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RESEARCH ARTICLE

THE BLACK SEED *(NIGELLA SATIVA)* AS AN IMMUNOMODULATOR AND ANTIMICROBIAL AGENT

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ABSTRACT

Objectives: The study objective was to examine immunomodulatory and antimicrobial potency of *Nigella sativa.*

Methods: Iron in *Nigella sativa* was studied by Spectrophotometer and molar absorptivity of metal concentration was by Calibration curve. Anti-microbial activity was evaluated by well and disc diffusion including its MIC and MBC values.

Findings: In calibration curve, molar absorptivity of metal concentration was calculated as 10,000/M/cm at 515nm and concentration average values of five samples were 133.83038ppm. Hydroquinone found to be highly reductants, followed by *Nigella sativa* and then hydroxyl ammonium chloride. In standard antibiotic discs testing, all microbes were showed resistance to Oxacillin. Inhibitory zones were measured by well and disc diffusion assay. Log of CFU/ml was recorded by Miles and Misra. *Streptococcus pyogenes* showed high sensitivity against methanolic and aqueous extracts while *Pseudomonas aeruginosa* highly sensitive against methanolic extract. Growth curve and Killing time of MRSA bared that aqueous extract posse's bacteriostatic effect while oil and methanolic extract showed bactericidal effect against few microbes. *Nigella sativa* oil showed more effectiveness as least concentration is required to kill microbes.

Conclusion: From this studies we conclude *Nigella sativa* serve as a source of iron and antioxidants and provide appropriate amount of iron, according to the body need.

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INTRODUCTION

Nigella sativa is the most promising plant that rich in religious and historic background. The most famous saying of Holy Prophet (S.A.W), "Hold on to use of the black seeds, for it has a remedy for every illness except death" and the word "Hold on" represent as long term use (Saleem, 2005). He himself (S.A.W.W) used to take black seeds along with honey for therapeutic purpose (Bakathir and Abbas, 2011). *Nigella sativa* belongs to Ranunculaceae family. It is an aromatic plant harvested in Southwest Asia and Mediterranean region (Rabbani *et al.,* 2011). *Nigella sativa* contain fixed oil and volatile oil, thymoquinone, thymohydroquinone, dithymoquinone, ascorbic acid, tocopherol, oleic acid, lipase, nigellone etc, some of these have antioxidant property (Hadjzadeh *et al*., 2012; Khan, 1999). Pharmacological effects include carminative, diuretic, antifertility, antibacterial, antifungal, antiparasitic etc (Khan, 1999). Black seeds have chemopreventive effect against ferric nitrilotriacetate, responsible for oxidative stress on kidney (Khan and Sultana, 2005). Nowadays the major cause of mortality and morbidity is microbial infections (Al-Bari *et al*., 2007). Resistance among organisms are either by intrinsic or by extrinsic mechanism. (Neu, 1992) Different clinical isolates have different consequences onto the human beings like Methicillin resistant *Staphylococcus aureus* (MRSA) caused sepsis and also effect the multiple organs of the body (Kokai-Kun *et al*., 2007), *Streptococcus pyogenes* relatively caused pharyngitis, impetigo, scarlet fever etc (Nobbs, 2009), respiratory infection caused by *Pseudomonas aeruginosa* (Klevens, 2006), *Staphylococcus epidermidis* and *Bacillus subtilis* are common colonizer of the body (Rolo *et al*., 2012; Abouseouda *et al*., 2008). *Klebsiella pneumoniae* and *Escherichia coli have acquired* Carbapenem resistance and difficult to treat (Abouseouda *et al*., 2008; Patel *et al*., 2008). *Salmonella typhi* was known to cause typhoid fever (Renuka, 2005).

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Different elements including Ca, Fe, K, Se, Na etc have an important role in maintaining the physiological and biochemical processes and control disease and health (Nergiz and Otle, 1992; Siddiqi and Kan, 1990). Most of them performed their role as a cofactor but among them iron has great importance (Crowan, 1997). *Nigella sativa* is known to be a source of Ca, Fe, K, Na, Mg, Zn and Se (Khan, 1999) with highest utilization of iron from this and also been used since many years as a source of medicine (Rabbani *et al.,* 2011). The aims and objective of the current study was to examine the medicinal properties of different extract of *Nigella sativa* (Black seeds) against different microorganisms and to investigate the availability of iron and antioxidant activity.

MATERIALS AND METHODS

Plant material

Nigella sativa seeds and oil have been purchased from local market of Karachi, Pakistan.

Glass Ware, Antibiotics, Media and Chemicals

Glass wares were bought from Brosil, Wartlab and Germany. Nutrient agar, Nutrient Broth, Mueller Hinton agar and antibiotics [Gentamicin 10µg (CN), Tetracycline 30µg (TE), Oxacillin 1µg (OX), Amoxicillin 10µg (AML), Chloramphenicol 30µg (C) and Streptomycin 10µg (S)] purchased from Oxoid. Corning® filter membrane of 0.20micron and 0.45micron were used. Methanol and different salts for phosphate buffered saline (PBS) obtained from Scharlau while UV spectrophotometer of Shimadzu model- 160 was used.

Cultures

In this study microbial isolates (both gram positive and gram negative) were used. Apart of that two ATCC strains and one CMCC strain were also used including *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922 and *Bacillus subtilis* [CMCC (B) 63501]. Clinical isolates, *K. pneumoniae, M. luteus, S.epidermidis*, *S.pyogenes, Salmonella typhi* and *P.aeruginosa* were provided by First Affiliated Hospital of Zhejiang University, Hangzhou. ATCC and CMCC cultures of *S. aureus* ATCC 43300, *E. coli* ATCC 25922 and *Bacillus subtilis* [CMCC (B) 63501] were purchased commercially. Organisms were maintained on nutrient agar slants and plates at 4^{o}C. For assuring the purity of cultures, Gram staining was done.

Extract preparation

For iron estimation, 5 gm of black seeds were soaked into 100ml of 1M HCl up to 24 hours, filtered it via Whattman filter paper 1 and finally made up to 250 ml volume with distilled water. Black seed oil was filter sterilized via 0.45 micron meter filter membrane for antimicrobial activity, 60% of aqueous extract was prepared. 50% methanolic extract of black seeds were prepared by soaking 150 gram of black seeds into methanol for 8 hours then filtered by Whattman filter paper 1 followed by rotatory evaporation. Concentrated extract filter sterilized via 0.45 micron meter filter membrane for antibacterial activity.

Iron estimation of Methanolic preparation

Fe-Optimum assay was used for the estimation of iron in *Nigella sativa*. First of all iron was standardized, 0.0101 gram of ammonium iron (II) sulphate $Fe(NH_4)_2(SO_4)_2.5H_2O$ was solubilized in 100ml of distilled deionized (DI) water then few drops of concentrated sulphuric acid was added, diluted up to 250ml and mixed appropriately ($1x10^{-4}$M). 1 molar solution of sodium acetate, 0.25% solution of 1, 10 phenanthroline monohydrate and 1% of hydroquinone solution in deionized distilled water were made. Variable amount of standard solutions were transferred into 25ml volumetric flasks, sodium acetate solution was added to keep pH to 3.5± 1.0, confirmed by pH meter (model Orion 720). 2ml of 1% solution of hydroquinone was added for the retention of iron into ferrous. 2.5 ml of 1:10 of orthophenanthroline was added for complexation, into the same flask. The absorbance was checked at 515nm on spectrophotometer (Shimadzu model-160, UV- VIS). In order to analyze the content of iron in the black seed extract, same method has followed.

Reducing effect of Methanolic preparation

$Fe(NO_3)_2.9H_2O$ reduction was examined with different types of reducing agents i.e. hydroxyl ammonium chloride, hydroquinone and black seed methanolic extract. Different volumes of 0.0001 molar solutions of Fe (III) were added into the 25ml volumetric flask. To detect the reduction, same method was followed by using different reductants. For the 1st set, 2ml of 1% of Hydroquinone, for the 2nd set, 2ml of 10% of hydroxyl ammonium chloride and for the 3rd set, 5ml of black seed was used.

Inhibitory activity of aqueous preparation

Antimicrobial activity of aqueous extract of black seeds was evaluated by well diffusion and disc diffusion method by using pour plate technique. In well diffusion method, 100 µl from 35% to 60% of black seed was added into each well. In disc diffusion assay, 18µl from same concentrations was added onto discs. All the plates were incubated at 37°C overnight. Experiment was run in duplicate and repeat thrice, n=6.

MIC and MBC of aqueous preparation

10ml volume of each concentration, 35% to 55% was prepared in nutrient broth. 100µl from 10^6 CFU/ml of culture suspension in PBS was added and incubated at 37°C overnight. All the tubes were streaked over nutrient agar plates and incubated at 37°C overnight.

MILES and MISRA of aqueous preparation

Miles and Misra was performed for the sake of detection of decline in Log of CFU /ml at the concentration of 34% to 55%. For that concern, 10 fold serial dilution of each concentration was prepared in nutrient broth from 10^{-1} to 10^{-6}. Add a drop of 100 µl of each dilution onto nutrient agar plates and incubated

at 37°C overnight. Number of colonies was counted and log of CFU/ml was calculated. Experiment was run in duplicate and repeat 4 time, n=8.

Inhibitory activity of Methanolic preparation

Antimicrobial activity of methanolic extract of black seeds was evaluated by well diffusion and disc diffusion method by using pour plate technique. In well diffusion method, 0.1mg, 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg, 3.0mg, 3.5mg and 4.0mg concentrations were assessed by following the same method as in case of aqueous extract. Experiment was run in duplicate and repeat thrice, n=6.

Inhibitory activity of black seeds oil

Antimicrobial activity of black seeds oil was evaluated by well diffusion and disc diffusion method by using pour plate technique. In well diffusion method, 10%, 20%, 40%, 60%, 80% and 100% in ethylene glycol were prepared and evaluated same as aqueous extract of black seeds. Experiment was run in triplicate, n=3.

MIC and MBC of methanolic preparation and oil

0.1mg, 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg, 3.0mg, 3.5mg and 4.0mg concentrations of oil and methanol extract of black seeds were prepared in Mueller Hinton agar at the temperature of 50°C. Plates were streaked by using different culture suspensions in PBS and incubated at 37°C overnight. After that results were recorded. Experiment was run in duplicate and repeat once, n=4.

Bacterial growth curve and killing time of clinical culture MRSA

Staphylococcus aureus was grow overnight and diluted by 10 fold up to 10^{-3}. Bacterial killing time was performed by using 55% of aqueous extract of black seed in nutrient broth in which final volume was 50ml. 1% of 10 fold diluted culture was inoculated into suspension and on the other side same percentage into 50ml of nutrient broth for the evaluation of bacterial growth curve. First reading was taken at o minute and for that 1ml form suspension was serially diluted from 10^{-1} up to 10^{-6}. From each dilution 10 µl transferred onto Mueller Hinton agar and incubated at 37°C overnight. Second reading was taken by following the same method after every 30 minutes. Experiment was run in duplicate, n=2.

Statistical analysis

Statistical analysis was also performed by using MS Excel in which standard deviation; average, standard error, Pearson's correlation and regression equation were used. Pearson's correlation was used to settle Statistical relations and those values which were nearest +1 was thought to be significant. Dynamic Model Fit (DM Fit) was also used.

RESULTS

Three different preparation of black seeds (aqueous, methanolic and oil) have tested against different microbes. Purity of cultures has proved by gram staining. Five gram positive and five gram negative cultures have used. Standard antibiotic discs have used to perform antibiotic sensitivity test. Iron has detected in *Nigella sativa* methanolic extract spectrophotometrically, by following the most suitable method, Iron (II) Optimum complexation. For that concern, reducing agent has added in the accurately measured amount of sample and as a chelating agent, orthophenanthroline has added. Iron Optimum complex has prepared in buffer of pH 4.0. Calibration curve has plotted with known concentration of Iron- Optimum solution. Molar absorptivity has calculated from this curve in order to estimate the level of metal in different prepared samples. Molar absorptivity was 10,000/ M/ cm at 515 nm. 5 different samples of *Nigella sativa* methanolic extracts have also treated with Iron Optimum complex by following the same procedure at 515nm absorbance. Concentrations of metal have estimated with the help of molar absorptivity value. Mean of averages of concentration for all 5 samples of *Nigella sativa* was 133.829ppm that was as good as reported value of iron in *Nigella sativa* seeds (Table 1).

Table 2 1, 10- Orthophenanthroline (Opt) = 0.25%; Sodium acetate = 2.5M; sample extract = 5gm/100ml in 1 molar HCl diluted up to 250ml of distilled water, ε = 10,000/ M /cm, λ max= 515nm.

To determine the antioxidant activity of *Nigella sativa*, Fe (III) solution was added to methanolic extract of *Nigella sativa* along and compared with two other active biological reducing agents i.e. hydroxyl ammonium chloride and hydroquinone. Sequence of reducing power was highest in hydroquinone followed by *Nigella sativa* extract and then hydroxyl ammonium chloride (Table 2).

Table 1. Calculation of values of Fe- Opt complex for Fe (II) determination in Nigella sativa

S #	Sample/25ml	Abs.	C=A/ε[M]	mmoles	gms/5gm	gm/gm	µg/mg	Overall factor
1	2.5	0.051	0.0000051	0.0128	0.000706	0.0001412	141.194	141.194
2	5.0	0.101	0.0000101	0.0126	0.000699	0.0001398	139.809	139.809
3	7.5	0.149	0.0000149	0.0124	0.0006875	0.0001375	137.502	137.502
4	10.0	0.201	0.0000201	0.0126	0.0006956	0.0001391	139.117	139.117
5	12.5	0.251	0.0000251	0.0126	0.0006949	0.0001390	138.979	138.979
6	15.0	0.301	0.0000301	0.0125	0.0006944	0.0001389	138.886	138.886
7	17.0	0.351	0.0000351	0.0129	0.0007145	0.0001429	142.903	142.903

Table 2. Reducing activity of Nigella sativa and other reducing agents on Fe(III)

	Absorbance of Fe(III) after addition of RA			Pearson's Correlation of Fe(III) in *Nigella sativa*
S #	Fe(II) solution/ 25ml	Hydroquinone	Hydroxyl Ammonium Chloride	Sample extract
1	2.50	0.271	0.101	0.205	
2	5.00	0.424	0.211	0.357	
3	7.50	0.629	0.325	0.465	
4	10.00	0.719	0.415	0.547	0.991289
5	12.50	0.825	0.505	0.629	
6	15.00	0.920	0.595	0.711	

Table 2 - concentration of Orthophenanthroline = 0.25%, Hydroxy ammonium Chloride = 10%, Fe(III) = 0.0001 molar, Hydroquinone = 1%, Sample extract = 2ml.RA= Reducing agents. Sample extract was prepared by dissolving 5 gms in 100 ml in 1 molar HCL and diluted up to 250ml of distilled water, λ max= 515nm.

Well and disc diffusion assay have performed to evaluated inhibitory action of aqueous, methanolic preparations and oil of black seeds. MIC and MBC have calculated by broth dilution assay while Miles and Misra has also performed in case of aqueous preparation, in order to calculate CFU/ ml.

Table 3. CFU/ml Log of gram positive and gram negative isolates by performing Miles and Misra using aqueous preparation of *Nigella sativa.* Initial inoculum was Log 6. Control for each organisms also run

Microbial Isolates	Concentrations %		
	45%	50%	55%
Staphylococcus aureus	>9	>9	9.795
Staphylococcus epidermidis	>9	>9	8.963
Streptococcus pyogenes	>9	>9	>9
Bacillus subtilis	>9	>9	>9
Micrococcus luteus	>9	>9	>9
Salmonella typhi	>9	>9	9.544
Escherichia coli	>9	>9	>9
Pseudomonas aeruginosa	>9	>9	>9
Klebsiella pneumoniae	8.033	7.949	7.724

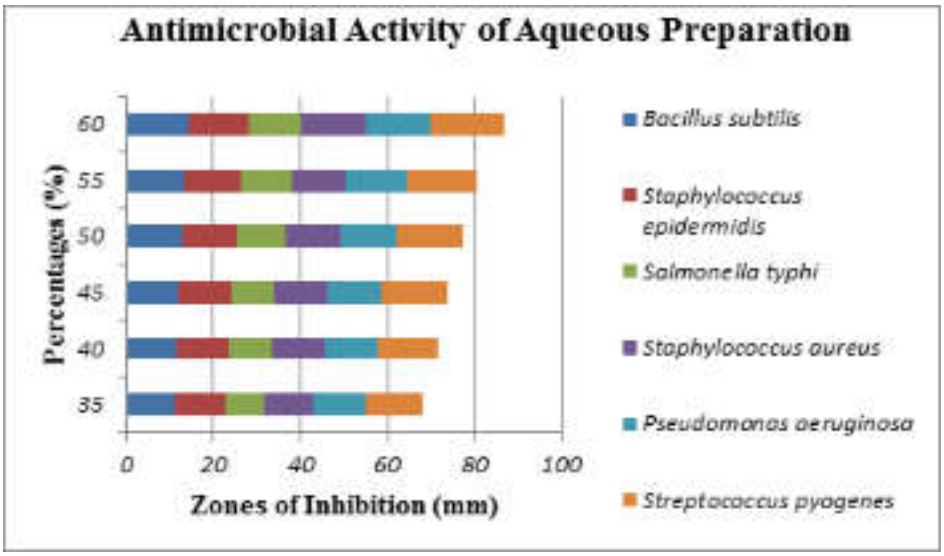

Figure 1. Antimicrobial effect of aqueous preparation of *Nigella sativa* against microbial isolates using well diffusion assay. Sample run in duplicate and repeat twice, n=4

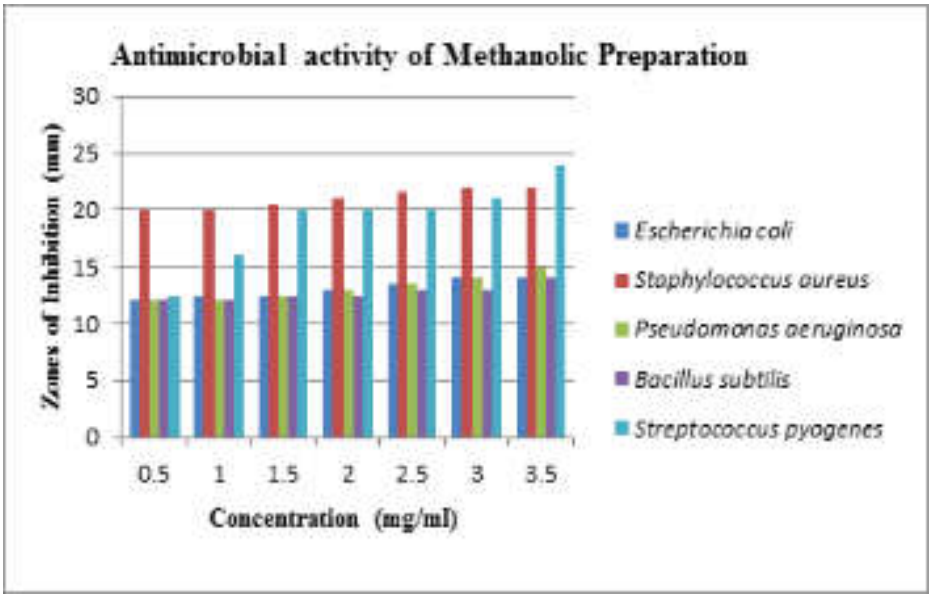

Figure 2. Antimicrobial effect of methanolic preparation of *Nigella sativa* against microbial isolates using well diffusion assay. Sample run in duplicate and repeat, n= 4

Table 4. MIC and MBC of methanolic preparation and oil of Nigella sativa against gram positive and gram negative microbial isolates

Microbial Isolates	MIC and MBC (mg/ml)			
	MIC (Oil)	MIC (Methanolic Preparation)	MBC (Oil)	MBC (Methanolic Preparation)
S. aureus	1.0	1.5	1.5	2
S. epidermidis	>4	>4	>4	>4
S. pyogenes	1	>4	1.5	>4
B. subtilis	>4	>4	>4	>4
M. luteus	1	1.5	1.5	2.0
S. typhi	>4	>4	>4	>4
E. coli	>4	>4	>4	>4
P. aeruginosa	>4	>4	>4	>4
K. pneumoniae	>4	>4	>4	>4

Table 4 The method used was agar dilution assay using 0.1mg/ml to 4.0mg/ml concentrations. Experiment run in duplicate and repeat (n= 4).

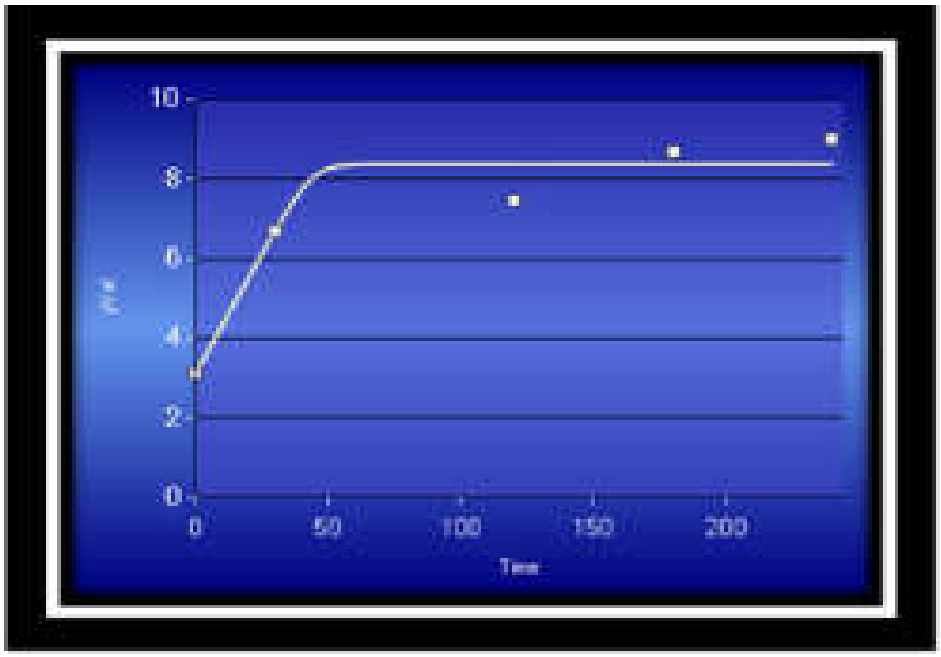

Figure 3. DM fit model of MRSA Killing time 55% concentration of aqueous preparation of *Nigella sativa*, used. Samples have taken after every 1 hour and continue up to 4 hours. Value of R^2= 0.9426. SE of Fit= 0.3325. Experiment run in duplicate (n=2)

In standard antibiotic testing assay, *Streptococcus pyogenes* and *Micrococcus luteus* have found to be sensitive to AML, C and TE while along with *Pseudomonas aeruginosa*, *Micrococcus luteus* also susceptible to C and S. AML, S and TE hadn't show any response against MRSA while OX hadn't active against any clinical isolates. *Staphylococcus epidermidis, Escherichia coli* and *Bacillus subtilis* resistant to AML and beside this *Pseudomonas aeruginosa* and *Micrococcus luteus,* to CN. In 60% aqueous preparation, among gram positives, in well diffusion method, *Streptococcus pyogenes* has showed 17.42mm i.e. maximum zones of inhibition then MRSA has given 15mm followed by *Bacillus subtilis* i.e. 14.11mm and the minimum zone has given by *Staphylococcus epidermidis* which has showed 13mm zone of inhibition. In gram negatives, *Pseudomonas aeruginosa* has given 17.41mm i.e. maximum zone of inhibition, then *Salmonella typhi,* showed 11.91mm (Figure 1) although *Escherichia coli* haven't given any zone. In disc diffusion method of aqueous preparation, within gram positives, maximum zone has given by *Bacillus subtilis* i.e. 9.5mm while MRSA has showed 8.25mm. Although among gram negatives, *Pseudomonas aeruginosa* has showed 10.91mm and *Salmonella typhi* has given 9.37mm zone of inhibition. MIC and MBC of aqueous preparation haven't observed by broth dilution assay but with the purpose of fall in CFU/ ml, Miles and Misra have performed. Within that, among gram positives, highest fall have given by *Staphylococcus epidermidis* i.e. Log 8.66, CFU/ml then Log 9.79 has given by MRSA. Other gram positives haven't given any response. Among gram negative clinical isolates, *Klebsiella pneumoniae* has given maximum fall in CFU/ml i.e. Log 7.724 then *Salmonella typhi* i.e. Log 9.54 although other gram negative haven't showed any inhibition (Table 3). In methanolic preparation, well diffusion assay analysis, largest zone has given by *Streptococcus pyogenes* i.e. 24.33mm at the concentration of 4mg/ml among gram positive, after that 22.33mm has given by MRSA, while *Bacillus subtilis* has given 14.66mm zone of inhibition. *Pseudomonas aeruginosa* has given largest zone i.e. 14.41mm among gram negative, then *Escherichia coli* has showed 13.66mm zone of inhibition (Figure 2). In disc diffusion assay analysis, 13mm zone has given by MRSA at the concentration of 4mg/ml then *Escherichia coli* has showed 10.5mm zone.

MIC for *Micrococcus luteus*, *Streptococcus pyogenes* and MRSA, in methanolic extract were 1mg/ml although 1.5mg/ml were MBC's. For other microbial isolates MIC and MBC were higher than 4mg/ml because the top figure of Methanolic extract was 4mg/ml. In *Nigella sativa* oil, MIC for *Micrococcus luteus* and MRSA were 1.5mg/ml while 2.0mg/ml was MBC. For other isolates MIC and MBC were higher than 4mg/ml because the top figure of *Nigella sativa* oil was 4mg/ml (Table 4). In MRSA growth curve steady increase in CFU/ml Log value was seen, initiate from Log 3 and after 4 hour sampling, increased up to Log 9. Once applied Dynamic (DM) fit model, value for R^2 =0.873 and SE of fit = 0.81828. In killing time, steady decline in CFU/ml Log value was seen in comparison to control. Initiate form Log 3, even if CFU/ml Log was increased but not higher than Log6 in comparison to control. Once applied dynamic (DM) fit model, value of R^2= 0.9426 and SE of Fit = 0.3325 (Figure 3).

DISCUSSION

In antibiotic sensitivity assay it was cleared that all the clinical isolates, which have used during this research were resistant to OX so that mean *Staphylococcus aureus* was MRSA. MRSA is very frequent and severe risk of illness and cause high rate of mortality just because of be deficient in sensitivity of available effective drug. The recent research has showed the quite visible anti MRSA activity even in aqueous form of *Nigella sativa*. According to previous research, vancomycin is a last drug of choice against MRSA. In 2002, resistant strains of *Staphylococcus aureus,* were also reported, USA (Khan and Sultana, 2005). Presence of iron in *Nigella sativa* has determined by colorimetric and atomic absorption Spectrophotometric method (Siong *et al*., 1989). Body needs iron in a little amount but such amount is essential as well. Calculated amount of iron into body is 140ppm (Duke, 1992). Ferrous and ferric are two different existing forms of iron into body but ferrous is utilizable form (Iffat *et al*., 2004, 2005). *Nigella sativa* contains both forms of iron along with reductants. Reducing agents serve as an iron provider, it keep the iron in utilizable form i.e. ferrous. Recent study discovered that *Nigella sativa* in aqueous form possessed bacteriostatic effect against clinical isolates that was comparable to reported data. Considerable inhibitory zones have observed at 60% concentration of aqueous extract in well diffusion method. At the same concentration, opportunely a considerable decline in CFU/ml Log has observed as compared to control but not least as compared to initial inocula i.e. Log 6 (Table 3). This proved that aqueous extract of *Nigella sativa* can decelerate the growth rate of bacteria but not kill them. The current study also revealed that *Streptococcus pyogenes* is more susceptible to aqueous form of *Nigella sativa* in comparison to other isolates (Figure 2). Among gram negative, *Pseudomonas aeruginosa* is more sensitive to aqueous extract even more than the *Bacillus subtilis* and MRSA gram positives (Figure 2) but *Escherichia coli* and resistant to aqueous extract. *Staphylococcus epidermidis,* in Miles and Misra showed the significant decline but in well diffusion method, smallest inhibitory zone has given that just because of improper diffusion of extract and organism over grow. So that results is more beneficent as previous data haven't given such type of *Staphylococcus epidermidis* inhibition.

Methanolic extract possess more antimicrobial activity that the aqueous preparation. *Streptococcus pyogenes* has given higher inhibitory zone against methanolic extract afterward MRSA and least by *Bacillus subtilis.* Among gram negative clinical isolates, *Pseudomonas aeruginosa* has given maximum inhibitory zone. At that time of experiment, inhibitory zones were not larger than the MRSA inhibitory zones against aqueous extract. Significant zones have also observed in the presence of *Escherichia coli*, against methanolic preparation of *Nigella sativa*. Well diffusion assay have performed to confirm the antimicrobial activity of *Nigella sativa* oil. Only some concentrations were examined but not recorded. MIC and MBC of *Nigella sativa* oil and methanolic preparation have examined. *Micrococcus luteus, Streptococcus pyogenes* and MRSA have given same value of MIC and MBC. Other clinical isolates were resistant to that. *Micrococcus luteus* and MRSA have given same inhibitory pattern against *Nigella sativa* oil. In accordance with result, it has proved that *Nigella sativa* oil has more antimicrobial activity than methanolic extract; against clinical isolates because a little concentration of oil is require to inhibit organisms in comparison to methanolic preparation. Interestingly, *Streptococcus pyogenes* has showed sensitivity against methanolic preparation while resistant against *Nigella sativa* oil.

MRSA killing time showed that *Nigella sativa* aqueous preparation can slow down the growth rate of clinical isolates because after four hours of incubation, considerable reduction in colony count has seen as compared to control (Figure 3).

Conclusion

From the above research we can conclude *Nigella sativa* serve as a source of iron and antioxidants and help to provide appropriate amount of iron, according to the body need. In antimicrobial study it was clear that *Streptococcus pyogenes* and *Pseudomonas aeruginosa* sensitive to aqueous and methanolic preparation of *Nigella sativa*. Against methanolic extract, *Pseudomonas aeruginosa* is more sensitive than MRSA. *Nigella sativa* needs little amount to inhibit organisms as compared to other preparations. *Nigella sativa* is actually the priceless medicine by nature.

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