Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 02, pp.12294-12299, February, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT, METHANOLIC EXTRACT AND OIL OF *NIGELLA SATIVA* (KALONJI) AGAINST GRAM POSITIVES AND GRAM NEGATIVES CLINICAL ISOLATES

[1]Komal Anjum, [1]Tanveer Abbas, [2]Mohib Raza Kazimi, [1]Syed Abdus Subhan, [1]Abdul Wahab, [1]Nayyar Mehmood, [1]Talat. Y. Mujahid and [1]Najmul Hasan

[1]Food Safety Research Group, Department of Microbiology, University of Karachi, Karachi 75270, Pakistan
[2]Department of Applied Chemistry, University of Karachi, Karachi 75270, Pakistan

ARTICLE INFO

Article History:
Received 27th November, 2014
Received in revised form
08th December, 2014
Accepted 05th January, 2015
Published online 26th February, 2015

Key words:
Nigella Sativa (Kalonji),
Antimicrobial activity.

ABSTRACT

Nigella sativa seed (Black cumin seed; Kalonji) was used since many years as medicinal product for the treatment of many infections. In the present study antibacterial effect of aqueous extract of seeds was studied against few of clinical isolates including both gram positive and gram negative species. Results were also compared with standard drugs. Most of the drugs were found to be non effective against isolates. Interestingly, during the recent study all isolates were resistant to oxacillin. Zones of inhibition were evaluated by disc diffusion and well diffusion method in all three preparation of *Nigella sativa*. Minimum inhibitory concentration was also determined by broth dilution method (aqueous extract) and agar dilution method (methanolic extract and Kalonji oil). Miles and Misra also performed and Log of cfu/ml calculated in aqueous preparation. *Streptococcus pyogene* found to be more sensitive to both aqueous and methanolic extract while among gram negatives *Pseudomonas aeruginosa* given larger zones of inhibition in the presence of methanolic extract, even in comparison of all gram positives. Killing time and growth curve of *Staphylococcus aureus* revealed that aqueous extract have bacteriostatic rather bactericidal activity while methanolic extract and oil given bactericidal effect against few isolates but Kalonji oil found to be more effective as it required least concentration to kill the organisms.

INTRODUCTION

The microbial infection is the major cause of morbidity and mortality throughout the world (**Fram *et al.*, 2014**). Presently organisms acquiring resistance my multiple ways against available antibiotics. Intrinsic resistance represents the inherited while acquired means secondary to other disease conditions i.e. because of change in the genetic makeup of pathogens. Some strategies which used by pathogens to acquire resistance include; limitation of intracellular concentration of antimicrobial drug by reduce the influx and accelerate the efflux mechanisms, alteration of the target site of drug situated over pathogen or to remove the target site but acquiring the alternative metabolic pathway (**Neu, 1992**). Misuse of antibiotics also responsible for resistance among pathogens (**Fram *et al.*, 2014**). The most common disease caused by methicillin resistant *Staphylococcus aureus* (MRSA) is sepsis. Multiple organs are affected by MRSA including skin, kidney and other organs, responsible to create life threatening conditions (Kokai-Kun *et al.,* 2007). *Streptococcus pyogenes* (group A streptococci) responsible for pharyngitis, tonsillitis, scarlet fever, otitis media, impetigo, necrotizing fasciitis and cellulitis (**Nobbs *et al.*, 2009**). *Pseudomonas aeruginosa* responsible to cause respiratory tract infection, type III secretary proteins contribute toward this infection (**Garau and Gomez, 2003**). *Staphylococcus epidermidis* is a common colonizer of different body sites in both human and animals (**Rolo *et al.*, 2012**). *Bacillus subtilis* able to produce biosurfactants when grown over oily surfaces and help in the adherence (**Abouseoud *et al.*, 2008**). Endospore forming *Bacillus subtilis* found to be produced more than two dozen of different varieties of antibiotics, peptide antibiotics was the predominant class (**Khan *et al.*, 2003**). Carbapenem resistant strains of *K pneumoniae* found to increase in proportion, major cause of different health related problem and high rate of mortality (**Patel *et al.*, 2008**). *A multidrug resistant* E. coli *posse's* bla NDM-1 metallo-β-lactamase gene which create resistant to Carbapenem (**Poirel *et al.*, 2010**). *Salmonella typhi* responsible to cause typhoid fever (**Renuka *et al.*, 2005**). Practices of using the herbsous plant as a medicinal use in ancient time also exist in this era, particularly in the rural areas of Pakistan as it provided the additional income support (**Rabbani *et al.*, 2011**).

The most promising plant is *Nigella sativa* with rich historical and religious background. The most famous saying of Holy

Corresponding author: Tanveer Abbas,
Department of Microbiology, Faculty of science, University of Karachi, 75270, Pakistan.

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 (**Salem, 2005**). He (SAW) himself used to take black seeds with honey syrup for therapeutic purpose (**Bakathir and Abbas, 2011**). Ranunculaceae is the family to which *Nigella sativa* belongs to. *Nigella sativa* is an aromatic plant naive to Southwest Asia and Mediterranean region. This specie is short lived, approximately 20 to 60cm in height with a grayish green linear leaves that are wispy and thread like (**Rabbani *et al.*, 2011**). *Nigella sativa* contain fixed oil and volatile oil, protein, alkaloid and saponins. Volatile oil contains 18.4% to 24% Thymoquinone and 43% monoterpenes like pcymene and a-piene **(Hadjzadeh *et al.*, 2012**).

Pharmacological effects of *Nigella sativa* seed involved anti inflammatory, anti parasitic, anti bacterial, anti fungal, anti cancer etc (**Khan, 1999**). Other therapeutic benefits include; relieve from bronchial asthma, dysentery, headache, gastrointestinal problem, eczema, hypertension, obesity etc (**Padhye *et al.*, 2008**). *Nigella sativa* also found to reduce the cisplatin induced toxicity (**Nematbakhsh and Re, 2013**). The organic fraction of *Nigella sativa* exhibits bronchodilator and spasmolytic properties by blacking the calcium channel in vivo, it is useful in diarrhea and asthma (**Gilani *et al.*, 2001**). Seeds of *Nigella sativa* have chemopreventive effect against ferric nitrilotriacetate (Fe-NTA), which is responsible for oxidative stress on kidney (**Andrews, 2001**). According to ***Salomi et al.,*** crude methanolic extract of *Nigella sativa* have strong cytotoxic activity on Erlich ascites carcinoma development, sarcoma 180 cells and Dalton's ascites lymphoma while least activity on normal human lymphocytes **(Khan and Sultana, 2005**). The methanol soluble portion of *Nigella sativa* represents the inhibitory effects on platelet aggregation and blood coagulation induced by arachidonic acid (AA) (**Khan *et al.*, 2005)**. The *Nigella sativa* oil has anti-inflammatory, antineoplastic, analgesic, antimicrobial and antipyretic activity. Oil reduces the blood pressure and increases the respiration. Oil can also increases the packed cell volume (PCV), and hemoglobin, and reduces the concentration of cholesterol, triglycerides, and glucose in plasma. The seeds have the low toxicity. Seeds found to represent least adverse effect on liver and kidney on human body **(Abouseoud *et al.*, 2008**). *Nigella sativa* oil also have anticancer, antiradical, antidiabetic, immunomodulatory, bronchodilator, heptoprotective, antihypertensive and renal protective activities **(Ramadan, 2007**). In diabetic patients *Nigella sativa* decrease the malondialdehyde (MDA) and glucose level and increase the level of glutathione (GSH) and ceruloplasmin and prevented liver damage by lipid peroxidation in diabetic patients (**Khan *et al.*, 2003**). *Nigella sativa* oil possesses anticestode and antinematode actions (**Mahmoud *et al.*, 2002**).

Thymoquinone, an active ingredient of *Nigella sativa* have anti tumor, anti proliferative, pro- apoptotic activities on cell lines derived from colon, larynx, lung, ovary, breast etc **(Rooney and Ryan, 2005**). Thymoquinone also proved to be used for adoptive T-cell therapy against cancer and infectious disease (Tesarova *et al.,* 2011). The objective of the present study was to evaluate the antimicrobial activity of aqueous extract, methanolic extract and oil of *nigella sativa* (Kalonji) against few gram positives and gram negatives clinical isolates.

MATERIALS AND METHODS

Plant material

Nigella sativa seeds and oil have been purchased from local herb store of Karachi.

Glass ware, Chemicals, Media and Antibiotics

Glass wares were purchased from Wartlab, Germany and Brosil. Media (Nutrient agar, Mueller Hinton agar, Nutrient Broth) and antibiotics (Oxacillin 1µg (OX), Tetracycline 30µg (TE), Gentamicin 10µg (CN), Chloramphenicol 30µg (C), Amoxicillin 10µg (AML) and Streptomycin 10µg (S)) obtained from Oxoid. 0.20micron and 0.45micron filter membrane corning® used. Methanol (organic solvent as diluent) and different salts for phosphate buffered saline (PBS) obtained from Scharlau.

Clinical isolates

Clinical isolates have been received from Darul Sehat Hospital, Karachi, Pakistan and from Microbiology Department, University of Karachi, Karachi, Pakistan. Clinical isolates which was used in that study: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa strain 1, Pseudomonas aeruginosa strain 2, Klebsiella pneumoniae, Escherichia coli, Micrococcus luteus and Salmonella typhi.*

Preparation of aqueous extract of *Nigella sativa* (Kalonji)

60% of aqueous extract of Kalonji have been prepared for that concerned 60 grams of Kalonji have been weighed, in a boiled water added weighed Kalonji and heat for 1 minute then removed the flask from flame, gave the rest to flask for 2 minutes and repeat the process for 6 times. Filter the extract by whattman filter paper 1 and filter sterilized the extract by passing it through 0.45µm pore size filter. keep it in refrigerator at 4°C until used.

Preparation of methanolic extract of *Nigella sativa* (Kalonji)

50% methanolic extract of Kalonji have been prepared, for that concerned 150 gram of weighed Kalonji soaked in 300 ml of absolute methanol for 8 hours then filter the content by whattman filter paper 1 and keep the extract in an air tight bottle.

Rotatory evaporation

Methanol filtered extract have been concentrated through rotator evaporator. Concentrated extract keep in wide mouth bottle and left over night in order to get completely dry extract. Passed the extract through 0.45micron pore size filter.

Nigella sativa oil

Nigella sativa have been purchase from local market and passed it through 0.45µm pore size filter in order to get sterilized oil.

Well diffusion assay of aqueous extract of *Nigella sativa* (Kalonji)

Well diffusion method of aqueous extract of *Nigella sativa* (Kalonji) have been performed in order to detect the antimicrobial activity, for that concerned different concentrations of aqueous extract have been made i.e. 35%, 40%, 45%, 50%, 55% and 60%. Pour plate method have been followed in which 100µl of each concentration in each well, all the plates were incubated at 37°C for 24 hours and zones were measured. Experiment was done in triplicate and repeat so n=6.

Disc diffusion assay of aqueous extract of *Nigella sativa* (Kalonji)

Same concentration of aqueous extract used as in well diffusion assay. Pour plate method have been followed in which 18µl of each concentration was in each filter paper disc, all the plates were incubated at 37°C for 24 hours and zones were measured. Experiment was done in duplicate and repeat so n=4.

Minimum inhibitory concentration (mic) of aqueous extract of *Nigella sativa* (Kalonji) by tube broth dilution method

A total of 10ml of volume used in which different concentration of aqueous extract of Kalonji i.e. 35%, 40%, 45%, 50%, and 55% have been prepared in nutrient broth. 100µl of 10^6 cfu/ml of freshly prepared culture suspension in PBS added in each tube and all the tubes were incubated at 37°C for 24 hours.

Minimum bactericidal concentration (mbc) of aqueous extract of *Nigella sativa* (Kalonji)

All the MIC's tubes incubated for 24 hours and streaked over nutrient agar plate. Plates were incubated at 37°C for 24 hours.

Miles and Misra

In order to detect the decline in Log of cfu/ml at each concentration i.e. 35%, 40%, 45%, 50% and 55% Miles and Misra was performed. To precede that method 10 fold serial dilution of each concentration have been prepared in nutrient broth from 10^{-1} upto 10^{-6}. Put a drop of 100µl from each dilution over nutrient agar plates then incubated at 37°C for 24 hours and next day count the number of colonies and calculated Log of cfu/ml.

Well diffusion assay of methanolic extract of *Nigella sativa* (Kalonji)

Different concentrations of methanolic extract have been prepared i.e. 0.1mg, 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg, 3.0mg, 3.5mg and 4.0mg. Same method has been followed as in well diffusion of aqueous extract. Experiment was performed in duplicate and repeat n=4.

Disc diffusion assay of methanolic extract of *Nigella sativa* (Kalonji)

Same concentrations of methanolic extract used as in well diffusion assay. Same method has been followed as in disc diffusion of aqueous extract. Experiment was performed in duplicate and repeat n=4.

Well diffusion assay of *Nigella sativa* (Kalonji) oil

Different concentrations of Kalonji oil have been prepared i.e.100%, 80%, 60%, 40%, 20% and 10% in ethylene glycol. Same method have been followed as in well diffusion of aqueous extract. Experiment run in triplicate n=3.

Disc diffusion assay of *Nigella sativa* (Kalonji) oil

Same concentration of Kalonji oil used as in well diffusion assay. Same method has been followed as in disc diffusion of aqueous extract. Experiment run in triplicate n=3.

Minimum inhibitory concentration (mic) and minimum bactericidal concentration (mbc) of methanolic extract and oil of *Nigella sativa* (Kalonji) by agar dilution method

Different concentrations of methanolic extract of Kalonji and oil i.e. 0.1mg, 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg, 3.0mg, 3.5mg and 4.0mg prepared by using liquefy Mueller Hinton agar at 50°C. All the plates were kept in refrigerator for 30 minutes in order to completely solidify. Then different suspensions of all clinical isolates were streaked over each concentration of methanolic extract and Kalonji oil plate. Plates were incubated at 37°C for 24 hours. Next day results were recorded.

Growth curve and killing time of *Staphylococcus aureus* (clinical isolate)

Overnight culture of *Staphylococcus aureus* diluted by 10 fold serial dilution in nutrient broth upto 10^{-3}. For killing time 55% concentration of aqueous extract of *Nigella sativa* prepared in nutrient broth and final volume was 50ml then 1% of 10 fold serially diluted culture was added in that suspension and same percentage of culture in 50ml of nutrient broth for growth curve and first reading taken at 0 min in which 1ml of sample have been collected and made a 10 fold serial dilution from 10^{-1} upto 10^{-6}. Then from each dilution 10µl inoculated onto the Mueller Hinton agar plate then incubated at 37°C for 24 hours. Immediately after taken the first sample at 0 minute, bottle was incubated at 37°C and second reading was taken by following the same method and process have followed for 4 hours in both cases. Experiment run in duplicate n=2.

Statistical analysis

In statistical analysis was also performed by using MS Excel software. Statistical relations were determine by Pearson's correlation and value nearest the +1 considered as significant. Dynamic model fit (DM fit) also concerned.

RESULTS

A total of ten different clinical isolates have been investigated against three different forms of *Nigella sativa* (Kalonji) seeds i.e. aqueous extract, methanolic extract and Kalonji oil. First of all purity of all clinical isolates were check by performing simple gram staining. Collectively 5 gram positive and 5 gram negative isolates used. Standard antibiotic susceptibility testing

also performed by using antibiotic discs. Antimicrobial activity of aqueous extract, methanolic extract and oil have been determined my well diffusion and disc diffusion. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all three forms also investigated. In case of aqueous extract drop in Log of cfu/ ml also calculated by Miles and Misra method. In standard antibiotic susceptibility testing it was observed that *Micrococcus luteus* and *Streptococcus pyogenes* sensitive to TE, AML and C. *Pseudomonas aeruginosa* and *Micrococcus luteus* sensitive to S and C. MRSA resistant to TE, AML and S. All the isolates found to be resistant to OX. While *Escherichia coli*, *Staphylococcus epidermidis, Bacillus subtilis* resistant to S and AML and along with *Micrococcus luteus* and *Pseudomonas aeruginosa* resistant to CN. In well diffusion assay in case of aqueous preparation of *Nigella sativa* at 60% concentration, *Streptococcus pyogene* found to be given maximum zone of inhibition i.e. 17.42mm followed by MRSA i.e. 15mm then *Bacillus subtilis* i.e. 14.11mm, and the least zones of inhibition were given by *Staphylococcus epidermidis* i.e. 13mm among gram positive. In case of gram negatives *Pseudomonas aeruginosa* strain 1 gave the maximum zones of inhibition i.e. 17.41mm followed by *Salmonella typhi* i.e. 11.91mm while no zones were given by *Escherichia coli* and *Pseudomonas aeruginosa* strain 2.

In case of disc diffusion assay of aqueous extract of *Nigella sativa*, among gram positives largest zone given by *Bacillus subtilis* i.e. 9.5mm and for MRSA it was 8.25mm. While among gram negatives *Pseudomonas aeruginosa* strain 1 given a zone of 10.91mm and for *Salmonella typhi* it was 9.37mm.
MIC and MBC of aqueous extract of *Nigella sativa* were not observed but in order to calculate the drop in the Log of cfu/ml, Miles and Misra have been performed. In that method among gram positives, *Staphylococcus epidermidis* found to have maximum drop in the Log of cfu/ml i.e. Log 8.96 followed by MRSA i.e. Log 9.79 while other gram positives didn't able to inhibit. In case of gram negative *Klebsiella pneumoniae* found to be given maximum decline i.e. Log 7.724 followed by *Salmonella typhi* i.e. Log 9.54 while other gram negatives didn't inhibit. In well diffusion assay of methanolic extract among gram positives maximum zones were given by *Streptococcus pyogenes* i.e. 24.33mm at 4mg/ml concentration followed by MRSA i.e. 22.33mm and for *Bacillus subtilis* it was 14.66mm. In case of gram negatives, maximum zones were given by *Pseudomonas aeruginosa* strain 1 i.e. 14.41mm followed by *Pseudomonas aeruginosa* strain 2 i.e. 14.25mm while in *Escherichia coli* it was 13.66mm. In disc diffusion assay of methanolic extract MRSA gave 13mm zones of inhibition at 4mg/ml concentration followed by *Pseudomonas aeruginosa* strain 2 i.e. 11.5mm and for *Escherichia coli* it was 10.5mm zones were measured.

Disc susceptibility testing of kalonji extract (aqueous) against gram positive clinical isolates, represents average zones of inhibitions in mm ± standard deviation

Clinical isolates	Percentages %						Pearson's correlation*
	35%	40%	45%	50%	55%	60%	
B. subtilis	7.166±	7.666±	8.5±	8.833±	9.083±	9.5±	0.984**
	0.288	0.381	0.661	1.010	0.877	1	
S. aureus	6.75±	7.0±	7.25±	7.75±	8.0 ±	8.25±	0.9983
	0.621	0.353	0.530	0.707	0.144	0.803	

Table Antimicrobial activity of Kalonji extract (aqueous) against gram positive clinical isolates by Disc Diffusion using 6mm disc, 18µl volume

SD= standard deviation. *Correlation between log dose and size of zones. All values are significant in Pearson's correlation. Experiment run in triplicate and repeat (n=6).

Disc susceptibility testing of kalonji extract (aqueous) against gram negative clinical isolates, represents average zones of inhibitions in mm ± standard deviation

Clinical isolates	Percentages (%)						Pearson's correlation *
	35%	40%	45%	50%	55%	60%	
P. aeruginosa	8.83± 0.520	9.333± 0.381	9.833± 0.629	9.916± 1.010	9.916± 0.144	10.916± 0.803	0.934
S typhi	6.75± 0.353	7.375± 0.530	7.75± 0.707	8.375± 0.883	8.375± 0.530	9.375± 0.530	0.971

In methanolic extract MIC's for MRSA, *Streptococcus pyogenes* and *Micrococcus luteus* were 1mg/ml while MBC's were 1.5mg/ml while for other isolates MIC's and MBC's values were greater than 4mg/ml, as the upper limit of Methanolic extract was 4mg/ml. In Kalonji oil MIC's for MRSA and *Micrococcus luteus* were 1.5mg/ml while MBC's were 2.0mg/ml while for other isolates MIC's and MBC's values were greater than 4mg/ml, as the upper limit of Kalonji oil was 4mg/ml.

Minimum inhibitory concentration (mic) and minimum bactericidal concentration (mbc) of methanolic extract of kalonji against gram positive and gram negative isolates

Clinical isolates	MIC(mg/ml)	MBC(mg/ml)
Staphylococcus aureus	1.0	1.5
Staphylococcus epidermidis	>4	>4
Streptococcus pyogenes	1.0	1.5
Bacillus subtilis	>4	>4
Micrococcus luteus	1.0	1.5
Salmonella typhi	>4	>4
Escherichia coli	>4	>4
Pseudomonas aeruginosa	>4	>4
*Pseudomonas aeruginosa**	>4	>4
Klebsiella pneumoniae	>4	>4

Table 3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of Kalonji against gram positive and gram negative isolates by agar dilution method. Using 0.1mg/ml to 4.0mg/ml concentrations, sample run in duplicate and repeat (n=4).

In growth curve of MRSA gradual increase in the Log of cfu/ml were observed i.e. start from Log 3 and increased upto 9 after 4 hours sample. After applying Dynamic Model (DM) fit model value of R^2 was 0.873 and SE of Fit was 0.8182.

In killing time of MRSA gradual decline in Log of cfu/ml were observed as compared to control. Start from Log 3, although Log of cfu/ml increase but not more than Log 6 as compared to control. After applying Dynamic Model (DM) fit model value of R^2 was 0.9426 and SE of Fit was 0.3325.

DISCUSSION

In antibiotic susceptibility testing, according to Clinical and Laboratory Centre Institute (CLSI) document it was observed that all the used clinical isolates resistant to oxacillin so it was cleared that *Staphylococcus aureus* actually methicillin resistant i.e. methicillin resistant Staphylococcus aureus (MRSA) which is now a day a quite common and serious threat of disease and responsible for high mortality rate because of lack of availability of medicine but in present study MRSA found to be sensitive even by aqueous extract.

Our present study revealed that aqueous extract of *Nigella sativa* has bacteriostatic activity against clinical isolates rather bactericidal. In well diffusion assay a significant zones of inhibition were observed at 60% concentration. At 60%, fortunately a significant decline in Log of cfu/ml were observed as compared to control but not as compared to initial inoculum i.e. Log 6. It indicated that, that concentration of aqueous extract able to slow down the multiplication of clinical isolates but not completely kill them because in control (free of extract), after overnight incubation colony count were Log >9 as compared to test (containing extract) in which after overnight count were Log <9. So it proved that aqueous extract of *Nigella sativa* (Kalonji) have bacteriostatic effect rather bactericidal. In that study it was found out that *Streptococcus pyogenes* more sensitive to aqueous extract as compared to other gram positives while in gram negatives *Pseudomonas aeruginosa* more sensitive, even in comparison of MRSA and *Bacillus subtilis* gram positives but *Pseudomonas aeruginosa* strain 2 and *Escherichia coli* resistant to aqueous extract. So in aqueous extract even gram negative isolates also significantly inhibited. In Miles and Misra, a maximum decline in Log of cfu/ml were given by *Staphylococcus epidermidis* but in well diffusion assay least zones were given by that organism, the reason behind that extract didn't able to properly diffused at that point and *Staphylococcus epidermidis* over grow.

In methanolic preparation of *Nigella sativa* (Kalonji) again *Streptococcus pyogene* given maximum zones of inhibition followed by MRSA then *Bacillus subtilis* among gram positives as in case of aqueous extract and in gram negatives again maximum zones were given by *Pseudomonas aeruginosa* strain 1 but at those time zones were not larger than the zones given by MRSA in the presence of aqueous extract. It indicated that MRSA more sensitive to methanolic extract as compared to aqueous preparation. Even *Pseudomonas aeruginosa* strain 2 and *Escherichia coli* also given significant zones in the presence of methanolic extract. In order to check whether *Nigella sativa* (Kalonji) oil posses antimicrobial activity or not, a few concentrations were examined by well diffusion assay and zones were observed but not recorded because we didn't want to examined that whether oil was more active as compared to aqueous extract or methanolic preparation.

MIC and MBC of methanolic extract and oil were determined in which MRSA, *Streptococcus pyogenes* and *Micrococcus luteus* found to be given same level of MIC and MBC while other strains resistant to methanolic preparation while in Kalonji oil MRSA and *Micrococcus luteus* were inhibited at same concentration. But according to results it was clear that Kalonji oil was more active against clinical isolates as compared to methanolic preparation because a least concentration was required for inhibition as compared methanolic extract. Although *Streptococcus pyogenes* didn't able to inhibited by Kalonji oil but found to be sensitive to methanol preparation. Killing time of MRSA proved that aqueous extract of *Nigella sativa* (Kalonji) able to ceased down the growth of clinical isolate because as compared to control/ growth curve after four hours of incubation a significant reduction in the colony count was observed.

Conclusion

The overall conclusion of our present study was that aqueous extract and methanolic extract of *Nigella sativa* more effective to *Streptococcus pyogenes* even *Pseudomonas aeruginosa* able to given a significant inhibition in the presence of aqueous extract. *Pseudomonas aeruginosa* found to be more sensitive to methanolic extract even in comparison of MRSA and other gram positives and Kalonji oil required a least concentration in order to completely inhibit the growth as compared to methanolic extract.

REFERENCES

Abouseoud, M., Maachi, R., Amrane, A., Boudergua, S. and Nabi, A. 2008. Evaluation of different carbon and nitrogen sources in production of biosurfactant by Pseudomonas fluorescens. *Desalination,* 223(1): 143-51.

Andrews, J.M. 2001. Determination of minimum inhibitory concentrations. *J. Antimicrob Chemother*, 48(1): 5-16.

Bakathir, H.A. and Abbas, N.A. 2011. Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. *Afr J Tradit Complement Altern Med,* 8(2): 159-64.

Fram, D., Taminato, M., Ponzio, V, *et al*. 2014. Risk factors for morbidity and mortality of bloodstream infection in patients undergoing hemodialysis: a nested case-control study. *BMC Res Notes,* Dec 7; 7(1): 882.

Garau, J. and Gomez, L. 2003. Pseudomonas aeruginosa pneumonia. *Curr Opin Infect Dis.,* 16(2): 135-43.

Gilani, A., Aziz, N., Khurram, I., Chaudhary, K. and Iqbal, A. 2001. Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): a traditional herbal product with multiple medicinal uses. *J. Pak Med Assoc.,* 51(3): 115-20.

Hadjzadeh, M.A., Keshavarzi, Z., Yazdi, S.A.T., Shirazi, M.G., Rajaei, Z. and Rad, A.K. 2012. Effect of Alcoholic Extract of *Nigella sativa* on Cisplatininduced Toxicity in Rat. *Iranian J. Kidney Dis.,* 6(2): 99-104.

Khan, M., Ashfaq, M., Zuberi, H., Mahmood, M. and Gilani, A. 2003. The in vivo antifungal activity of the aqueous extract from *Nigella sativa* seeds. *Phytother Res.,* 17(2): 183-6.

Khan, M.A. 1999. Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology,* 7(1): 15-35.

Khan, N. and Sultana, S. 2005. Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*. *Eur J. Cancer Prev.,* 14(2): 159-68.

Kokai-Kun, J.F., Chanturiya, T. and Mond, J.J. 2007. Lysostaphin as a treatment for systemic Staphylococcus aureus infection in a mouse model. *J. Antimicrob Chemoth.,* 60(5): 1051-9.

Mahmoud, M., El-Abhar, H. and Saleh, S. 2002. The effect of< i> *Nigella sativa*</i> oil against the liver damage induced by Schistosoma mansoni infection in mice. *J. Ethnopharmacol.,* 79(1): 1-11.

Nematbakhsh, M. and Re, N. 2013. Effect of alcoholic extract of *Nigella sativa* on cisplatin-induced toxicity in rat. *Iran J. Kidney Dis.,* 7: 165-6.

Neu, H.C. 1992. The crisis in antibiotic resistance. Science. 257(5073): 1064-73.

Nobbs, A.H., Lamont, R.J. and Jenkinson, H.F. 2009. Streptococcus adherence and colonization. *Microbiol Mol Biol Rev.,* 73(3): 407-50.

Padhye, S., Banerjee, S., Ahmad, A., Mohammad and Rand Sarkar, F.H. 2008. From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. *Cancer Ther.,* 6(b): 495-510.

Patel, G., Huprikar, S., Factor, S.H., Jenkins, S.G. and Calfee, D.P. 2008. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Cont Hosp Ep., 29(12): 1099-106.

Poirel, L., Lagrutta, E., Taylor, P., Pham, J. and Nordmann, P. 2010. Emergence of metallo-β-lactamase NDM-1-producing multidrug-resistant Escherichia coli in Australia. *Antimicrob Agents Chemoth.,* 54(11): 4914-6.

Rabbani, M.A., Ghafoor, A. and Masood, M.S. 2011. NARC-kalonji: an early maturing and high yielding variety of *Nigella sativa* released for cultivation in Pakistan. *Pak. J. Bot.,* 43: 191-5.

Ramadan, M.F. 2007. Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. *Int J. Food Sci Technol.,* 42(10): 1208-18.

Renuka, K., Sood, S., Das, B.K. and Kapil, A. 2005. High-level ciprofloxacin resistance in Salmonella enterica serotype Typhi in India. *J. Med Microbiol.,* 54(10): 999-1000.

Rolo, J., de Lencastre, H. and Miragaia, M. 2012. Strategies of adaptation of Staphylococcus epidermidis to hospital and community: amplification and diversification of SCCmec. *J. Antimicrob Chemoth.,* 67(6): 1333-41.

Rooney, S. and Ryan, M. 2005. Effects of alpha-hederin and thymoquinone, constituents of *Nigella sativa*, on human cancer cell lines. *Anticancer Res.,* 25(3B): 2199-204.

Salem, M.L. 2005. Immunomodulatory and therapeutic properties of the< i> *Nigella sativa*</i> L. seed. *Int Immunopharm.,* 5(13): 1749-70.

Tesarova, H., Svobodova, B., Kokoska, L. *et al*. 2011. Determination of oxygen radical absorbance capacity of black cumin (*Nigella sativa*) seed quinone compounds. *Nat Prod Commun.,* 6(2): 213-6.

****