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COMPARATIVE STUDY OF ANTIMICROBIAL EFFECT OF *NIGELLA SATIVA* SEED EXTRACTS FROM DIFFERENT GEOGRAPHIES

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Keywords:

Nigella sativa, Kalongi, Herbal medicinal ingredients, Antimicrobial activity, Herbal extracts

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ABSTRACT: Prophet Mohammed calls *Nigella sativa* seed as a medicine of blessings. *Nigella sativa* is an herbaceous plant and cultivated in the Middle East, South East Asia and some part of Europe. *Nigella sativa* seed has been in use in the Middle East, South East Asia and some part of Europe over 2000 years. Until date lot of research, studies are conducted to prove the therapeutic utility of *Nigella sativa* seeds. In these various studies, *Nigella sativa* seeds and its derivative showed therapeutic activity against allergy, cough, and diabetes, different types of cancer and skin infections. It is one of the widely used spices in the Middle East, South East Asia and some part of the Europe *Nigella sativa* seed is intensively studied for its chemical composition. It is reported to contain Thymoquinone, Nigellimine-N-oxide, Nigellicine, Nigellidine, Nigellone, Dithymoquinone, Thymohydroquinone, Thymol, Arvacrol, 6-methoxy-coumarin, 7-hydroxycoumarin, Oxy-coumarin, Alpha-hedrin, Steryl-glucoside, Tannins, Flavinoids, Essential fatty acids, Essential amino acids, Ascorbic Acid, Iron and Calcium. The presence of these natural actives makes *Nigella sativa* seed as a super medicinal herb. The antimicrobial property of *Nigella sativa* seed is studied by former researchers are confined to the particular solvent and *Nigella sativa* seed of specific origin. There is practically no research available which compare the anti-microbial potential of *Nigella sativa* seed from various geographies. This study was planned to test and compare the antimicrobial property of different solvent extracts of *Nigella sativa* seed from Egypt, Tunisia, Syria, Saudi Arabia, Pakistan, Turkey, Oman, and India. The solvent used were n-hexane, methanol, and water. All these extracts were subjected to zone of Inhibition on Mueller-Hinton agar using Kirby-Bauer Disk Diffusion Susceptibility Test Protocol against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Streptococcus mitis* ATCC 49456, *Streptococcus mutans* ATCC 25175, *Aspergillus brasiliensis* ATCC 16404. The result showed that methanol extract, n-hexane extract and water decoction of *Nigella sativa* seed had antimicrobial property against the *Staphylococcus aureus, Streptococcus mitis, Streptococcus mutants* but not effective against *Aspergillus brasiliensis, Pseudomonas aeruginosa, Escherichia coli, Candida albicans.* This antimicrobial activity could be due to the presence of constituents like Thymoquinone, Dithymoquinone, Thymohydroquinone, Thymol, Tannins, etc in *Nigella sativa* extracts. Further, it is observed that antimicrobiological potential of *Nigella sativa* seed from India found to be far superior to other *Nigella sativa* seeds from different geographies.

INTRODUCTION: Abu Huraira recited that Prophet Mohammed described black seed as the seed of healing for all diseases except death (As-Sam) [1]. *Nigella sativa* seeds are available with different names in various geographies.

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Nigella sativa seed is commonly known as Habbah Sauda, Kalonji, Fennel flower seed, Onion Seed, Nutmeg flower seed, Black cumin seed, black cumin, black caraway seed [2].

In reality, there is no botanical relation with *Nigella sativa* seed and other mentioned seeds like an onion seed (*Allium cepa*) Seed, Sesame Black (*Sesamum indicum*) and Seeds of *Argemone Mexicana*, although they are quite similar and easily confused with *Nigella sativa* seed [3]. These same looking seeds are found to be part of commercially available *Nigella sativa* seed stock and used as adulterants.

These adulteration and substitution impact the efficacy of *Nigella sativa* seed adversely. It is also observed that *Nigella sativa* seed consignments adulterated with exhausted seeds after CO_2 supercritical extraction, such type of seed do not have any medicinal value

Nigella sativa herb is 20-25 cm tall, with finely divided, linear leaves. Leaves are divided into linear segments 2 to 3 cm long. Leaves are opposite in pairs on either side of the stem. Upper leaves are long as compared to lower leaves, and they are petiolate, and flowers grow terminally on its branches. The flowers are colored pale blue and white, with 5 to 10 petals and are quite delicate. The fruit is a large inflated capsule composed of three to seven united follicles. Seeds are black, triangular in shape, 2 to 3 mm long. The fruit has the pungent odor when crushed, contains a good amount of fixed and essential oil.

FIG. 1: FLOWERS OF *NIGELLA SATIVA* [4]

FIG. 2: FRUIT OF *NIGELLA SATIVA* [5]

FIG. 3: BROKEN FRUIT WITH *NIGELLA SATIVA* SEED [6]

FIG. 4: SEED OF *NIGELLA SATIVA*

Until date there is 21, distinct species of black seed reported [7]. However, *Nigella sativa* is the most studied species, followed by *Nigella damascene* and *Nigella arvensis*.

Recently few researcher reported the presence and isolation of chemical actives like Thymol, Thymo quinone, Dithymoquinone, Thymohydroquinone, Nigellicine, nigellidine, nigellimine - N-oxide, Nigellone, Arvacrol, oxy-coumarin, 6-methoxy-coumarin and 7-hydroxycoumarin, alpha-hedrin, Steryl-glucoside, tannins, essential amino acids, essential fatty acids, ascorbic acid [8, 9, 10, 11, 12].

In various studies, *Nigella sativa* seeds have proved to be anti-inflammatory, analgesic, anti-histaminic, anti-allergic, anti-cancer, anti-oxidant, immune stimulant, anti-hypertensive, anti-asthmatic, hypoglycemic, anti-bacterial, antiviral, anti-fungal and anti-parasitic [13, 14, 15, 16, 17, 18]. There are varies study available, which provides antimicrobial activity of *Nigella sativa*, where particular extract was investigated for activity against specific microorganism [19, 20, 21, 22].

Until date there no single study which had attempted to compare the antimicrobial potential of various solvent extracts of *Nigella sativa* seeds from different geographies.

Present study is an attempt to test and compare antimicrobial potential of various solvent extracts of *Nigella sativa* seed from Egypt, Tunisia, Syria, Saudi Arabia, Pakistan, Turkey, Oman, and India against human pathogens namely *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* , *Streptococcus mitis*, *Streptococcus* mutans, *Aspergillus Brazilians.*

We used the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol [23]. This protocol is quite popular amongst the researchers to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds. The antimicrobial liquid /solution is delivered on growth media plate using filter paper immersed in a testing solution. The inhibition of growth can be readily determined by the ability of a compound to develop a circle or zone around the disks. Bigger the zone of inhibition, better the antimicrobial potential.

MATERIAL AND METHOD:

***Nigella Sativa* Seeds:** We have collected *Nigella sativa* seed from India, Pakistan, Tunisia, Egypt, Saudi Arabia, Turkey, and Syria. Chief botanist identifies all samples. The portion of the samples is identified and kept in university herbarium for further reference. All samples are well cleaned, washed with water and disinfected with isopropyl alcohol to remove all biological contaminants. Sterilized seeds are dried and kept for cleaned samples bottle at cool and dry places until its use for study.

Solvents Used: Methanol and n-Hexane solvents utilized in the experiments were of analytical grade from M/s Merck. Water used in experiments was distilled water.

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.

Microbial Strains: All the culture used in the experiments were arranged from American Type Culture Collection (ATCC) namely *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Streptococcus mitis* ATCC 49456, *Streptococcus mutans* ATCC 25175, *Aspergillus brasiliensis ATCC 16404*.

Media: Media used for the experiment was arranged from M/s Hi-media, Mumbai, India namely Soybean Casein Digest Medium, Soybean Casein Digest Agar (Tryptone Soya Agar) (Casein Soybean Digest Agar), Sabouraud Dextrose Agar and Muller-Hinton agar.

Methods: All samples of seeds are cleaned, disinfected and dried. All dried samples were crushed using a grinder. 20 g 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 ºC and Round bottom flask was placed in the heating mantle. All Soxhlet extraction [24] were carried out till clear

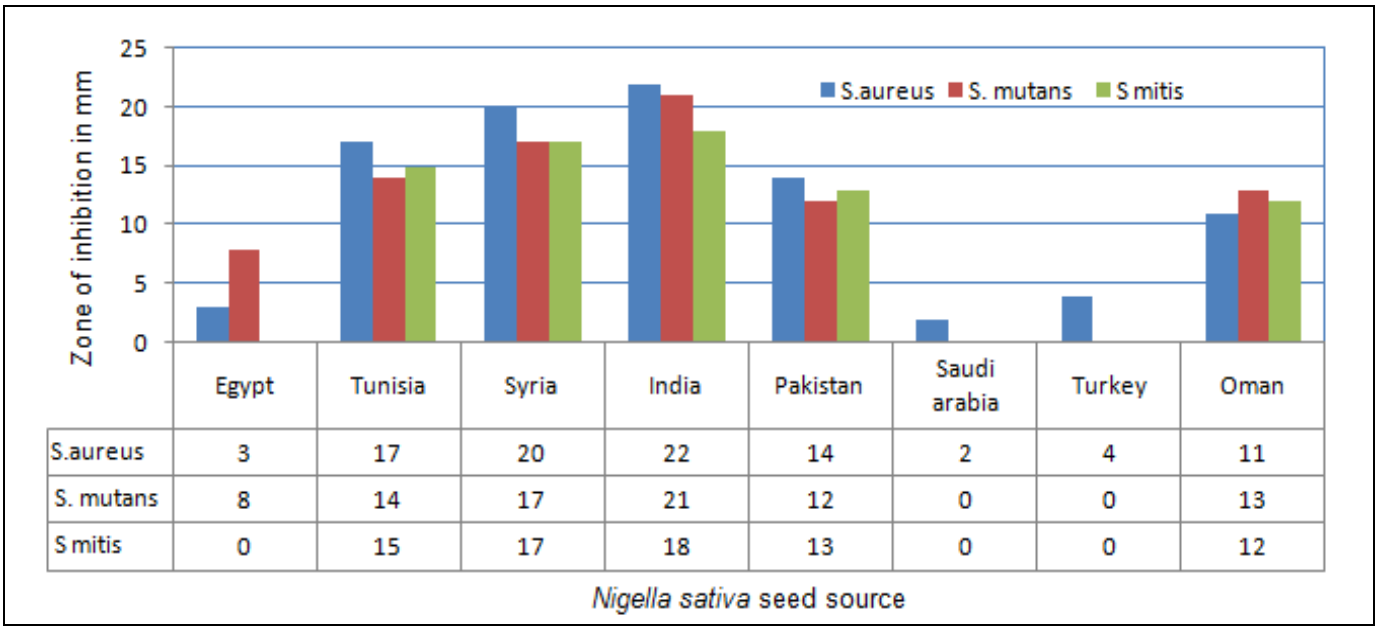
solvent is observed during reflux. For n-Hexane and hydrodistillation, only 4 h distillation was found to be sufficient, while for methanol distillation it took 48 h to complete distillation. Water decoction was prepared by keeping crushed seeds in distilled water in a beaker for 4 h with stirring.

Zone of Inhibition using Kirby-Bauer Disk Diffusion Susceptibility Test Protocol: Antimicrobial potential of *Nigella sativa* seed and its extracts are studied by using zone of inhibition using Kirby-Bauer Disk Diffusion Susceptibility Test Protocol on Mueller-Hinton MH agar plate. This is a viable standardized alternative to broth dilution methods. Methanol, n-hexane, distilled water solvents are used negative control while Ofloxacin solution is used as positive control in the experiment. ATCC microbial strains were added in Soybean Casein Digest Medium and incubated for 24 h at a temperature of 35 ºC. Soybean Casein Digest Medium solution got turbid after 24 h of incubation. Using micro streamer, a loopful of the solution was streaked on the plate with Soybean Casein Digest Agar (Tryptone Soya Agar) (Casein Soybean Digest Agar) plates (which are prepared as manufacturer's instruction) for bacterial culture and Sabouraud Dextrose Agar for Yeast and Mould culture. The inoculated Soybean Casein Digest Agar (for bacteria) plate was incubated at 35 ºC and Sabouraud Dextrose Agar (For Y+M) at 28 ºC. Colonies were observed on both the plates, we had taken one colony from each plate using micro streamer into distilled water and swirled for proper mixing. We adjusted the turbidity at 0.5 McFarland standard (Equivalent to 1.5×10^8 suspension bacterial colonies) [25]. Mueller-Hinton MH agar plates were streaked using micro streaker. Plates are allowed to settle for some time.

Simultaneously, the extracts were diluted with Dimethylsulfoxide (DMSO) to the concentration of 50 µg per mL of Methanol and n-Hexane seed extract, while 100µg per mL of Hydrodistillate and water decoction. Filter paper discs are then impregnated with 1 mL each of diluted extracts. The antimicrobial constituents diffused from the filter paper disc into the agar. A zone of inhibition was created due to the sensitivity of microbial agents from the extract in the Mueller-Hinton MH agar plate. The Clear zone which gets developed due to the sensitivity of antimicrobial agent was measured using a measuring scale.

Observation:

Antimicrobial Property of n-Hexane Extract of *Nigella sativa* Seeds:

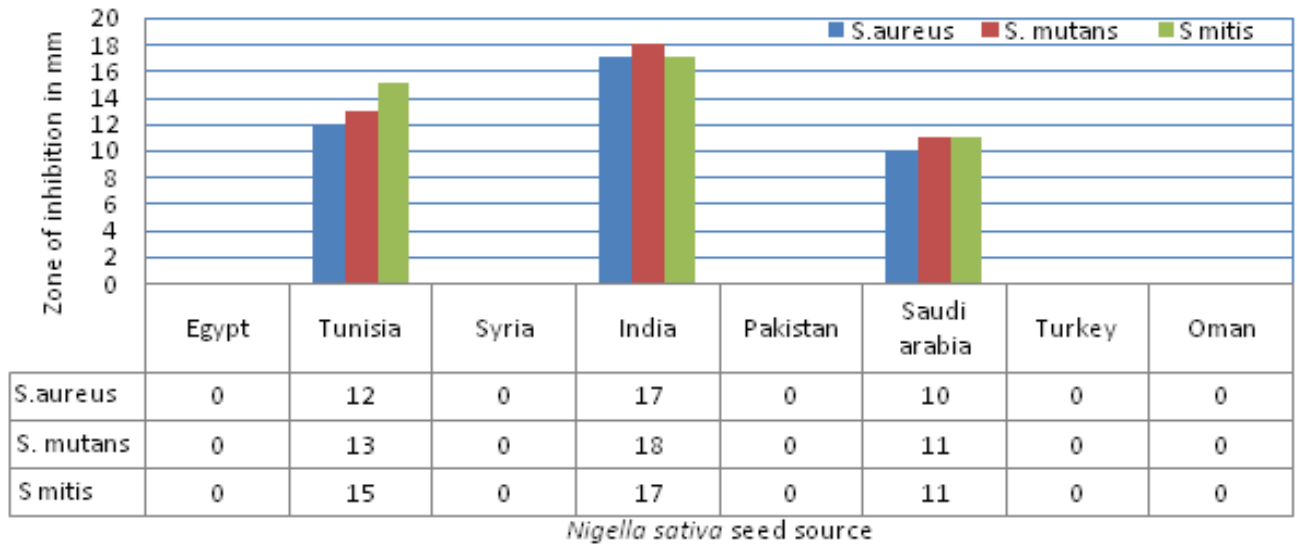


	Egypt	Tunisia	Syria	India	Pakistan	Saudi arabia	Turkey	Oman
S.aureus	3	17	20	22	14	2	4	11
S. mutans	8	14	17	21	12	0	0	13
S mitis	0	15	17	18	13	0	0	12

FIG. 2: ZONE OF INHIBITION OF *NIGELLA SATIVA* SEEDS n-HEXANE EXTRACTS FROM DIFFERENT GEOGRAPHIES

From the above graph, it is quite clear that zone of Inhibition of *Nigella sativa* seed extract in n-Hexane showed the following degree of sensitivity. Indian *Nigella sativa* > Syrian *Nigella sativa* > Tunisia *Nigella sativa* > Pakistan *Nigella sativa* > Oman *Nigella sativa* > Egypt *Nigella sativa* > Turkey *Nigella sativa* > Saudi Arabia *Nigella sativa***.**

Antimicrobial Property of Water Decoction of *Nigella sativa* Seeds:

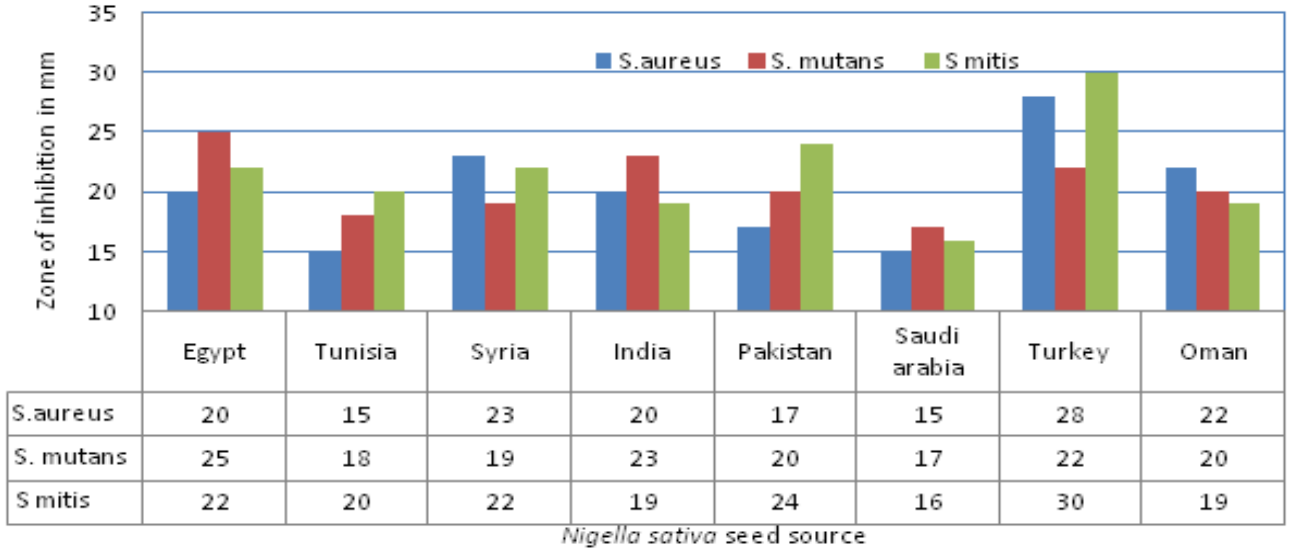


	Egypt	Tunisia	Syria	India	Pakistan	Saudi arabia	Turkey	Oman
S.aureus	0	12	0	17	0	10	0	0
S. mutans	0	13	0	18	0	11	0	0
S mitis	0	15	0	17	0	11	0	0

FIG. 3: ZONE OF INHIBITION OF *NIGELLA SATIVA* SEED WATER DECOCTION

From the above graph, it is quite clear that zone of Inhibition of *Nigella sativa* seed extract as water decoction showed the following degree of sensitivity. Indian *Nigella sativa* > Saudi Arabia *Nigella sativa* > Tunisia *Nigella sativa*, while Syrian *Nigella sativa*, Pakistan *Nigella sativa*, Oman *Nigella sativa*, Egypt *Nigella sativa*, Turkey *Nigella sativa* seed extract in water decoction not shown any antimicrobial activity.

Antimicrobial Property of Methanol Extract of *Nigella sativa* Seeds:



	Egypt	Tunisia	Syria	India	Pakistan	Saudi arabia	Turkey	Oman
S.aureus	20	15	23	20	17	15	28	22
S. mutans	25	18	19	23	20	17	22	20
S mitis	22	20	22	19	24	16	30	19

FIG. 4: ZONE OF INHIBITION OF *NIGELLA SATIVA* SEEDS METHANOL EXTRACT FROM DIFFERENT GEOGRAPHIES

From the above graph, it is quite clear that zone of Inhibition of *Nigella sativa* seed extract in methanol showed the following degree of sensitivity. Turkey *Nigella sativa* > Egypt *Nigella sativa* > Syrian *Nigella sativa* > Indian *Nigella sativa* > Oman *Nigella sativa* > Pakistan *Nigella sativa* > Saudi Arabia *Nigella sativa* > Tunisia *Nigella sativa.*

None Sensitive Extracts of *Nigella sativa:* During the experiment n-Hexane, methanol and water extract of seed of Indian *Nigella sativa,* Tunisian *Nigella sativa*, Syrian *Nigella sativa,* Pakistan *Nigella sativa*, Oman *Nigella sativa,* Saudi Arabia *Nigella sativa,* Turkey *Nigella sativa* and Egypt *Nigella sativa* found non-effective against the *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis ATCC 16404.*

Zone of Inhibition for Positive Control: As a positive control, Ofloxacin (50 µg per mL) solution was used, it showed a strong zone of inhibition on Muller Hinton agar against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Streptococcus mitis* ATCC 49456, Streptococcus mutans ATCC 25175, *Aspergillus brasiliensis ATCC 16404.*

Zone of Inhibition for Negative Control: As a negative control Methanol, n-Hexane, and water were used, all of them showed no zone of inhibition on Muller Hinton agar against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231, *Streptococcus mitis* ATCC 49456, *Streptococcus mutans* ATCC 25175, *Aspergillus brasiliensis ATCC 16404.*

RESULTS AND DISCUSSION: During this study methanol extract, n-Hexane extract and water decoction of *Nigella sativa* seed had shown highest antimicrobial potential against the *Staphylococcus aureus, Streptococcus mitis*, Streptococcus mutans but found not effective against *Aspergillus brasiliensis*, *Pseudomonas aeruginos, Escherichia coli*, *Candida albicans.* This means all these extracts of *Nigella sativa* are quite effective against gram-positive bacteria but not effective against gram-negative bacteria and fungi. Constituents like Thymoquinone, Dithymoquinone, Thymohydro-quinone, Thymol, Tannins could provide anti-microbial property to *Nigella sativa* seed extracts.

Staphylococcus aureus is observed as one of the bacteria responsible for various clinical conditions including skin infection. Antibacterial activity against *Staphylococcus aureus* qualifies *Nigella sativa* seed extract for use in skin cosmetics like skin cream, skin lotion, skin cream gel, *etc*.

Streptococcus mitis and *Streptococcus mutans* are important cariogenic bacteria. They are responsible for dental cavities in human being [26]. The effectiveness of n-hexane, methanol, and water decoction adequately support of the use of *Nigella sativa* seed and its extract in preparation meant for improving oral health in a human being. This could prompt a great shift from the use of synthetic agents to nature derived actives.

One of the prime objectives of this study was to compare antimicrobiological potential of methanol extract, n-Hexane extract and water decoction of *Nigella sativa* seed from different geographies. It is observed that there are major differences in antimicrobial activity amongst the same solvent extract from a different origin.

Extracts of *Nigella sativa* seed from India found to be far superior to other *Nigella sativa* seeds from other geographies. Differences in efficacy could be due to various reasons such as exposure to environmental conditions in that particular geographies. The effects of exposure to heat, light, and age of seed can also contribute to variation in active constituents, which in terms would impact its antimicrobial activity.

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CONFLICT OF INTEREST: Nil

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