-MS analysis was carried out in CSIR- Central Electrochemical Research Institute, Karaikudi, Tamil Nadu, India. One micro litre of the filtrate was injected into the GC-column. Then the sample get evaporated and carried away by the carrier gas, helium and it got segregated into individual fraction. The sample fraction coming out of the column was let into the mass detector and the mass spectrum of each compound was recorded. The mass spectrum of the unknown compound was compared with the spectrum was accomplished using data base dictionaries.

Identification of Component: The database in the WILEY online library has been used for the interpretation on GC-MS. The spectrum of the unknown component was compared with the spectrum of the unknown component stored in the WILEY online library. Then the molecular formula and molecular weight of component were identified accordingly.

Antioxidant Activity: DPPH radical scavenging activity of extract was determined according to the method reported by Blois (1958). An aliquot of 0.5 ml of sample solution in methonal was mixed with 2.5ml of 0.5 mm ethanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.

% of Antioxidant activity = absorbance of control - absorbance of sample / absorbance of control × 100.

RESULTS: The ethanol extract of *Nigella sativa* seed showed maximum activity against *Klebsiella pneumoniae* (12 mm) and *Bacillus spizizenii* (12 mm). The methanol extract of *Nigella sativa* seed exhibited highest activity against *Klebsiella pneumoniae* (20mm).

The acetone extract of *Nigella sativa* seed shown highest activity against *Escherichia coli* (20mm) The water extract of *Nigella sativa* seed showed highest activity against *Klebsiella pneumoniae* (23mm) .Among the 4 solvent extracts, water extracts of *Nigella sativa* seed showed maximum inhibition against *Klebsiella pneumoniae* (23mm) **Table 1, Fig. 2**, **3.**

The mass chromatogram of the methanol extract of *Nigella sativa* seeds showed 34 different phytochemical compounds **Fig. 4**. Twenty major compounds were identified such as Piperidine, 1-(2-phenylethyl)-, p-cymene, cis-4-methoxy thujane, Thymoquinone, Phenol, 2-methyl-5-(1-methylethyl) -, Tricyclo[5.4.0.0(2, 8)]undec-9-ene, 2,6,6,9- tetramethyl-, (1R, 2S, 7R, 8R)-, Longifolene, p-cymene-2, 5-diol, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z, Z)-, methyl ester, Methyl stearate, 9,12-Octadecadienoic acid(Z-Z)-, 11-14-Eicosadienoic acid, Glycerol 1-palmitate, 9,12-Octadecadienoic acid (Z, Z) -, 2-hydroxy1-(hydroxymet, Ethanol, 2-(9,12-Octadecadienyloxy)-, (Z, Z) - and 2-Dodecen-1-yl(-) succinic anhydride.

In the chromotogram the height of each peak is in proportion to the amount of particular compound present in the sample. The methanol seed extract of *Nigella sativa* showed the % of antioxidant activity using DPPH assay was 19.88% where the positive control ascorbic acid showed 98.68% (IC_{50} 500ug/ml).

FIG. 1: *NIGELLA SATIVA* SEEDS

TABLE 1: ANTIBACTERIAL ACTIVITY OF NIGELLA SATIVA SEED EXTRACTS AGAINST ATCC CULTURES

S. no.	ATCC culture	Zone of inhibition (mm)			
		Ethanol	Methanol	Acetone	Water
1	*Staphylococcus aureus*	8	-	8	-
2	*Klebsiella pneumoniae*	12	20	-	23
3	*Escherichia coli*	-	8	20	8
4	*Clostridium sporogenes*	-	9	11	-
5	*Pseudomonas aeruginosa*	8	8	8	9
6	*Bacillus spizizenii*	12	-	10	-

mm-Millimeter – No activity.

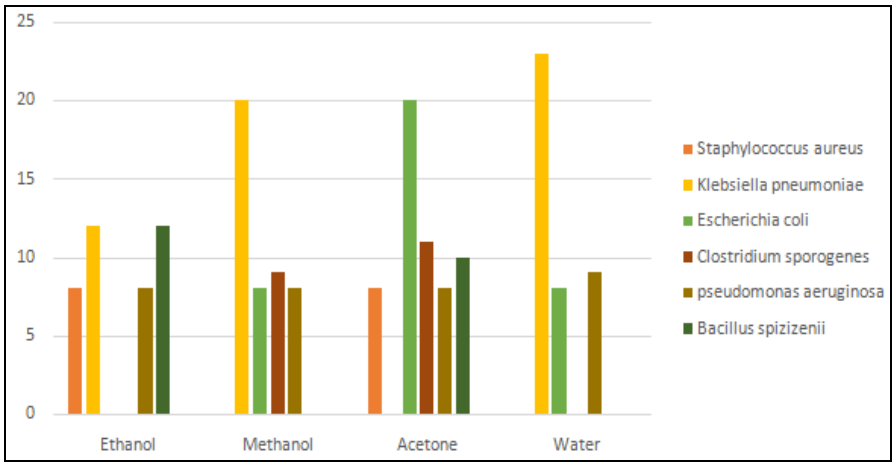

FIG. 2: ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* SEED EXTRACTS AGAINST ATCC CULTURES

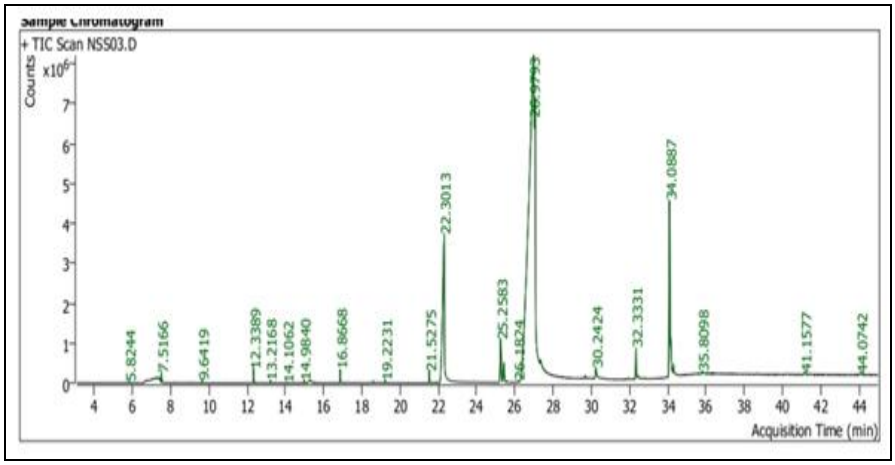

FIG. 3: MASS CHROMATOGRAM OF *NIGELLA SATIVA* SEED EXTRACT

KLEBSIELLA PNEUMONIAE ***ESCHERICHIA COLI***

FIG. 4: ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* SEEDS EXTRACT AGAINST ATCC CULTURES

DISCUSSION: Esra Kocoglu *et al.,* (2019) carried out the antibacterial activity of *Nigella sativa* seeds extract against the most frequently isolated infectious bacteria of the middle and external ear. It showed the antibactericidal activity against *H. influenzae, M. catarrhalis* and *S. pneumoniae.* However the *Nigella sativa* was not found to be effective against *P. aeruginosa* at any concentration. In the present study, the antibacterial activity of *Nigella sativa* seed extracts (ethanol, methanol, acetone and water) was carried out against 6 ATCC cultures such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Clostridium sporogenes, Bacillus spizizenii* and *Pseudomonas aeruginosa* by disc diffusion method and maximum inhibition was noted with the water extract of *Nigella sativa* seed against *Klebsiella pneumoniae* with a zone of inhibition of 23mm.

Gurdip singh *et al.,* (2005) revealed the antioxidant effect of acetone extract of *Nigella sativa* which has good scavenging activity against DPPH radicals in comparison with synthetic antioxidants. The scavenging effect of essential oil is 82.1%. In the present study, the methanol seed extract of *Nigella sativa* showed the % of antioxidant activity using DPPH assay was 19.88% where the positive control ascorbic acid showed 98.68% (IC 50 500ug/ml).

Salima Tiji *et al*., (2021) performed the phytochemical analysis of *Nigella sativa* seeds by using GC-MS method which contains secondary metabolities such as polyphenols, flavanoids, alkaloids, steroids, terpenes, coumarins, tannins and saponins. In the present study, the mass chromotogram of the methanol extract of *Nigella sativa* seed showed 34 different phytochemical compounds. Twenty major compounds were identified.

CONCLUSION: Microorganisms gain resistance against the commercial antibiotics. Plant based compounds have promising activity to treat various type of illness without any side effects. In the present study Ethanol, methanol, acetone and water extracts of *Nigella sativa* seeds were prepared and were tested against clinically important pathogens such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus spizizenii, Pseudomonas aeruginosa* and *Clostridium sporogenes.* It showed inhibitory activities against all pathogens. It also showed considerable antioxidant potential.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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