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Original Research Article

Alteration in Antibacterial Potential of *Nigella sativa* L. Seed during Different Phases of Germination

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A B S T R A C T

An alarming increase in bacterial strains resistant to existing antimicrobial agents demands a renewed effort to seek agents effective against pathogenic bacteria resistant to current antimicrobials. Different extracts of successive stages of *Nigella sativa* Linn. was studied for antibacterial activity against various bacteria resistant to a number of antibiotics, in varying concentrations by Agar well diffusion technique on nutrient agar plates. The extracts showed pronounced dose or day dependent antibacterial activity against Gram positive and Gram negative bacteria. Out of 5 strains tested, most of which were resistant to a number of antibiotics, were inhibited by the extracts of black cumin, Gram positive *Staphylococcus epidermidis* (NCIM 2493) was sensitive to the various extracts . while Gram –ve bacteria *Pseudomonas aeruginosa* (NCIM 5029), *Klebsiella neumonia*(NCIM 2957) *Enterobacter aerogenes* (NCIM 5139) *Salmonella typhymurium*(NCIM 2501)were sensitive to different extracts. The minimum inhibitory concentration (MIC) values were determined by using the modified broth dilution method. The isopropanol extracts showed the best antimicrobial potential. It significantly inhibited the growth of almost all the pathogenic bacteria tested. The methanol and ethanol extracts showed the highest activity on 5th and 9th day of germination. Some of the results showed that the extracts were better antibacterial agents when compared with commercially available broad-spectrum antibiotics. The results of this study revealed clear potentiality of *N. sativa* extracts as a source for anti-microbial drug.

Keywords

Antibiotics; antimicrobials; biopharmaceutic als; minimum inhibitory concentration (MIC).

Introduction

Nigella sativa L. is commonly known as black seed and belongs to the botanical family of Ranunculacea. *N. sativa* seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world (Al-Hader et al., 1993; El-Dakhakhani et al., 2000; Al-Ghamadi, 2001). *N. sativa* is considered a natural food additive and a condiment. It had also been included in the list of the natural drugs of Tibb-Al-Nabawi as it was

recommended by the Prophet Mohammed (PBUH), "*N. sativa* is the medicine for every disease except death" (Ghosheh et al., 1999; Takuri, 2003). Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries for acquiring good health and treating of many ailments including fever, common cold, headache, asthma, rheumatic diseases and various microbial infections and to expel worms from the intestine (Akhtar and Riffat, 1991; Al-Jassir, 1992, Al-Ghamadi, 2001).

The antimicrobial effects of *N. sativa* seeds against different pathogenic microbes were investigated by many researchers. The diethyl ether extract was found to cause concentration-dependent inhibition of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and a pathogenic yeast *Candida albicans* (Hanafy and Hatem, 1991). The methanol and chloroform extracts also showed high inhibitory effects against *S. aureus*, *P. aeruginosa* and *C. albicans* (Mashhadian and Rakhshandeh, 2005). The essential oil of the seeds have also shown dose-dependent antibacterial effects on Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Hosseinzadeh et al., 2007). The volatile oil and crude extracts of *N. sativa* seeds was also proved to be effective against many strains of bacteria, including those known to be highly resistant to drugs (Salman et al., 2008; Joe et al., 2009; Kamal et al., 2010; Rahman et al., 2011).

The developing microbial resistance to the existing antimicrobial agents has become a serious problem. Therefore, production of new potent agents is urgently needed, especially for hospitals and health centers, keeping in mind that studies on strains of pathogenic microbes are scarce. The present study was carried out to investigate the antimicrobial effects of *N. sativa* crude (aqueous and organic) extracts against five pathogenic bacterial strains in successive germinating phases.

Materials and Methods

Collection of *N. sativa*

Seeds of *N. sativa* were procured from a local grocery store in Lucknow and was certified by the National Botanical Research Institute, Lucknow, India.

Germination of seeds

Seed lots used for the different experiments showed germination capacities ranging from 80 to 98%. For germination studies, seeds were placed on four folds of damp filter paper at 25°C and incubated in dark till the initiation of sprouting after which they were placed at a light intensity of 100 µmol m^{-2} $s^{-1}$and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained. Germination, defined as 1 mm radicle emergence, was followed for 11d; no contamination by microorganisms was observed during this time.

Extraction method

A modification of reflux extraction and wetting procedure by Mashhadian and Rakhshandeh (2005) was used. 500 mg of *N. sativa* seeds in 20 ml of each of methanol, ethanol, isopropanol, chloroform, diethyl ether and hexane were incubated for 48h at 25°C with at least 5 times vibration per day. The viscous mixture was centrifuged for 10 min at 12000 rpm to collect the supernatant. The extracts were filtered using Whatman filter paper and evaporated using rotary

distillation apparatus and finally kept at 4°C until further testing.

Determination of *in vitro* antimicrobial activity:

Microbial strain

The aqueous and organic extracts of different germinating stages of *Nigella sativa* seeds were tested against five bacterial strains, namely *Staphylococcus epidermidis* (NCIM 2493), *Pseudomonas aeruginosa* (NCIM 5029), *Klebsiella neumonia* (NCIM 2957), *Enterobacter aerogenes* (NCIM 5139) *and Salmonella typhymurium* (NCIM 2501). The study included both Gram positive and Gram negative strains.

Inoculum preparation

The test microorganisms were maintained at 4°C on nutrient agar slants. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10 cfu ml^{-1} (Duraipandiyan et al., 2010).

Broth dilution assay

The minimum inhibitory concentration (MIC) values were determined by using the modified broth dilution method. Overnight culture of bacteria grown in nutrient both cultures were diluted 100 folds in NB (100µl bacterial cultures in 10ml NB).Gradually increasing volumes of the extracts were added to test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhabiting the bacterial growth. The tubes were incubated at 37^0C for 18-24 hours. The tubes were examined for visible turbidity and optical density of cultures was determined at 620nm using NB as control. The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC (Perez et al., 1990; Singleton P., 1999).

Agar well diffusion assay

The agar well diffusion method (Okeke et al., 2001) with some modification was used to test the antimicrobial effect of *N. sativa* crude extracts in different stages of germination**.** All media plates (9 cm in diameter) were prepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of four wells per plate. The extracts were prepared by dissolving them in 99.5% analytical Dimethyl Sulphoxide (DMSO) as an organic solvent. Streptomycin (30 µg), gentamycin (10µg), doxycycline (30µg), ampicillin (10µg), penicillin (10µg), tetracycline (10ug) were used as positive control. 100µL of each diluted microbial suspension (10^8-10^9 cfu mL^{-1}) were inoculated on nutrient agar plates using sterile cotton swab. Then, 100 µL (100µg mL^{-1}) of each extract solution, blank (DMSO) and positive control was added separately to each well of agar plate and allowed to diffuse at room temperature for 15-20 minutes and were incubated at 37°C for 24 h, all plates were examined for zones of growth inhibition and the diameter of these zones was measured. The assay was repeated thrice for each extract and bacteria. The antimicrobial effect was recorded as the mean diameter of the resulting inhibition zones of growth in millimeter.

Results and Discussion

In this study, the antibacterial effect of methanol, ethanol, isopropanol, chloroform, hexane and diethyl ether extracts of successive stages of germinating seeds of *N.sativa* was investigated on both gram positive and gram negative bacterial strains. The results of antibacterial studies indicated that different extracts of *N. sativa* showed different degrees of growth inhibition depending on the day of germination, dose and bacterial strains.

The preliminary assessment of the *in-vitro* antimicrobial effect of different germinating stages of *N. sativa* crude extracts revealed that the methanol extracts of *N. sativa* seed during different germination phases showed significant inhibitory effects on both Gram positive and Gram negative bacteria. The activity was found to be more prominent during earlier germination stages against all standard strains as compared to later stages of germination (Tables 2 and 3).The methanolic extract was found to be more effective on *K. pneumonia, P. aeruginosa and S. epidermidis.* The highest activity was observed in the seed extract against *P. aeruginosa* (18±0.35mm) at the MIC value 5.5mg ml^{-1}.

The ethanolic extract of *N. sativa* also showed considerable inhibition, it kept on rising with the increasing concentration and days of germination (Tables 4 and 5). *E. aerogenes* and *S. epidermidis* were more sensitive to the extract as compared *K. pneumonia, S. typhimurium* and *P. aeruginosa*. *P. aeruginosa* was least sensitive to this extract. The ethanol extracts showed highest activity on 9d of germination. The extract at a dose of 0.5-2.5mg ml^{-1} were proved to be effective under the conditions of the present investigation.

However, isopropanol extracts were proved to be the most powerful one against these bacteria (Table 6 and 7). It proved to be effective even at a dose of 0.2 mg ml^{-1}. It did not show day-dependent activity as it gave uniform results in all the stages of germination. The largest inhibitory zone found against all the tested strains was of 20.8 ± 0.99 mm against *K. pneumonia.*

This study also revealed that chloroform extracts of seed of *N. sativa* did not show any significant inhibitory effect but they showed some inhibition against all standard strains in later stages of germination (Table 8 and 9) while the hexane extracts proved to be an effective antibacterial agent against the tested bacterial strains (Table 10 and 11). The extract of 7d and 8d of seed germination found to be most effective on most of the tested bacterial strains. The maximum inhibition zone (18±0.12 mm) was observed against *P. aeruginosa* by the extract of 7d and 8d of seed germination. These observations were reflected in the MIC values of these extracts for different bacteria. MIC value of the hexane extract for different bacteria was lowest (5.5mg ml^{-1}) for *P. aeruginosa*.

The diethyl ether extracts of different germination phases of *N. sativa* seeds exhibited significant antibacterial activity (Table 12 and 13). The diethyl ether extracts of *N. sativa* showed good activity against *E. aerogenes* showing inhibition zones of 11- 19 mm. The diethyl ether extract showed concentration dependent activity rather than day dependent activity. The extract exhibited maximum inhibition against *E. aerogenes* at a MIC value

Table.1 Activity of standard antibiotics against selected bacterial strains

S. No.	Organism	ZONE OF INHIBITION (mm)					
		ST	GN	DO	AM	PN	TC
1	*E. aerogenes*	23±0.37	18±0.43	12±0.39	0	0	19±0.44
2	*K. pneumoniae*	21±0.57	18±0.49	19±0.33	0	0	25±0.59
3	*P. aeruginosa*	26±0.38	18±0.62	12±0.58	0	0	10±0.39
4	*S. epidermidis*	23±0.58	18±0.49	12±0.69	0	0	19±0.43
5	*S. typhimurium*	22±0.75	18±0.38	12±0.58	0	0	10±0.39

*All the tested bacterial strains were resistant to penicillin and ampicillin.

Table.2 Antimicrobial activity of methanol extract of *N. sativa*

S. No.	Organism	Zone of Inhibition (mm) Days									
		DMSO	0	4	5	6	7	8	9	10	11
1	*E. aerogenes*	_	--	10±0.66	10±0.33	_	12±0.57	10±0.66	_	_	_
2	*K. pneumoniae*	_	10±0.57	10±0.20	10±0.88	10±0.33	12±0.38	10±0.85	10±0.76	10±0.23	_
3	*P. aeruginosa*	_	18±0.35	17±0.56	14±0.42	14±0.35	14±0.50	12±0.99	10±0.98	11±0.49	10±0.76
4	*S. epidermidis*	_	11±0.33	11±0.35	11±0.65	10±0.76	12±0.23	13±0.14	14±0.29	13±0.34	13±0.83
5	*S. typhimurium*	_	12±0.45	12±0.45	12±0.45	_	_	_	_	_	_

* Data is a mean of three replications. *Values are mean inhibition zone (mm) ± S.D of three replicates
* 10% DMSO - Negative control. *"-" No inhibition observed.

Table.3 Minimum inhibitory concentration of methanol extract of *N. sativa*

S. No	Organism	Minimum inhibitory concentration (mg ml^{-1}) Days								
		0	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	_	6.5±0.57	5.5±0.55	_	7±0.65	8.5±0.57	_	_	_
2	*K. pneumoniae*	9±0.91	8.5±0.87	_	8±0.32	5.5±0.56	_	9±0.41	8.5±0.53	_
3	*P. aeruginosa*	5.5±0.54	6±0.89	5.5±0.56	_	_	_	6.5±0.63	6±0.21	5.5±0.91
4	*S. epidermidis*	5.5±0.87	6±0.83	5.5±0.91	7±0.32	5.5±0.25	6.5±0.57	6.5±0.65	6±0.93	6±0.57
5	*S. typhimurium*	6.5±0.75	_	6.5±0.57	_	_	_	_	_	_

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.4 Antimicrobial activity of ethanol extract of *N. sativa*

S. No.	Organism		Zone of Inhibition (mm) Days								
		DMSO	**0**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	--	11±0.57	12±0.37	12±0.57	12±0.81	12±0.37	12±0.57	14±0.51	12±0.35	12±0.91
2	*K. pneumoniae*	--	12±0.37	12±0.57	10±0.81	10±0.57	10±0.37	10±0.37	13±0.95	12±0.25	11±0.27
3	*P. aeruginosa*	--	10±0.57	10±0.81	--	--	--	10±0.37	13±0.81	--	--
4	*S. epidermidis*	--	12±0.57	11±0.57	12±0.81	11±0.81	11±0.47	12±0.35	15±0.29	14±0.57	11±0.81
5	*S. typhimurium*	--	--	10±0.57	10±0.57	10±0.81	10±0.57	10±0.57	13±0.57	13±0.57	13±0.37

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.5 Minimum inhibitory concentration of ethanol extract of *N. sativa*

S. No	Organism	Minimum inhibitory concentration (mg ml^{-1}) Days								
		0	4	5	6	7	8	9	10	11
1	*E. aerogenes*	2.5±0.57	2.5±0.57	2.5±0.37	2.0±0.81	2.0±0.81	2.0±0.57	2.5±0.57	2.5±0.35	2.5±0.81
2	*K. pneumonia*	3±0.32	3.5±0.22	3±0.57	2.5±0.38	2.5±0.62	3±0.75	2.5±0.99	2.5±0.57	3±0.81
3	*P. aeruginosa*	2.5±0.44	3±0.47	_	_	_	3.5±0.57	3.5±0.81	_	_
4	*S. epidermidis*	2.5±0.57	2.5±0.81	2.5±0.21	3±0.57	2.5±0.32	3±0.81	3.5±0.57	3±0.57	2.5±0.81
5	*S. typhimurium*	_	0.5±0.79	0.5±0.81	1.5±0.37	1.0±0.25	1.5±0.71	0.5±0.35	0.5±0.23	0.5±0.57

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.6 Antimicrobial activity of isopropanol extract of *N. sativa*

S. No.	Organism	DMSO	ZONE OF INHIBITION (mm) Days								
			0	4	5	6	7	8	9	10	11
1	*E. aerogenes*	--	15±0.21	17±0.35	17±0.21	17±0.81	17±0.57	16±0.21	17±0.57	18±0.99	17±0.57
2	*K. pneumonia*	--	20±0.57	20±0.37	18±0.77	18±0.81	17±0.27	17±0.35	20±0.99	20±0.29	18±0.21
3	*P. aeruginosa*	--	18±0.57	19±0.81	18±0.57	18±0.37	17±0.21	16±0.21	18±0.21	19±0.57	18±0.57
4	*S. epidermidis*	--	18±0.81	19±0.35	18±0.57	17±0.15	16±0.28	16±0.57	16±0.81	18±0.15	18±0.19
5	*S. typhimurium*	--	20±0.55	20±0.79	19±0.57	19±0.35	17±0.35	18±0.81	19±0.57	17±0.35	17±0.23

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.7 Minimum inhibitory concentration of isopropanol extract of *N. sativa*

S. No.	Organism	Minimum inhibitory concentration (mg ml^{-1}) Days								
		0	4	5	6	7	8	9	10	11
1	*E. aerogenes*	0.2±0.13	0.3±0.56	0.3±0.47	0.3±0.12	0.3±0.53	0.3±0.81	0.2±0.55	0.2±0.57	0.2±0.47
2	*K. pneumoniae*	0.2±0.15	0.2±0.81	0.3±0.57	0.3±0.99	0.3±0.31	0.3±0.57	0.2±0.45	0.2±0.99	0.2±0.81
3	*P. aeruginosa*	0.3±0.19	0.3±0.57	0.4±0.81	0.5±0.13	0.5±0.95	0.5±0.87	0.5±0.17	0.5±0.12	0.5±0.09
4	*S. epidermidis*	0.2±0.12	0.2±0.09	0.4±0.57	0.4±0.65	0.4±0.85	0.4±0.12	0.4±0.08	0.3±0.09	0.2±0.00
5	*S. typhimurium*	0.2±0.19	0.2±0.77	0.3±0.91	0.3±0.09	0.3±0.05	0.2±0.15	0.2±0.12	0.2±0.95	0.2±0.82

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed

Table.8 Antimicrobial activity of chloroform extract of *N. sativa*

S. No.	Organism	DMSO	ZONE OF INHIBITION (mm) Days								
			0	4	5	6	7	8	9	10	11
1	*E. aerogenes*	--	--	--	--	10±0.51	11±0.09	--	--	--	--
2	*K. pneumoniae*	--	--	10±0.05	10±0.25	11±0.15	12±0.55	--	--	--	--
3	*P. aeruginosa*	--	--	10±0.91	11±0.11	11±0.57	10±0.91	--	--	--	--
4	*S. epidermidis*	--	--	--	--	--	--	--	--	--	--
5	*S. typhimurium*	--	--	10±0.57	12±0.13	11±0.81	10±0.39	--	--	--	--

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates.

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.9 Minimum inhibitory concentration of chloroform extract of *N. sativa*

S. No.	Organism	Minimum inhibitory concentration (mg ml^{-1}) Days								
		0	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	_	_	_	10±0.57	9.5±0.11	_	_	_	_
2	*K. pneumoniae*	_	9±0.75	9±0.99	9±0.05	8.5±0.99	_	_	_	_
3	*P. aeruginosa*	_	8.5±0.66	9±0.15	10±0.99	8.5±0.81	_	_	_	_
4	*S. epidermidis*	_	_	_	_	_	_	_	_	_
5	*S. typhimurium*	_	9±0.23	8±0.35	8.5±0.57	10±0.91	_	_	_	_

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.10 Antimicrobial activity of hexane extract of *N. sativa*

S. No.	Organism	Zone of inhibition (mm) Days									
		DMSO	**0**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	--	_	10±0.11	10±0.85	10±0.75	10±0.57	11±0.19	10±0.12	10±0.57	_
2	*K. pneumoniae*	--	12±0.31	13±0.45	13±0.12	14±0.97	12±0.81	17±0.55	12±0.89	11±0.49	10±0.11
3	*P. aeruginosa*	--	_	12±0.65	12±0.77	12±0.89	16±0.19	18±0.12	12±0.51	17±0.51	_
4	*S. epidermidis*	--	11±0.75	12±0.66	11±0.15	10±0.05	11±0.09	11±0.65	10±0.67	_	_
5	*S. typhimurium*	--	12±0.11	10±0.09	_	10±0.19	10±0.42	10±0.77	10±0.95	10±0.81	10±0.81

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table 11: Minimum inhibitory concentration of hexane extract of *N. sativa*

S. No	Organism	Minimum inhibitory concentration (mg ml^{-1}) Days								
		0	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	_	8.5±0.55	6±0.49	6.5±0.71	7.5±0.31	5.5±0.34	6±0.51	6.5±0.23	_
2	*K. pneumoniae*	8.5±0.71	6.5±0.76	6±0.34	7±0.54	5.5±0.23	6.5±0.34	7±0.21	5.5±0.55	5.5±0.45
3	*P. aeruginosa*	_	6.5±0.83	6.5±0.65	6.5±0.98	5.5±0.45	5.5±0.55	5.5±0.75	6.5±0.43	_
4	*S. epidermidis*	7.5±0.33	5.5±0.67	7±0.32	7±0.98	6±0.44	6.5±0.87	6±0.65	_	_
5	*S. typhimurium*	6.5±0.55	6.5±0.56	7±0.77	8±0.34	6.5±0.28	7±0.27	6.5±0.78	6±0.63	5.5±0.48

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table 12: Antimicrobial activity of diethyl ether extract of *N. sativa.*

S. No.	Organism	Zone of inhibition (mm)Days									
		DMSO	**0**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	--	_	16±0.78	13±0.50	14±0.11	15±0.39	13±0.45	16±0.91	13±0.12	13±0.34
2	*K. pneumoniae*	--	11±0.49	13±0.79	14±0.54	14±0.13	10±0.42	10±0.48	14±0.92	13±0.14	15±0.36
	P. aeruginosa	--	12±0.51	14±0.81	14±0.56	14±0.16	14±0.46	15±0.49	14±0.94	15±0.16	15±0.38
4	*S. epidermidis*	--	11±0.54	16±0.83	15±0.57	10±0.19	13±0.48	13±0.52	13±0.96	11±0.18	11±0.39
5	*S. typhimurium*	--	12±0.57	10±0.85	10±0.59	10±0.20	10±0.45	10±0.45	11±0.99	11±0.20	14±0.41

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates

∗ 10% DMSO - Negative control. ∗"-" No inhibition observed.

Table.13 Minimum inhibitory concentration of diethyl ether extract of *N. sativa*

S. No	Organism	Minimum inhibitory concentration (mg ml^{-1}) Days								
		0	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*E. aerogenes*	1.0±0.75	0.25±0.31	0.25±0.67	0.25±0.78	0.5±0.42	0.5±0.56	0.25±0.22	0.25±0.87	0.5±0.97
2	*K. pneumoniae*	0.5±0.21	0.5±0.34	0.75±0.69	0.75±0.71	1.0±0.35	1.0±0.58	0.75±0.14	0.5±0.71	0.5±0.23
3	*P. aeruginosa*	1.0±0.21	0.5±0.37	0.5±0.69	0.5±0.82	0.5±0.37	0.5±0.99	0.5±0.16	0.5±0.89	1.0±0.35
4	*S. epidermidis*	0.75±0.23	1.0±0.65	1.0±0.31	0.5±0.84	0.5±0.39	0.5±0.18	0.5±0.18	1.0±0.55	1.0±0.65
5	*S. typhimurium*	1.0±0.25	1.0±0.41	0.75±0.73	0.75±0.86	0.75±0.41	0.75±0.63	0.75±0.20	1.0±0.92	1.0±0.25

∗ Data is a mean of three replications. ∗Values are mean inhibition zone (mm) ± S.D of three replicates * 10% DMSO - Negative control. ∗"-" No inhibition observed.

0.25mg ml^{-1} and the least activity was observed against *Salmonella typhimurium*.

Nigella sativa is frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar, 2004). Several investigations have been directed towards their antibacterial properties (Voravuthikunchai et al., 2005).

The data of the preliminary assessment of the *in vitro* antimicrobial effect of successive germinating stages of *N. sativa* seeds revealed some basic outcomes. First, the aqueous extracts showed significant antibacterial potential against all the tested strains only during the later stages of germination. High tannin and flavonoid content might also be responsible for the antibacterial activity in later stages of thymoquinone. Thymol is a phenolic alcohol present in the essential oil of *N.* germination (Ahmad et al., 2010) which are in fully agreement with other studies on the same plant species. While in a study the extract was found to be ineffective on standard strains (Mashhadian and Rakhshandeh, 2005). However, other studies have shown that extracts of these seeds could have an inhibitory effect on the growth of the yeast, *C. albicans* in the liver, spleen and kidneys of infected mice (Khan et al. 2003).

Second, the organic extracts at a dose of 0.2-5mg ml^{-1} were proved to be effective under the conditions of the present investigation. The methanol and ethanol extracts were found active against both Gram-positive and Gram-negative bacteria. The activity shown by the methanol extract may be due to the presence of some active phytoconstituents such as thymol and *sativa* (Randhawa and Al- Ghamdi, 2002) that has been reported to possess

antibacterial activity (Karapinar and Aktug, 1987). The presence of tannins was also reported in seeds of *N. sativa*, which could be extracted by methanol (Eloff, 1998). A number of studies have reported antimicrobial properties of tannins (Scalbert, 1991). Alhaj et al. (2008) and Masood (2008) tested the antibacterial activity of methanolic extracts of *N. sativa* seeds against number of bacterial strains and found excellent results. Salman et al. (2008) also reported that the methanolic extract showed remarkable dose dependant antibacterial activity against the tested strains up to a dilution of 1:50 as evident from the zones of inhibition. Khalid et al. (2011) showed that methanolic extracts of *N. sativa* seeds showed maximum zone of inhibition of 18 mm against *B. subtilis* and *S. aureus*, 12 mm against *P. aeruginosa* while it exhibited no activity against *E. faecalis* and *S. typhimurium.*

The ethanol extracts showed highest activity on 9d of germination. Previous work on *N. sativa* has also shown that the ethanol, ethyl acetate and water extracts of the seeds showed only moderate activity against Gram-positive strains (Awadh Ali et al.. 2001). It was also reported that all tested strains of Methicillin resistant *Staphylococcus aureus* (MRSA) were sensitive to *N. sativa* extract at a concentration of 4 mg/disc while the extract had an MIC range of 0.2-0.5 mg ml^{-1} (Hannan et al., 2008).The results was also coincides with the findings of Zahra et al. (2011) who showed that ethanolic extract showed some activity against *B. subtilis and E. coli.*.

However, isopropanol extracts were proved to be the most powerful one against these bacteria. It proved to be effective even at a dose of 2.5mg ml^{-1}. It inhibited the growth of all the tested pathogenic bacterial strains. It did not show day -dependent activity as it gave uniform results in all the stages of germination. Diethyl ether extracts were next in order followed by hexane, methanol and ethanol extracts. Aqueous and chloroform extracts showed negligible activity. These observations were in agreement of the work of Landa et al. (2009) and Mashhadian & Rakhshandeh (2005) who reported weak antimicrobial activity for the chloroform extract of *N. sativa* against various microorganisms.

The organic solvents used to prepare extracts of different germinating stages of *N. sativa*, were methanol, ethanol, isopropanol, chloroform, hexane and diethyl ether. The methanol, ethanol, choloroform, hexane and diethyl ether have been used widely (Hanafy and Hatem, 1991; Mashhadian and Rakhshandeh ,2005; Hosseinzadeh et. al., 2007), while the isopropanol was never used before at least with seeds of *N. sativa*. The results showed that the isopropanol extract of *N. sativa* seeds in different germinating stages had the best antimicrobial activity and the chloroform extract had a weaker effect. Although the extracts used were in crude form, they proved superior over some of the standard antibiotics like penicillin and ampicillin as the strains were resistant to these antibiotics (Table 1). This could be explained by the presence of potent antibacterial constituents in high concentrations in these extracts. No previous reports have been published for evaluating the efficiency of isopropanol extracts on the different bacterial infections. The potency of the extracts to inhibit the growth of different strains was significantly high, which provides an evidence for the presence of highly active antibacterial agents in that extracts.

Therefore, further studies should be followed to isolate pure active antimicrobial agents for testing specific antimicrobial effect.

Third, almost all the extracts showed highest activity on 7d and 9d of germination. This might be due to the higher levels of bioactive compounds, protein and carbohydrate content of the extract (Wafaa et al., 2007, Kamal et al., 2010). Flavonoid content might also be responsible for the antibacterial activity in later stages of germination.

Finally, the extract also revealed superior inhibitory effect over the standard drug ampicillin (10 µg), penicillin (10 µg), and tetracycline (10ug). Our results are in agreement with others who showed that *N. sativa* extracts produce antimicrobial activity against a broad range of microbes and especially on multiple antibiotic resistant bacteria. (Morsi, 2000.). Further studies on the activity-directed fractionation for the isolation of respective pure compounds from the extracts may result in interesting results.

N. sativa seed possesses antimicrobial activity against several multidrug resistant pathogenic bacteria and germination increased the biological activity. Moreover, its edibility might increase its use as a potent antimicrobial drug on wider scale as it is used as a common spice. Further studies are required to advocate its systemic use in infectious diseases.

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